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ABOMASAL SECRETION AND MOTILITY IN SHEEP

Effect of diet and digesta components

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Abstract

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In sheep fitted with re-entrant cannulas in the proximal duodenum, secretory rates of acid in the abomasum increased with protein or buffering content of the rations.

In sheep fitted with an abomasal infusion tube and with duodenal re-entrant cannulas, proteins and inorganic buffers continuously infused into the abomasum stimulated acid secretion. Intra-abomasal infusion of amino acids had little effect on secretion of acid. Soya protein, partly hydrolysed in advance, was a more active stimulator of acid secretion than unhydrolysed soya protein. Volatile fatty acids and L-lactic acid stimulated acid secretion slightly.

After infusion into the abomasum of soya protein, partly hydrolysed in advance, or of a $KHCO_3$ buffer, concentration of gastrin in blood plasma increased. Infusion of α-alanine, β-alanine and glycine did not alter gastrin concentration.

Pepsin activity of duodenal digesta did not correspond with that to be expected from abomasal secretion of pepsinogen. In tests in vitro, proteins inhibited pepsin activity.

Blood flow in the abomasal mucosa, as measured by aminopyrine clearance, was not related to abomasal secretion of acid. Mucosal blood flow was measured in a feeding trial and after intra-abomasal infusion of soya protein or of a $KHCO_3$ buffer into the abomasum.

Abomasal antral and pyloric contractile activity, recorded in sheep fitted with strain gauges, were regular. The pattern depended little on feeding regime, and was not affected by continuous infusion of soya protein into the abomasum. Discontinuous infusions of soya protein, of a $KHCO_3$ buffer, of amino acids, and of fatty acids into the abomasum showed minor effects on antral and pyloric contractile activity. The motility data indicated that propulsion of abomasal digesta into the duodenum cannot be explained by frequency and intensity of antral and pyloric contractile activity only.

Free descriptors: gastrin, pepsin and blood flow.

Contents

1 General introduction

Functionally, the role of the abomasum in ruminants is analogous to that of the conventional mammalian stomach of monogastric animals. Digesta leaving the forestomachs are attacked and digested under acid conditions by the proteolytic enzymes of the abomasal juice. In monogastric animals, gastric secretion has been studied thoroughly by means of oesophageal tubes or simple gastric cannulas. In ruminants, these approaches cannot be used since the abomasum cannot be reached by an oesophageal tube, and since no pure abomasal juice can be collected from an abomasal cannula, because digesta enter the abomasum in a rather regular pattern.

At the onset of the experiments the regulation of the function of the abomasum under steady-state conditions was poorly understood. A distinct need for such information was derived from the proposed relationship between gastric function and gastric mucosal injury (Davenport, 1972), regarding the increasing occurrence of abomasal ulcers in adult cattle with fatal haemorrhage (Aukema, 1971; Aukema & Breukink, 1974).

Hill (1960) accommodated the problem of the continuous presence of digesta in the abomasum by emptying the forestomachs. Ash (1961) and McLeay & Titchen (1970, 1973, 1974, 1975) studied abomasal secretion in sheep by preparing vagally innervated fundic pouches, surgically isolated fundic compartments. The first approach has the disadvantage that it is hardly possible to work under steady-state physiological conditions, when forestomach digesta are removed. Vagally innervated fundic pouches are capable to secrete abomasal juice induced by neural or hormonal stimuli. From these pouches pure abomasal juice can be collected, but since digesta are prevented to enter these pouches, degeneration of the mucosa may occur. Furthermore, it is difficult to extrapolate fundic pouch secretion to total abomasal secretion, because of the difficulties in quantifying the respective fundic mucosal areas. Besides, the vagal innervation of fundic pouches is supposed to be saved, but the intrinsic plexuses are cut.

In the present experiments, abomasal secretion of acid, as affected by ration composition and by continuous intra-abomasal infusion of various substances, additionally to the digesta present in the abomasum, was studied by determination of the amounts of total acid and of chloride leaving the abomasum. For these experiments, sheep were chronically fitted with duodenal re-entrant cannulas for sampling of duodenal digesta. With these sheep, abomasal secretion could be studied under steady-state physiological conditions during prolonged periods.

In monogastric animals, regulation of gastric secretion of acid is achieved by neural and hormonal mechanisms. In ruminants, it is obvious that abomasal secretion of acid is governed by similar mechanisms. As far as the hormonal aspects of abomasal secretion of acid are concerned, it became obvious in the course of our research that the release of gastrin by the mucosa of the abomasal antrum plays an essential role in the regulation of

abomasal secretion of acid, since in sheep antrectomy resulted in a marked reduction of abomasal secretion of acid (McLeay & Titchen, 1975). Data on gastrin concentrations in blood plasma in ruminants are still lacking. Therefore we studied gastrin concentrations in serum of sheep, as influenced by continuous intra-abomasal infusion of various substances.

Secretion of hydrogen and chloride ions by the glands of the abomasal fundus succeeds against an electrochemical gradient. Hydrogen ions and energy are proposed to be delivered by the intracellular oxidative metabolism. For this reason a relation between blood flow in the gastric mucosa and gastric secretion of acid in non-ruminants has been suggested, although literature reports on this subject are rather contradictory. In ruminants, no data on blood flow in the abomasal mucosa are available. In the present experiments, the relation between abomasal secretion of acid and blood flow in the abomasal mucosa in sheep was studied.

Abomasal digesta are propelled into the duodenum of sheep in a rather regular way. It is likely that propulsion of abomasal digesta is achieved by peristaltic antral activity. Ehrlein & Hill (1970) studied abomasal antral and pyloric motility in goats, as affected by amount and composition of duodenal contents. Recording of abomasal motility in sheep during more prolonged periods was carried out by Laplace (1970). It was suggested that abomasal hypermotility coincides with periods of abomasal emptying. In the present experiments, abomasal antral and pyloric motility, as influenced by amount and composition of abomasal digesta, were recorded during prolonged periods in order to study the function of abomasal motility in abomasal emptying.

2 Survey of the literature

2.1 INTRODUCTION

Ruminants eat plant food mainly, and like other herbivores they developed a digestive system, in which micro-organisms are present in order to break down certain plant structural polymers, such as cellulose and hemicellulose. In non-ruminants, microbial fermentation of plant food constituents may take place in caecum or colon. Ecologically, ruminants developed a compound stomach, subdivided in four compartments, designated as rumen, reticulum, omasum and abomasum.

During food intake ruminants masticate roughages incompletely. However, after a meal regurgitation and remastication of rumino-reticular contents occurs. According to the physical structure of the ration, one or more cycles of rumination are needed in order to degrade roughage particles physically, and thus to increase the availability of the roughage particles for the rumino-reticular microbial fermentation process (Stevens & Sellers, 1968).

Under normal conditions, not only carbohydrates, i.e. starch, but also dietary proteins are fermented largely, resulting in a production of volatile fatty acids, carbondioxide, methane and ammonia. Carbohydrate and protein breakdown products may also be utilized for the synthesis of microbial cell constituents. The rate of degradation of dietary protein is influenced by different factors, such as dietary protein level, protein availability, digesta retention time in the rumino-reticulum and rumino-reticular fermentation rate. Ammonia, originating from the fermentation process, may be utilized by the rumino-reticular micro-organisms, or may be absorbed through the rumino-reticular wall, transported to the liver, where it is converted to urea. At low dietary protein levels the major part of the degraded dietary nitrogen may be converted to microbial nitrogen, in the form of bacterial or protozoal protein. Ingested non-protein nitrogen, or recycled endogenous urea nitrogen may be utilized as well (Hungate, 1968; Moir, 1968).

Rumino-reticular digesta pass fairly continuously from the reticulum to the omasum. In the omasum absorption of water, volatile fatty acids and bicarbonate occurs to a certain extent. In goats and sheep, the rate of fermentation was found to be lower in the omasum than in the rumino-reticulum (von Engelhardt & Giesecke, 1972).

From the omasum digesta enter the abomasum, which corresponds morphologically and functionally to the gastric secretory stomach of the non-ruminant mammals.

2.2 FUNCTIONAL ANATOMY OF THE RUMINANT STOMACH

Food enters the rumino-reticulum through the oesophagus at the cardia. Rumen and reticulum are partially separated by the rumino-reticular fold, a pillar which does not totally

restrict flow of digesta between these two compartments. The reticulum communicates with the omasum through the reticulo-omasal orifice, located at the end of the oesophageal groove.

In cattle as well as in the smaller ruminants, the epithelial cells of the three forestomach compartments can be classified as keratinized, stratified, squamous cells, which do not produce mucus and are non-glandular. The whole of the internal surface of the rumen is covered with papillae, which are most dense in the ventral part of the rumen in both cattle and sheep. The reticular epithelial membrane is raised into a honeycomb pattern and covered with small conical papillae (Comline et al., 1968). To the greater curvature of the omasum a large number of laminae is attached, which vary in size. The free borders of these laminae lie parallel with the omasal canal at the lesser curvature, which is covered by papillae pointing in the direction of the abomasum, and extends mainly downward from the inlet of the omasum to the outlet, the large omaso-abomasal orifice. At the surface, the omasal laminae bear small horny papillae and longer ones at the free borders. Becker et al. (1963) found in cattle the total number of omasal leaves to vary from 89-192, with an average of 152 at the greater curvature. In the ovine omasum the laminae are less numerous. Stevens & Stettler (1966) calculated in bovines a total area of the omasal laminae, which was equivalent to about one third of the total forestomach area.

The relative size of the various parts of the ruminant stomach varies between species. On the basis of water fill, Warner & Flatt (1965) mentioned percentages of total stomach volume for the bovine and ovine omasum of 6-7 and 2-3, and for the bovine and ovine abomasum of 6-8 and 11-12, respectively.

The abomasum constitutes the glandular compartment of the ruminant stomach and in the adult ruminants it nearly resembles the simple stomach of non-ruminants. Digesta enter the abomasum through the omaso-abomasal orifice and leave it through the pylorus to the duodenum. The abomasal mucosa is subdivided into a small region of cardiac glands adjacent to the omaso-abomasal junction, a much larger fundic part with the mucous membrane thrown into 10-15 large longitudinal spiral folds, and an antral area in the narrow tubular part towards the pylorus.

Transition from omasum to abomasum is sharply defined by the change from stratified to simple columnar epithelium. The cardiac mucosa is composed of mucus secreting glands. In the fundic part, the mucosa is also lined by a layer of simple columnar cells. The fundic surface has numerous invaginations, the gastric foveolae, which are also covered by the same type of mucous cells. At the bottom of these foveolae the fundic tubular glands empty. These fundic glands contain specialized secretory cells, the hydrochloric acid secreting oxyntic or parietal cells and the pepsinogen secreting peptic or chief cells. In the distal part of the abomasum, the antral part, the mucosa is composed of mucus secreting glands (Hill, 1951). Although the major secretion from the antral glands is mucus, in sheep peptic activity was demonstrated in juice collected from an innervated antral pouch by McLeay & Titchen (1975), and Grossman & Marks (1960) convincingly confirmed the presence of some pepsinogen in dog antral pouches, whereas Hanley et al. (1966) isolated pepsinogen from human, pig and rabbit antral mucosa.

The major blood supply of the compound ruminant stomach is derived from the coeliac

artery, which supplies the hepatic artery, the left and right ruminal artery and the omaso-abomasal artery. This omaso-abomasal artery divides into a ventral and dorsal branch. The dorsal branch supplies the greater curvature of the omasum and then runs in the lesser omentum along the lesser curvature of the abomasum (left gastric artery). The ventral omaso-abomasal artery lies between the omasum and reticulum and gives off a cranial branch to the reticulum. The main artery continues ventrally and supplies the lesser curvature of the omasum and the greater curvature of the abomasum (left gastro-epiploic artery). Two branches of the hepatic artery, the right gastric artery and the right gastro-epiploic artery supply the proximal part of the duodenum and anastomose in the antral region of the abomasum with the left gastric artery and the left gastro-epiploic artery, respectively. The veins normally accompany the arteries and empty in the portal vein (Comline et al., 1968).

Like all splanchnic organs the compound ruminant stomach is innervated both parasympatheticly and sympatheticly. The parasympathetic innervation is derived from the dorsal and ventral vagus nerve, which arise after a variable number of anastomoses from the two vagus nerves, located left and right to the oesophagus. The dorsal vagal trunk supplies the prevertebral coeliaco-mesenteric plexus, the rumen, reticulum and omasum, and the visceral abomasal surface. The ventral vagal trunk also contributes to the coeliaco-mesenteric plexus and to the rumen, reticulum and omasum, and innervates the parietal abomasal surface. From the ventral vagus nerve the long pyloric nerve originates, which gives off branches to the hepatic plexus near the porta of the liver and turns ventrad parallel to the duodenum and right gastric artery to innervate the duodenum and the antral region of the abomasum. The vagus nerves contain both afferent and efferent fibres. Leek (1968) estimated both vagal trunks to consist for about 90 percent of afferent fibres. All efferent vagal fibres are preganglionic and cholinergic.

The coeliaco-mesenteric plexus is supplied by branches of the dorsal and ventral vagus nerve and by the splanchnic nerves, arising from the distal thoracal, the lumbal and the proximal sacral sympathetic ganglions. The efferent splanchnic nerve fibres originate in the spinal cord and synapse for the first time in the coeliaco-mesenteric plexus. Therefore these splanchnic nerve fibres are preganglionic and cholinergic. After leaving the plexus, the postganglionic adrenergic fibres join mainly the arteries and innervate the splanchnic organs sympatheticly (Habel, 1956; Comline et al., 1968).

The parasympathetic and sympathetic fibres are supposed to terminate in or near by the ganglions of the intramural plexuses of the ruminant stomach wall. In non-ruminants, Daniel (1969) postulated the intramural postganglionic fibres to be non-adrenergic. The sympathetic fibres are not supposed to innervate the gastro-intestinal musculature directly. Hence sympathetic stimulation is thought to act indirectly by inhibition of parasympathetic activity or by decreasing blood flow in the gastro-intestinal mucosa (Daniel, 1968). In the non-glandular part of the ruminant stomach the intramural plexus ganglions are mainly located between the smooth muscular layers. This plexus is called the myenteric plexus. In the wall of the abomasum a second intramural plexus, the submucosal plexus, is present. Habel (1956) postulated the ganglions of this plexus to act on the abomasal secretory cells, and the myenteric ganglions to exhibit a motor or receptor function.

In general, sympathetic and parasympathetic activities are regarded to behave as each

others antagonists, parasympathetic activation stimulating, and sympathetic activation inhibiting gastro-intestinal activity. Several deviations of such a model, however, have been reported (Comline & Titchen, 1951; Duncan, 1953; Daniel, 1969; Sharma et al., 1972; Sanders, 1976).

2.3 SECRETORY FUNCTION OF THE ABOMASUM

2.3.1 Introduction

The role of the abomasum, the glandular part of the ruminant stomach, is strictly comparable to that of the mammalian simple stomach. Digesta reach the abomasum from the omasum and are attacked and digested under acid conditions by pepsin present in the abomasal juice.

In comparison with the limited number of reports on secretory function of the abomasum in ruminants, numerous reports on non-ruminant gastric secretion are available in the literature. No evidence is available that secretion of abomasal juice in ruminants is functionally different from the secretion of gastric juice in non-ruminants. Therefore several reports from the literature on gastric secretion in non-ruminants are cited below.

In monogastric animals, food enters the stomach discontinuously, depending on the eating pattern. After partial digestion, digesta leave the stomach in a more regular pattern, which depends on different factors, such as composition and amount of the gastric contents. When the stomach is almost emptied or when the pH of the gastric contents is low, the gastric glands secrete at a basal rate. Just before, during and after food intake, four phases of gastric secretion are distinguishable.

In trained animals, it is possible to stimulate gastric secretion by conditional stimuli. Also when food is seen or smelled gastric secretion starts. This first phase is called the psychic phase of gastric secretion. During food intake, food is masticated, salivary secretion is stimulated, and again secretion of gastric juice increases. Using experimental animals, fitted with an oesophageal cannula, swallowed food can be prevented to reach the stomach (sham feeding), and under these experimental conditions pure gastric juice can be collected from a gastric cannula. This gastric juice is secreted due to the second phase of gastric secretion, the cephalic phase. Both gastric secretory phases, the psychic and cephalic phase, are mediated through neural pathways. After vagotomy the gastric secretory response to both psychic stimulation and sham feeding is abolished (Davenport, 1971).

When food enters the stomach, gastric secretion is stimulated by the third secretory phase, the gastric phase. The gastric fundus is distented and under experimental conditions distension of the gastric fundus or antrum, for instance after inflation of a balloon, was found to stimulate gastric secretion in humans and dogs (Grossman, 1962; Gedde-Dahl, 1974; Walsh, 1975). This secretory response is thought to be mediated partly through neural pathways after excitation of tension receptors, which act on the gastric glands through centrally or intramurally mediated reflexes. Stimulation of abomasal secretion of acid by distension of the sheep abomasum was postulated by Leek & Harding (1975) to arise from vagally mediated reflexes. Blair et al. (1975) found a reduced gastric secretory

activity of acid after stimulation of sympathetic fibres in the anaesthetized cat.

Composition of gastric contents has also been shown to be involved in the regulation of gastric secretory activity, partly mediated through neural transmissions responding to chemo-receptors (Konturek et al., 1976). On the other hand, specific chemical agents have been mentioned as potent releasers of the hormone gastrin. This hormone stimulates gastric secretion and is released by specific cells that are mainly present in the mucosa of the gastric antrum (Davenport, 1971).

Propulsion of the gastric contents from the stomach into the duodenum starts the fourth phase of gastric secretion, the intestinal phase, causing mainly inhibition of gastric secretion. Perfusion of the duodenum with acid, with proteins or fats, or with hypertonic solutions, inhibits gastric secretory activity. This inhibitory response is partly mediated neurally, but also by hormones released by the duodenal mucosa, such as secretin or cholecystokinin (Sircus 1958; Alday & Goldsmith, 1973).

2.3.2 Psychic and cephalic stimulation of acid secretion

In monogastric animals, the gastric glands start secreting just prior to feeding or when animals are fed. Hill (1960), however, did not notice an obvious relation between food intake and secretory activity of innervated pouches of the abomasal fundus in sheep, fed ad libitum. Ingestion of food after food depreviation for a period of 24 h caused a secretory response with a maximal output of acid and pepsin within 1-1.5 h after feeding commenced. In earlier experiments with sheep fed ad libitum, Masson & Phillipson (1952) did not observe either an immediate abomasal secretory response to feeding.

Hill (1960) was not able to stimulate abomasal secretion of acid in sheep by sham feeding or feeding when the forestomachs were free from digesta. Based on these results, Hill concluded that a psychic or cephalic phase of abomasal secretion is absent in sheep. In monogastric animals, vagal stimulation of gastric acid secretion may be tested by insulin-induced hypoglycaemia. In sheep, such a response of abomasal secretion of acid was found, but was thought to be mainly attributable to an increase in the rate of passage of digesta to the abomasum caused by rumino-reticular hypermotility. No increased acid output from innervated pouches of the abomasal fundus was noticed after insulin-induced vagal stimulation when the forestomachs were empty. An increased pepsin output was demonstrated under these conditions, and therefore the conclusion was drawn that if a cephalic phase of abomasal secretion is of importance, it should be confined mainly to stimulation of pepsinogen secretion.

McLeay & Titchen (1970) demonstrated a secretion response of innervated pouches of the abomasal fundus in sheep to teasing with food and to feeding after a period of fasting. Under restricted feeding conditions a comparable response was found. They did not agree with the conclusions of Hill, that this increased secretory response should be entirely due to an increased inflow rate of digesta into the abomasum, as induced by an increased rumino-reticular motility. They observed an abomasal secretory activity, in response to feeding or teasing with food, which did not parallel rumino-reticular motility. Whereas frequency of rumino-reticular motility progressively declined as feeding continued, an initial peak of abomasal secretion of acid was commonly found within 30-45 min after eating

commenced. A second sustained and greater increase in acid secretion was noticed from 60 min onwards after eating. This second abomasal secretory response of acid was ascribed to an increased passage rate of digesta into the abomasum, starting 1-3 h after feeding under a restricted feeding regime, as was also reported by Phillipson & Ash (1965) in sheep. The initial secretion peak was postulated to indicate a contribution of the psychic or cephalic phase to abomasal secretion, especially to the secretion of pepsinogen, since in later experiments (McLeay & Titchen, 1974) an increased pepsin concentration in abomasal juice was found after feeding, which was related to amount and taste of the material fed.

2.3.3 Secretion of acid as induced by a continuous passage of digesta from the forestomachs

Although the abomasum in ruminants and the simple stomach in monogastric animals act principally in the same way, the abomasum secretes more continuously, despite the intake of intermittent meals. In ruminants, food is partly fermented microbially in the rumen, before it reaches the abomasum. Digesta retention time in the rumen reservoir is variable and depends on different factors. According to the results of Hogan & Weston (1969) and of Hogan et al. (1969), the average retention time of a water soluble marker in the rumen of sheep, fed approximately at ad libitum level, varied from 8-14 h. In these experiments, marker retention time was influenced by the maturity of the crops before harvest, the protein content of the ration and of chopping or grinding of the material fed. On the basis of disappearance of polyethylene glycol from rumen contents, Hydén (1961) estimated an average flow rate of digesta from the rumino-reticulum of 300 ml/h in sheep fed twice daily on hay or on hay and concentrates at maintenance level. A volume of rumen fluid of about 4.5 litre and consequently an average retention time of fluid in the rumen of about 15 h were calculated. McManus (1961) estimated an average saliva production in sheep of about 7 litre/24 h, and therefore the volume of rumen contents is not that fluctuating, as might be expected, when sheep are fed once or twice daily.

Nevertheless, on restricted feeding regimes a diurnal pattern in digesta flow from the abomasum, and thus from the rumino-reticulum, in relation to feeding time, has been observed, especially when smaller particle size rations were supplied. Phillips & Dyck (1964) found in sheep fed on pelletted rations once daily the highest passage rates of digesta in the duodenum just prior to and during feeding and the lowest rates 6-12 h after feeding. However, Thompson & Lamming (1972) found the highest flow rates of digesta in the duodenum 4-12 h after feeding in sheep fed on barley straw and concentrates once daily. In earlier experiments with sheep fed on hay at 7:00 h and 16:00 h and on concentrates at 11:00 h, a decreased duodenal flow rate of digesta was noticed when hay was offered. After this period duodenal flow returned to its previous level. After feeding on concentrates similar immediate responses were found, followed by an increased duodenal flow rate after 3 h (Phillipson, 1952). Van 't Klooster et al. (1969), however, were not able to detect a dependence of the duodenal flow rate of digesta on the times of feeding in sheep, fed on straw pellets and concentrates twice a day.

Even when sheep or goats were starved for periods of up to 48 h, digesta still enter-

ed the abomasum (Hill, 1955), and abomasal juice of high acidity continued to be secreted by innervated pouches of the abomasal fundus, although volume and acidity were less than under normal conditions. If digesta were prevented to enter the abomasum, by emptying the forestomachs through a rumen cannula, secretion of abomasal juice ceased. Therefore the continuous secretion of abomasal juice, observed under normal feeding conditions, was concluded not to be due to the innate ability of the abomasal glands to secrete spontaneously, but to a continuous stimulation of acid secretion by the continuous passage of digesta from the forestomachs into the abomasum.

Ash (1962) proposed that rumino-reticular outflow of digesta to the omasum through the reticulo-omasal orifice is determined by reticular motility, which is affected by amount and composition of the rumino-reticular contents. Laplace (1970), however, suggested that the type of omasal motility is also involved, according to whether omasal canal or omasal body contractile activity being predominant. Phillipson (1939) demonstrated radiographically that rumino-reticular contents pass fairly continuously from the reticulum through the omasum into the abomasum. After intake of barium sulphate, it was shown that blobs and trickles of barium pass from the omasum to the abomasum. The passage of blobs of barium occurred after each reticular contraction cycle, but the passage of the quick moving trickles did occur irregularly and independently of reticular motility. Such a pattern was also described by Ash (1962a), using omaso-abomasal re-entrant cannulas in sheep.

Stevens et al. (1960) also suggested that reticular digesta enter the omasum during contractions of the reticulum, the second phase of which is associated with relaxation of both the omasal canal and the omasal body. Part of the digesta then passes immediately into the abomasum while most of the solid matter is retained between the leaves of the omasum. Using polyethylene glycol as a water soluble marker and ^{144}Ce-labelled hay particles as a water insoluble marker, Hauffe & von Engelhardt (1975) showed that the retention time in the omasum of sheep of solid particles is higher than of the fluid phase.

Digesta entering the omasum are highly buffered with salts of saliva components and of volatile fatty acids. In the omasum, partial absorption of electrolytes occurs. Oyaert & Bouckaert (1961) found absorption of sodium, potassium, ammonia and bicarbonate in sheep, according to initial concentrations, but with chloride an increase was observed. Von Engelhardt & Giesecke (1972) found an absorption of sodium and potassium of 18% and 5% of the amounts entering the omasum, respectively. They also observed an absorption of volatile fatty acids, 33% of acetic acid, 37% of propionic acid and 52% of butyric acid. For chloride they found an increase (89%), and in comparison with the reticular contents, in digesta leaving the omasum chloride concentration was approximately doubled.

Based on dry matter percentages of omasal contents in slaughtered sheep, Badawy et al. (1958) postulated an absorption of water of about 50% of the amount entering the omasum. There is no doubt, however, that digesta leaving the omasum under normal conditions do not resemble the contents found in the omasum of slaughtered animals. In fistulated small ruminants, percentages of water absorption were shown to be far much lower, ranging from 7-20% (Oyaert & Bouckaert, 1961; von Engelhardt & Ehrlein, 1968; von Engelhardt & Giesecke, 1972; Hauffe & von Engelhardt, 1975a). Therefore dry matter content of digesta entering the abomasum will be slightly higher than of the material leaving the reticulum.

The pH of the material leaving the omasum was found to be a little higher after the partial absorption of buffering compounds, such as bicarbonate and volatile fatty acids. The pH of the material entering the abomasum was shown to approximate neutrality. (Oyaert & Bouckaert, 1961; Prins et al. 1972; von Engelhardt & Hauffe, 1975).

The solid particles emerging from the omasum are in a much finer state of division than those in the rumen, probably due to a selective inflow of reticular contents into the omasum. After introduction of ^{144}Ce-labelled hay particles of approximately 5 mm into the reticulo-omasal orifice, no radioactivity was detectable in the reticulum afterwards in experiments with small ruminants, carried out by Hauffe & von Engelhardt (1975). However, after using labelled hay particles up to 10 mm, a considerable radioactivity of the reticular contents was observed several hours after introduction of the marker. So it seems likely that bigger particles are retained between the papillae in the proximal part of the omasal body, and are returned at irregular intervals after contraction of the omasal body during closure of the omaso-abomasal orifice, as was also suggested by Stevens et al. (1960).

Both inflow of digesta into and outflow of digesta from the abomasum are regulated by potent feedback mechanisms, originating in abomasum and small intestine. In part these feedback mechanisms have been proposed to be mediated by neural pathways, after excitation of mechano- or chemo-receptors (Leek & Harding, 1975). Recently it did become clear that enterohormones are involved as well in this context (Bruce & Huber, 1973; Horn & Huber, 1975). Due to this rather regular inflow and outflow of digesta the volume of the abomasal contents is probably kept within narrow limits. Data on the volume of the abomasal contents are limited, however. In own experiments with sheep fed on hay and concentrates, an average volume of abomasal contents of about 500 ml was calculated from the dilution rate of polyethylene glycol in abomasal contents, as measured in proximal duodenal digesta.

The abomasum will never be empty and will never secrete at a basal rate during interdigestive periods, since abomasal conditions affect the passage rate of digesta through the omasum, as determined by reticular and omasal contractile activity. Oyaert & Bouckaert (1961) and Ash (1962a) showed that omasal outflow of digesta was high, when after collection of digesta leaving the omasum, digesta were not reintroduced into the abomasum. Omasal outflow was found to be low or to cease temporarily after distension of the abomasum. Phillipson (1939), Iggo & Leek (1967) and Ehrlein & Hill (1970) in sheep, and Ruckebush & Kay (1971) in cattle observed a decreased frequency and intensity of reticular motility after distension of the abomasum. Harding & Leek (1973) demonstrated in the abomasal wall of sheep the existence of slowly adapting tension receptors and of rapidly adapting tactile receptors. The slowly adapting tension receptors were excitated after abomasal distension, abolishing reticular motility. Afferent vagal activity of the rapidly adapting receptors was evoked by tactile excitation, resulting in an increased frequency but a decreased intensity of reticular motility. This type of receptor was suggested to be located in or near the abomasal mucosa. Excitation of these rapidly adapting receptors was also achieved at an intra-abomasal pH lower than 3.5 or higher than 8.0, which might explain the results of Ehrlein & Hill (1970) intensity of reticular motility in sheep to decrease when the pH of the abomasal contents was high. In earlier studies, Ash (1959a) noticed an increase in the amplitude and frequency of the reticulum contractions when the

abomasal contents were acidified to about pH 1.

Bruce & Huber (1973) concluded that enterohormones are also involved in this feedback mechanism. After intravenous infusion of secretin and after duodenal acidification (pH 2), both frequency and intensity of rumen motility were inhibited. This inhibition was also noticed in sheep receiving portal blood of sheep with their duodenum acidified. Horn & Huber (1975) demonstrated that acidification of the duodenum of sheep is a more potent stimulus for the release of secretin than of cholecystokinin.

Omasal motility is influenced by abomasal conditions in the same direction as reticular motility. When the abomasum was emptied, both the omasal canal and body contracted very actively, while the opposite effect was seen after distension of the abomasum (Stevens et al., 1960).

Secretion of acid by the abomasal glands is affected by volume and composition of abomasal contents. Hill (1960) and Ash (1961) showed that volume of abomasal contents is important in regulating abomasal secretory activity. Distension of both the abomasal fundic or antral region in sheep did produce an increase in acid production within a few minutes. This response is thought to be mediated through neural pathways being stimulated by tension receptors located in the abomasal wall (Leek & Harding, 1975), probably acting through intramurally or centrally mediated reflexes on abomasal secretion of acid or through a stimulated release of gastrin (Grossman, 1967).

Different chemical substances are known to stimulate abomasal secretion, partly mediated by stimulation of chemo-receptors and partly by stimulation of the release of gastrin. Chemical factors, which are involved in the regulation of abomasal secretion of acid, are discussed below.

2.3.4 Chemo-stimulation of acid secretion

Of monogastric animals many reports are available on the effect of several chemical compounds on gastric secretion of acid, as reviewed by Grossman (1967). In non-ruminants, composition of the gastric contents varies with diet composition. In ruminants, composition of the material flowing into the abomasum is not only dependent on ration composition but also on the events taking place in the forestomachs. Microbial fermentation leads to the production of organic acids, the chief end products being short-chain fatty acids. Dietary protein is mainly broken down, and replaced by microbial and protozoal protein.

Due to this fermentation process, composition of digesta entering the abomasum is partly determined by ration composition and partly by fermentation products produced in the rumen. Among these fermentation products, volatile fatty acids were found to stimulate abomasal secretion of acid (Ash, 1961). Introduction of acetic acid into the emptied rumen of sheep was shown to produce a secretagogue effect on abomasal secretion of acid. Therefore Hill (1960) postulated that volatile fatty acids may stimulate abomasal secretion of acid after absorption from the rumen contents into the blood stream. It was also demonstrated that abomasal secretion of acid was stimulated after introduction of acetic, propionic and butyric acids into the abomasum, acetic acid being the most potent stimulus. McLeay & Titchen (1974) showed that irrigation of an innervated pouch of the abomasal antrum with volatile fatty acids also leads to stimulation of acid secretion by an inner-

vated fundic pouch. This effect may be achieved through a stimulated release of gastrin, but since these results were obtained with a vagally innervated antral pouch, a neurally mediated response cannot be ruled out. In these experiments, propionate and butyrate were found to be more effective than acetate.

Under the acid abomasal conditions volatile fatty acids are not ionized. Therefore they are fat soluble and thus readily absorbed from the abomasal contents. Once diffused into the abomasal mucosa, volatile fatty acids ionize and in consequence accumulation of these substances in the abomasal mucosa may occur. Of the individual volatile fatty acids omasal absorption percentages, which in general did not exceed 50% of the amounts entering the omasum, were reported (von Engelhardt & Giesecke, 1972; von Engelhardt & Hauffe, 1975). Masson & Phillipson (1952), however, found concentrations of volatile fatty acids in the proximal duodenum of sheep of 7-20 times less than in rumen fluid. Von Engelhardt et al. (1968) found in the abomasum of goats clearance rates for the volatile fatty acids, which were even higher than for tritiated water. Therefore the secretagogue effect of volatile fatty acids might be produced after absorption of these substances, possibly not only through a stimulated release of gastrin. Excitation of mucosal receptors is possibly involved too, as was suggested by Davenport (1967) as an explanation for the effect of a topical application of acetic acid on canine gastric motility. In these experiments, intramurally mediated reflexes were involved, since the results were obtained with vagally denervated fundic pouches.

The pH of digesta leaving the abomasum is fairly constant. Ash (1961a) found in sheep fed on hay and flaked maize a pH range of the abomasal digesta between 1.6 and 2.5 and in other reports, an abomasal pH was determined between 2.2 and 4.2 with experimental averages of pH 2.6-3.1, depending on ration composition (Weston & Hogan, 1968; Hogan & Weston, 1969). The pH of the abomasal contents was shown to be an important factor in regulating the abomasal secretory activity. The stimulatory effect of volatile fatty acids, introduced into the abomasum, on abomasal secretion of acid was dependent on the pH of the test solutions (Ash, 1961). At an abomasal acidity level lower than pH 2.0-2.5, abomasal secretory activity of acid seemed to be inhibited. Ash (1961a) prevented abomasal emptying in sheep by blocking the abomaso-duodenal re-entrant cannulas, and observed a sustained abomasal secretion of acid until a pH of the abomasal contents of about 2.0 was reached. This inhibitory effect is possibly mediated through neural pathways, since the existence of acid-sensitive abomasal mucosal receptors in sheep has been reported (Harding & Leek, 1973), but also by an inhibition of the antral release of gastrin when antral contents reach an acidity level of about pH 2.0 (McLeay & Titchen, 1975).

The pH of the abomasal contents is not only determined by amount and retention time of the abomasal contents, but also by the amount of hydrochloric acid secreted by the abomasal glands in relation to the buffering capacity of the digesta entering the abomasum. Digesta flowing into the abomasum are buffered by saliva bicarbonate and phosphate ions, by volatile fatty acid ions and by proteins. Introduction of a phosphate or bicarbonate buffer into the abomasum of sheep was shown to stimulate abomasal secretion of acid (Ash, 1961). Volatile fatty acids and proteins, however, may act not only because of their buffering capacity, but possibly also as more specific stimulators of abomasal secretion of acid.

The ability of specific agents to stimulate acid secretion is confined partly to a specific stimulative effect on the release of gastrin, as is discussed in Section 2.3.5. Besides, it is generally assumed that stimulation of abomasal or gastric secretion of acid by specific chemical agents is also mediated by neural pathways after excitation of chemoreceptors. Davison (1972) cited the experimental results of Delov, who found an increased afferent discharge activity in vagal gastric branches of cats as digestion proceeded. This effect was concluded to be caused by the products of digestion, since a casein hydrolysate was found to evoke the same activity. Sirotin (1961) demonstrated an increased electrical activity at the peripheral ends of vagal or mesenteric nerves of a resected human stomach after perfusion or topical mucosal application of glucose or protein hydrolysates. These studies indicate that vagally mediated reflexes may be involved. Other effects of chemical stimulation on gastric secretion of acid were suggested to involve intramural reflexes. Konturek et al. (1976) demonstrated that perfusion of vagally denervated fundic pouches in dogs with L-amino acids stimulated acid secretion, whereas the D-isomers were completely inert in this respect. Among the amino acids, histidine and glycine exhibited the strongest stimulation, the secretory response decreasing with the pH of the test solution (pH 5.0-1.0) and with local application of xylocaine or intravenous infusion of atropine or metiamide, a histamine H_2-receptor antagonist. These studies indicate that acid secretion may be stimulated by a gastrin-independent local neural mechanism, which is sensitive to the pH of the gastric contents.

2.3.5 Hormonal control of acid secretion

During the last two decades information did become available on the hormonal aspects of gastro-intestinal secretion and motility. Several polypeptide hormones were isolated from gastro-intestinal mucosal tissue. The criteria, which have to be fulfilled, before such hormones can be accepted as true gastro-intestinal hormones or enterohormones, were extensively discussed by Andersson (1973). At present sufficient evidence is available for gastrin, secretin and cholecystokinin to be involved in the regulation of gastro-intestinal function.

Recently, several new substances were isolated from intestinal mucosa with marked hormonal activities on gastro-intestinal target structures or organs. Of some of these candidate enterohormones the chemical structure has been elucidated, but chemical or immunological identification in the blood stream has not yet been achieved. For other hormones no evidence is available on the regulation of their release. Some candidate hormones are defined only as a principle on the basis of physiological criteria, but their chemical structure is still obscure.

Acidification of vagally innervated pouches of the duodenal bulb in dogs caused a marked inhibition of gastric secretion of acid in dog innervated fundic pouches (Andersson & Uvnäs, 1961). Andersson et al. (1967) demonstrated the same response in dogs provided with denervated fundic pouches. Inhibition of gastric secretion of acid is therefore probably achieved through a hormonal pathway, since duodenal bulb extracts were shown to induce the same effect, and since the inhibitory effect persisted after transection of the pylorus in dogs with innervated fundic pouches (Sjodin & Andersson, 1972). In later exper-

iments, acidification of the duodenal bulb in dogs produced a significant inhibition of gastric output of acid, in response to pentagastrin, the butyl-oxy-carbonyl-β-alanyl derivative of the C-terminal tetrapeptide of gastrin with comparable physiological activities as gastrin, or to a test meal, without any detectable stimulation of the production of pancreatic juice, one of the targets of secretin and cholecystokinin (Andersson, 1972; Nilsson, 1975). Therefore acidification of the duodenal bulb was concluded to release an agent different from both secretin and cholecystokinin, called bulbogastrone. Bulbogastrone is believed to be important under physiological conditions, since the pH-threshold for release is well within normal pH variations in the duodenal bulb. At a bulbar pH of around 4 in dogs inhibition of gastric secretion of acid, induced by sham feeding, could be detected (Nilsson, 1969). The chemical structure of this possible enterohormone has still to be elucidated.

Kosaka & Lim (1930) postulated the existence of the enterohormone enterogastrone, defined as the hormonal principle that is released from the small intestine by neutral fats and their digestion products. More recently Konturek & Grossman (1965) demonstrated in dogs that the degree of inhibition of acid secretion by denervated gastric pouches was closely associated with the rate of fat absorption and was most effective from the jejunum. Johnson & Grossman (1969) found in dogs inhibition of a histamine-stimulated gastric secretion of acid, when fat was instilled into the duodenum, whereas intravenous infusion of secretin and cholecystokinin were ineffective. Brown et al. (1970) purified a polypeptide having potent enterogastrone activity. The isolated material was highly inhibitory for acid secretion, for antral and fundic motor activity and for pepsin secretion in dogs, and did not possess any significant cholecystokinin or secretin activity. Brown & Dryburgh (1971) reported the chemical structure of gastric inhibitory polypeptide (GIP), originally isolated from impure preparations of cholecystokinin. In later studies (Brown, 1974), porcine GIP was described as having enterogastrone-like activity. In dogs, inhibition of acid secretion was achieved after stimulation of acid secretion with pentagastrin, synthetic human gastrin, histamine and insulin-induced hypoglycaemia. Immunologically, GIP could be identified and blood levels were found to be increased after duodenal perfusion with corn oil or with 20% dextrose, but not after perfusion with HCl 0.15 mol/litre. Immunofluorescence studies in the gastro-intestinal tract of dog and man showed GIP to be present in mucosal cells of the duodenum and to a lesser extent in the jejunum (Polak et al., 1973). In dogs, O'Dorisio et al. (1976) showed that radioimmunoassayable plasma GIP increased upon intraduodenal perfusion with an amino acid mixture or with medium-chain triglycerides. In these studies, the highest amounts of GIP could be extracted from duodenal and jejunal mucosa, when compared with antral or ileal mucosa. With these results, all criteria defined by Andersson (1973) are fulfilled. In consequence GIP has to be accepted as a true gastro-intestinal hormone, except that no data are available on the importance of this hormone under normal physiological conditions.

Of other candidate enterohormones the chemical structure and their physiological activities are known, but their physiological role during normal digestion has still to be elucidated. Motilin is a polypeptide of 22 amino acid residues. It was isolated from the small intestinal mucosa of the hog as a by-product in the purification of secretin (Brown et al., 1972). It stimulates gastric motility and therefore motilin has been sug-

gested to be released when the pH of the duodenal contents is high, since alkalinization of the duodenum in dogs has been shown to produce comparable powerful motor activity changes in transplanted fundic pouches (Brown et al., 1966). Another polypeptide, isolated from porcine small intestinal mucosa, has been called vasoactive intestinal polypeptide (VIP) (Said & Mutt, 1972). It is composed of 28 amino acid residues and stimulates peripheral and splanchnic blood flow.

So far, gastro-intestinal hormonal substances were discussed, discovered and partly identified chemically or physiologically during the last decade. Meanwhile, however, other substances have been well established as true enterohormones. The first evidence for gastro-intestinal hormones was provided by Bayliss & Starling (1902) for secretin and by Edkins (1905) for gastrin. But it took another fifty years before these hormones could be identified, before their specific biological actions could be demonstrated and before the real existence of these enterohormones was accepted.

Gregory & Tracy (1964) isolated two kinds of gastrin from hog antra. Both types of gastrin were shown to consist of the same 17 amino acid residues, differing in the same species only in the presence of an esterified SO_3H-group on the tyrosine residue in Position 12, gastrin I and gastrin II. Minor differences between species were shown in the amino acid composition of the gastrin molecules. In comparison with porcine gastrin, the structure of ovine gastrin differed only in the substitution of valine and alanine for leucine in Position 5 and glutamic acid in Position 10, respectively. In later experiments, however, evidence showed that immunoreactive gastrin activity in the blood stream was not confined only to both of these heptadecapeptide gastrin molecules. Yalow & Berson (1970) demonstrated that the major fraction of the immunoreactive endogenous plasma gastrin in human peripheral blood differed in size and charge from the heptadecapeptide gastrins. They suggested that the antral heptadecapeptide gastrin was linked to a more basic peptide. This 'big gastrin' was demonstrated by Durkin et al. (1972) to exhibit a greater potency in stimulating acid secretion in dogs, when human heptadecapeptide gastrin and human big gastrin were subcutaneously injected or intravenously infused at immunoreactively identical doses. The higher biological activity was suggested to be related to a lower metabolic clearance rate, which might also explain the results of Yalow & Berson (1970) in man and of Dockray et al. (1975) in dogs that the major fraction of immunoreactive plasma gastrin consisted of big gastrin.

Berson & Yalow (1971) demonstrated immunoreactive materials in tissue extracts from mucosa of human antrum, duodenum and jejunum, with properties identical to those of plasma big gastrin and heptadecapeptide gastrin, the proportion of big gastrin increasing but the total extractable immunoreactive gastrin decreasing with increasing distance from the antrum. Gregory & Tracy (1972) succeeded in purifying a pair of gastrin peptides having the size and charge characteristics of big gastrin. Both gastrin molecules were found to contain the same amino acids, but differed only in the presence or absence of a SO_3H-group on the tyrosine residue, the same difference as was noticed between both heptadecapeptide gastrins. The 17 C-terminal amino acid residues were found to be identical with those of the heptadecapeptide gastrins.

In general, the major source of gastrin is thought to be the mucosa of the antral gland area of the stomach, although immunoreactive gastrin has also been isolated from

duodenal and jejunal mucosa of various species, e.g. man, dog, cat and hog. The human duodenal mucosa was shown to contain almost as much gastrin as the human antrum (Walsh, 1975).

In sheep abomasal mucosa, gastrin showed to be present in the fundic mucosa as well (Anderson et al., 1962). This finding is in close agreement with the results of McLeay & Titchen (1973), who demonstrated that abomasal secretory responses in sheep in relation to quality and quantity of food were not substantially affected by antrectomy. They concluded other stimuli, such as vagally mediated reflexes, to mobilize gastrin from residual sources or to act directly on abomasal secretion of acid. More recently, however, McLeay & Titchen (1975) reported a decreased acid output from vagally innervated fundic pouches in sheep after antrectomy, indicating that fundic gastrin is not secreted in sufficient amounts to compensate for the loss of antral gastrin.

Gastrin has been found mainly in cells located in the deeper portion of the mucosa. Using an immunofluorescence technique, in mucosa of humans, hogs and cats, McGuigan (1968) and Bussolati & Canese (1972) demonstrated gastrin granules in the 'G-cells'. These cells were reported by Solcia et al. (1967) to possess a microvillous luminal neck, communicating with the lumen of an antral gland, and possibly containing receptors for chemical substances affecting the release of gastrin.

The physiological release of gastrin from antral mucosa is controlled by two main stimulatory mechanisms. The release of gastrin may be stimulated by neural mechanisms and by chemical agents present in gastric contents. In monogastric animals, and possibly also in ruminants, intake of food gives rise to cephalic stimulation, which causes release of gastrin by means of vagal reflexes. So the cephalic phase of gastric secretion is realized after direct stimulation of the gastric glands and indirectly after stimulative effects on the gastrin releasing cells. In dogs after sham feeding (Nilsson et al., 1972) and insulin-induced hypoglycaemia (Jaffe et al., 1970), a comparable vagally mediated increased gastrin release was noticed. In cats (Fyrö, 1967) and in dogs (Smith et al., 1975), gastrin release increased upon electrical stimulation of the vagus nerves. The release of gastrin may also be neurally stimulated by distension of the antral or fundic region (Gedde-Dahl, 1974; Walsh, 1975). Splanchnic nerve stimulation in chloralose-anaesthetized cats resulted in a decreased arterial gastrin concentration (Blair et al., 1975).

Gastric digesta components may stimulate the release of gastrin as well. Proteins and large peptides are believed to be the most potent natural stimuli for gastrin release. Bovine serum albumin was found to be ineffective as a gastrin releaser until it was partially digested with pepsin, the gastrin releasing ability being not removed by dialysis (Walsh, 1975). Among the various fractions of a protein hydrolysate also the small-molecular fractions were shown to stimulate the release of gastrin (Elwin, 1974). In dogs, perfusion of an antral pouch with glycine or with acetylcholine was demonstrated to stimulate acid secretion (Cooke & Grossman, 1968). Among the various amino acids a clear structure-activity relationship was found. Whereas glycine and β-alanine were shown to be potent gastrin releasers, α-alanine was less effective (Elwin, 1974). Between the stereo-isomers of serine and alanine no difference in gastrin releasing potency was found (Csendes & Grossman, 1972). Also less physiological chemical substances such as lower aliphatic alcohols showed to be active gastrin releasing agents (Elwin, 1969). Topical application

of atropine inhibits gastrin release in response to chemical stimuli, supporting the concept that stimulation of gastrin release is mediated by a local cholinergic reflex mechanism (Csendes et al., 1972; Andersson, 1973).

The release of gastrin is regulated by a potent feedback mechanism related to the degree of acidity within the antrum. Pe Thein & Schofield (1959) demonstrated that acidification of antral pouches diminished acid secretion from denervated fundic pouches in dogs in response to sham feeding. The optimal acidity range for gastrin release is between pH 5-7. Below pH 5 gastrin release is gradually inhibited and at pH 2 plasma gastrin levels in dogs were shown to be greatly reduced, and the stimulatory effect of ethanol or sham feeding and of fundic or antral distension on gastrin release was blocked completely (Andersson & Elwin, 1971; Nilsson et al., 1972). In sheep, acidification (pH 2) of antral pouches was also demonstrated to inhibit fundic pouch secretion of acid (McLeay & Titchen, 1975).

Under normal feeding conditions, the increase in serum gastrin level after food intake was shown to depend on diet composition. Korman et al. (1971) observed in man an increase in serum gastrin level especially after ingestion of high protein diets, whereas fats or carbohydrates were ineffective. Serum gastrin concentrations as low as 5 pg/ml are detectable radioimmunologically, and after stimulation of gastrin release serum levels up to 300 pg/ml were noticed (Blair et al., 1975; Malagelada et al., 1976) in cats and humans.

The metabolic clearance rate of gastrin was shown to be high. A mean half-life for synthetic human gastrin in dogs of 3.7 min was found by Schrumpf & Semb (1973), which was not affected by simultaneous infusion of secretin. Walsh et al. (1974) found for human heptadecapeptide gastrin in dogs a comparable value of 3 min, but for big gastrin a half-life of about 15 min was demonstrated. According to the results of Booth et al. (1973), the major site of gastrin inactivation in dogs was located in the kidneys. Substantial differences in gastrin level were demonstrated between renal arterial and venous blood after antral perfusion with acetylcholine. After bilateral nephrectomy half-life of gastrin was approximately doubled. Evidence has also been obtained demonstrating that the small intestine plays an important role in gastrin catabolism. Small intestinal mucosa and kidney homogenates were shown to contain enzymes capable of hydrolysing the C-terminal amide and internal peptide bonds of gastrin (Walsh & Laster, 1973).

The main targets of gastrin are stimulation of gastric secretion of acid and gastric motility. Gastrin was postulated to act on specific receptor sites, together with secretin and cholecystokinin (Grossman, 1970). In what way these receptors affect the activity of the mucosal gland cells after excitation, has not yet been fully elucidated. Vizi et al. (1973) proposed a release of acetylcholine to be involved. In other papers, a stimulative effect of gastrin on histidine decarboxylase has been described (Hakanson et al., 1973; Lundell, 1974), suggesting that histamine is involved. Histamine as a possible intermediate in the stimulation of gastric secretion of acid, was also mentioned by Gibson et al. (1974), since metiamide, a histamine H_2-receptor antagonist, inhibited dog gastric secretion of acid, stimulated by pentagastrin.

Enterohormones, released by the small intestinal mucosa, in general inhibit gastric secretory activity. Of secretin, the enterohormone, which was discovered first (Bayliss & Starling, 1902), the complete amino acid sequence was determined by Mutt et al. (1970).

Solcia et al. (1972) proposed secretin to be produced by the 'S-cells'. In pig intestinal mucosa, these cells were localized in the villous epithelium of the duodenum and upper jejunum.

In general, acid is regarded as the most potent stimulus for secretin release. Meyer et al. (1970) found in dogs a close relationship between duodenal pH and rate of secretin release within the range from the threshold pH 4.5 down to pH 3. Below pH 3, secretin release was shown to be related to the amount of acid introduced into the duodenum and to the length of the duodenum acidified. Lee et al. (1976) found in dogs no plasma secretin response after a meat meal at a postprandial duodenal pH of 4.5. Intraduodenal infusion of HCl 0.1 mol/litre, however, caused a significant increase in plasma secretin levels, while gastrin levels decreased. In anaesthetized dogs, Boden et al. (1975) found intraduodenal infusion of hydrochloric acid to increase immunoreactive serum secretin level, whereas application of an amino acid mixture, of fatty acids and of hypertonic solutions was ineffective. In sheep, release of secretin by the duodenal mucosa after duodenal acidification was shown by Horn & Huber (1975). Cholinergic stimulation of secretin release has also been reported. In conscious dogs, Henriksen & Rune (1969) reported that cholinergic stimulation potentiated the duodenal secretin release after topical application of hydrochloric acid.

Release of secretin causes an increase in exocrine pancreatic flow rate and bicarbonate secretion. It inhibits gastric secretion of acid and gastric motility, but stimulates gastric pepsinogen and mucus secretion (Nakajima & Magee, 1970; Vagne et al., 1970). Secretin inhibits gastric secretion of acid through a dual mechanism. It acts as a non-competitive inhibitor on gastrin-stimulated acid secretion, but secretin has also been mentioned as a negative modulator of the release of gastrin in man and dog (Grossman, 1970; Hansky et al., 1971; Sjodin & Miura, 1974; Walsh, 1975).

In dogs, the metabolic clearance of secretin was shown to be high. Boden et al. (1974) determined a mean half-life of 2.8 min.

The identity of the enterohormones pancreozymin, which stimulates the exocrine pancreatic zymogen output, and cholecystokinin, which causes contractions of the gallbladder, was proved to be the same. Cholecystokinin-pancreozymin (CCK-PZ) is a polypeptide hormone, composed of 33 amino acid residues, the C-terminal pentapeptide sequence being identical with that of the gastrin molecules (Mutt & Jorpes, 1971). In dogs, the concentration of releasable CCK-PZ was proposed to be high in the proximal duodenum and to decrease with the distance from the pylorus (Konturek et al., 1972). Solcia et al. (1972) and Polak et al. (1975) proposed CCK-PZ to originate from the 'I-cells'. These cells are mainly present in the upper intestine but a very few were also identified in the ileum.

Peptides, amino acids and fatty acids are generally accepted as the physiological most potent releasers of CCK-PZ. Fatty acids with more than eight carbon atoms were found to be potent releasers (Andersson, 1973). Of the amino acids, phenylalanine, valine and methionine were indicated as active CCK-PZ releasers in man. Of tryptophan the effect was indeterminate (Go et al., 1970). Meyer & Grossman (1970) demonstrated that in dogs only the L-isomers of neutral amino acids are effective and that the amount of released CCK-PZ is related to the amino acid load and to the length of the intestine exposed to amino acid. According to the results of Konturek et al. (1973) in dogs, the highest activity for re-

lease of CCK-PZ was exhibited by tryptophan and phenylalanine. Hydrochloric acid was also reported as a stimulant of CCK-PZ-release. Barbezat & Grossman (1971) demonstrated a stimulated pancreatic zymogen output after duodenal acidification.

CCK-PZ has been shown to be inactivated by blood plasma enzymes (Greengard et al., 1941).

Gastrin, cholecystokinin and secretin were postulated to act at one receptor with two interacting receptor sites, one site with affinity for the C-terminal pentapeptide of gastrin and cholecystokinin, and the other with affinity for secretin (Grossman, 1970). According to this hypothesis, CCK-PZ inhibits gastrin stimulated gastric secretion of acid competitively and secretin non-competitively. In the same way, gastrin and CCK-PZ augment pancreatic enzyme secretion competitively, and secretin and gastrin pancreatic bicarbonate output non-competitively.

The intestinal phase of gastric secretion is mainly inhibitory and is supposed to be mainly attributable to the release of enterohormones, such as secretin and cholecystokinin. The release of these hormones was shown to be potentiated by several digestive products. Besides, neural mechanisms are involved in the regulative mechanism of gastric secretion of acid. Cholinergic stimulation was discussed to act directly on gastric acid secretory cells, but also through a stimulated release of gastrin and possibly also of secretin. Splanchnic stimulation was found to exhibit the opposite effect and thus the neural and hormonal participants in the regulation of gastric secretion of acid may potentiate or inhibit each other, resulting in a gastric secretory activity kept within physiological limits.

2.3.6- Physiological role of histamine in acid secretion

Histamine is a potent stimulant of gastric secretion of acid in all species that have been studied, but its possible role in the normal physiological control of secretion has long been controversial. Accumulating evidence, however, indicates histamine to participate in the regulation of gastric secretion of acid. Code (1965) suggested vagal or gastrin stimulation of gastric secretion of acid to act through a specific histamine link. A conventional anti-histamine, mepyramine, however, fails to inhibit histamine-stimulated gastric secretion of acid. The mepyramine-sensitive histamine receptors, present in the bronchi and in the gastro-intestinal smooth musculature, have been called histamine H_1-receptors. Other histamine receptors, histamine H_2-receptors, are refractory to mepyramine and related anti-histamines, but not to buramimide and metiamide. These histamine H_2-receptors are involved in the stimulative effect of histamine on the heart rate, contractions of the uterus and on gastric secretion of acid (Black et al., 1972).

In rats and rabbits, a specific histidine decarboxylase has been identified (Weissbach et al., 1961; Werle & Lorenz, 1964; Leinweber & Braun, 1969), which is different from aromatic amino acid decarboxylase (dopa decarboxylase), for which histidine has a much lower affinity. This specific histidine decarboxylase was found in the fundic but not in the antral part of the stomach, and in the fundic part it was present in het mucosa. Thunberg (1967) demonstrated that in rat fundic mucosa histamine and histidine decarboxylase are located in the same specific cells, which occurred in the base of the fundic

glands. He proposed that histamine, released or newly formed, could rapidly be transferred from the site of origin to the acid secreting parietal cells. In rats, inhibitors of histidine decarboxylase were found to decrease both gastric mucosal histamine content and gastric secretion of acid (Castelluci & Megazzini, 1976).

Kahlson et al. (1964) and Rosengren & Svensson (1969) proposed vagal as well as gastrin stimulation of gastric secretion of acid in rats to be mediated by histamine. Feeding, injection of gastrin, distension of the stomach and vagal stimulation caused, besides initiation of acid secretion, a steep and prolonged acceleration of formation and turnover of histamine in the fundic gastric mucosa. Besides, vagal denervation, antrectomy and antral acidification, which depress the release of gastrin, caused a substantial lowering of mucosal histidine decarboxylase activity. These findings were confirmed by the results of Aures et al. (1970). In these experiments with rats, a suppressed histidine decarboxylase activity was noticed after antrectomy, but stimulation occurred after administration of pentagastrin, of cholecystokinin or of the vagomimetic drug 2-deoxy-D-glucose. Stimulation of mucosal histidine decarboxylase after gastrin administration was also noticed by Hakanson et al. (1973) in rats, whereas Lundell (1974) found mobilization of gastric mucosal histamine to increase after both vagal and pentagastrin stimulation. In dogs, metiamide, a histamine H_2-receptor antagonist, inhibited gastric secretion of acid, evoked by histamine, pentagastrin and cholinergic stimulation (Gibson et al., 1974). In isolated frog gastric mucosa and in an isolated rat stomach, metiamide was also found to inhibit histamine and pentagastrin stimulated acid secretion (Wan et al., 1974).

Contradictory reports, which do not support the physiological role of histamine in gastric secretion of acid are also available. According to the working hypothesis that histamine is an essential intermediate for gastric secretion of acid, secretin was emphasized to inhibit histidine decarboxylase and cholecystokinin was supposed to stimulate histidine decarboxylase to a lesser extent than gastrin does. Caren et al. (1969), however, did not observe an effect of secretin nor of cholecystokinin, both inhibitors of pentagastrin-stimulated gastric secretion of acid, on pentagastrin-stimulated histidine decarboxylase activity in rats. Johnson (1971) did not observe either a depression of rat fundic histidine decarboxylase activity by secretin, and exogenous histamine-stimulated gastric secretion of acid was not blocked by secretin. He concluded that histamine is not an essential intermediate, but that gastric secretion of acid may be stimulated through separate pathways, involving or not involving histamine. Lin & Evans (1970) were not able to demonstrate a direct relationship between gastric mucosal histamine content and gastric secretion of acid in rats, after pentagastrin stimulation. They also suggested that gastrin can trigger an increased mucosal synthesis of histamine, but can also directly stimulate the acid secreting parietal cells.

In sheep, histamine was also found as a stimulator of abomasal secretion of acid (Hill, 1965; McLeay & Titchen, 1975). In ruminants, however, no evidence is available on the relationship between endogenous histamine and abomasal secretion of acid.

2.3.7 Role of cyclic 3¦5'-adenosine monophosphate in acid secretion

Cyclic 3¦5'-adenosine monophosphate (cyclic AMP) is well known as a 'second messenger' on different hormonal actions. It has also been suggested as a possibility that this nucleotide plays a role in gastric secretion of acid.

Ruoff & Sewing (1973) found in rats that gastric mucosal cyclic AMP content decreased after starvation. After refeeding, however, they did not observe an immediately increased cyclic AMP level, whereas an intraperitoneal injection of pentagastrin was followed by a substantial increase in mucosal cyclic AMP within the first hour. The same workers (1974) found a double peak in rat gastric mucosal cyclic AMP content after cholinergic stimulation with either carbachol, insulin or 2-deoxy-D-glucose. A first peak was reached within 15 min and a second 75 min post intraperitoneal injection of these chemicals. The first peak preceded the gastric secretion response of acid, and was therefore suggested to be involved in the initiation of acid secretion. The second outlasted acid secretion, and was supposed to be related to other functions, showing that a high mucosal cyclic AMP level is not necessarily related to a high acid secretory rate. This double peak cyclic AMP response after cholinergic stimulation was not found after histamine injection, when both mucosal cyclic AMP level and gastric secretion of acid paralleled each other.

Domschke et al. (1972) demonstrated in rats a concomitant diurnal rhythm for both acid output and mucosal cyclic AMP levels. In humans, however, no relation was found between gastric secretion of acid and mucosal cyclic AMP content, after pentagastrin or gastrin stimulation (Domschke et al., 1974).

Levine & Wilson (1971) demonstrated in humans and dogs that exogenous cyclic AMP inhibits gastric secretion of acid. Because of the proposed poor penetration rate of the nucleotide into the cells, the authors suggested these effects on gastric secretion to be based on secondary influences, which overwhelmed the primary metabolic activity, since in the dogs cyclic AMP was shown to decrease blood flow in the gastric mucosa. Harris & Alonso (1965) and Way & Durbin (1969) in isolated frog mucosa, and Ramwell & Shaw (1968) in perfused rat stomach in vivo, were able to show a stimulatory effect of exogenous cyclic AMP on acid secretion.

Imidazole, which decreases mucosal cyclic AMP content by activating its specific phosphodiesterase, was found to inhibit acid secretion in frog gastric mucosa (Nakajima et al., 1970). Harris et al. (1969) found that theophylline in isolated frog gastric mucosa stimulates hydrochloric acid production by inhibiting the phosphodiesterase that degrades cyclic AMP. The cyclic AMP increase preceded the acid secretory response, and thereafter cyclic AMP and acid secretion paralleled each other. In rabbit isolated fundic mucosa, both cyclic AMP and theophylline were demonstrated to stimulate acid secretion (Fromm et al., 1975).

Amer (1972, 1974) suggested that cyclic 3¦5'-guanosine monophosphate (cyclic GMP) is also involved. He proposed that guanylcyclase activity is stimulated by gastrin, pentagastrin, cholecystokinin, acetylcholine and histamine. In dogs, rats and rabbits, gastric secretion of acid was stimulated by theophylline, which was suggested to be mediated through cyclic GMP, since the inhibitor constant (K_i) of theophylline is lower for cyclic GMP-phosphodiesterase than for cyclic AMP-phosphodiesterase. This proposed intermediate

role of cyclic GMP in gastric secretion of acid was not confirmed by Thompson & Jacobson (1975). These workers found that rat gastric guanylcyclase activity was depressed by histamine and by the cholinergic drug carbachol, was increased by secretin, whereas pentagastrin had no effect.

Additional supporting evidence for an intermediate role of cyclic AMP in gastric secretion of acid was supplied by Nakajima et al. (1971), who found that frog gastric mucosal adenylcyclase activity was stimulated by pentagastrin and histamine. Narumi & Maki (1973) found a comparable effect in the rat in vivo. In these experiments, an increased gastric mucosal intracellular cyclic AMP level was demonstrated after intravenous administration of gastric acid secretory stimulants such as histamine, pentagastrin and carbachol. Dousa & Code (1974) confirmed the effect of histamine on gastric mucosal cyclic AMP content, whereas the histamine H_2-receptor antagonist metiamide was found to depress intracellular cyclic AMP levels by inhibition of adenylcyclase.

Cyclic AMP is supposed to act on gastric secretion of acid through a stimulated liberation of substrates. Ohkura & Hattori (1975) found in rats gastrin and histamine to stimulate acid secretion through a cyclic AMP mediated increased protein kinase activity. In frog gastric mucosa, such a protein kinase was found to stimulate the conversion of inactive phosphorylase b to active phosphorylase a, thus promoting glycogenolysis (Nigon & Harris, 1968). In this regard, such a cyclic AMP dependent protein kinase system was also identified in bovine gastric mucosa by Kuo et al. (1970), whereas cyclic AMP has also been mentioned to be involved in the activation of adipose tissue lipase through a stimulated protein kinase activity in rabbits (Corbin et al., 1970).

2.3.8 Mechanism of acid secretion

Hydrochloric acid in the mammalian simple stomach as well as in the ruminant abomasum is secreted by the parietal cells, present in the tubular glands of the fundic mucosa. The ionic composition of pure gastric juice has been shown to depend on secretory activity. Hydrogen ion concentration is relatively low under unstimulated conditions, and increases as a function of the rate of secretion. Hunt & Wan (1967) and Davenport (1971) mentioned a lower limit of 20 mmol/litre and an upper limit of 140-160 mmol/litre in mammalian gastric juice. Chloride is the main anion of gastric juice. Its concentration is less variable than hydrogen ion concentration. Linde & Obrink (1950) showed that chloride concentration in dog gastric juice was approximately constant at 130-140 mmol/litre until the concentration of acid reached about 110 mmol/litre. At higher acid concentrations, a linear relationship between hydrogen and chloride ion concentration was noticed, with an upper limit for hydrogen ion concentration of about 150 mmol/litre and for chloride ion concentration of about 160 mmol/litre. In sheep, a maximal hydrogen ion concentration in fundic abomasal juice of 150 mmol/litre was reported (McLeay & Titchen, 1975). Under less stimulated conditions, lower acid concentrations were found. Hill (1968) reported a range for hydrogen ion concentration in sheep abomasal juice of 20-100 mmol/litre with an average chloride concentration of 155 mmol/litre.

In man, sodium concentration was found to be about 80 mmol/litre when acidity is low (Davenport, 1971). The relationship between sodium and hydrogen ion concentration was

reciprocal until an acidity level of about 110 mmol/litre was reached. At this acidity level, sodium concentration was lower than 20 mmol/litre and remained about stable with a further increase in acidity. The potassium concentration in gastric juice ranged from 10-15 mmol/litre and was hardly affected by gastric secretory rate (Davenport, 1971).

Based on the concentrations of the main inorganic ions in relation to gastric juice acidity, different hypotheses concerning the mechanism of gastric secretion of acid have been proposed. Hollander (1952) suggested that gastric juice is composed of two components, an acid component with a high hydrogen ion concentration produced by the parietal cells, and a neutral component with sodium as the main cation from the chief or peptic and from the mucous cells. This non-parietal component was supposed to be rather constant and stimulation of gastric secretion of acid should be mainly achieved by an increased parietal cell secretion. This two-component view explains that sodium and hydrogen ion concentration are inversely related, according to gastric secretory rate. Hirschowitz (1961) proposed that part of the reciprocal relationship between sodium and hydrogen ion concentration could be caused by an exchange of sodium ions against secreted hydrogen ions, which process would be more important at a lower gastric secretory rate.

Histological studies have shown that the mammalian parietal cells are characterized by 'intracellular' secretory canaliculi, networks of channels lined with microvilli, which extend almost to the base of the cells and are continuous with the apical cell membranes. The parietal cell cytoplasm is densely packed with mitochondria, and contains an extensive agranular reticulum of branching and anastomosing tubules. The secreting parietal cell may also contain large clear vacuoles, which were proposed to consist of distended elements of the reticulum filled with fluid to be secreted into the canaliculi (Davenport, 1971). After treatment of a gastric mucosal preparation with special kinds of indicators, Bradford & Davies (1950) demonstrated that intracanalicular pH may be lower than 1.4. In isolated rabbit parietal cells, accumulation of aminopyrine (Section 2.4) was found, which increased upon stimulation with histamine, indicating that in stimulated parietal cells but also in resting cells acid sites are present (Berglindh et al., 1976).

It has been established that in vivo the blood side of the gastric mucosa is about 20 mV positive against the lumen side. Therefore chloride ions have to be transported against both a chemical and an electrical gradient. Hydrogen ions as well have to be secreted against an electrochemical gradient, although the electrical gradient facilitates secretion of hydrogen ions. Rehm et al. (1970) supposed that water transport was linked to ion transport as a result of the osmotic gradient between the lumen of the gastric glands and the interstitial fluid.

Sanders et al. (1972) proposed that inorganic ions are transported through the apical and basic parietal cell membranes according to two different transport mechanisms. Hydrogen and chloride ions were suggested to pass the apical membrane through two separate electrogenic pump mechanisms, in the sense that both transport mechanisms can produce a net transport of electric charge. At the basic cell membrane, they proposed that chloride ions pass the membrane through a chloride-bicarbonate neutral carrier exchange mechanism, the bicarbonate ions being produced in the parietal cells during active secretion.

Since hydrogen ions can be produced at a very high rate, the existence of a metabolic machine for the production of hydrogen ions and of metabolic energy for the active trans-

port of both hydrogen and chloride ions has been generally accepted (Rehm, 1972). Therefore the gastric acid secretory mechanism is proposed undeniably to interact with respiratory chain activity. This has been evidenced in general by the dependence of acid secretion on aerobic metabolism. Under anoxic conditions acid secretion was shown to be rapidly inhibited and in a preparation of isolated canine parietal cells administration of histamine, which stimulates acid secretion, was found to increase oxygen consumption (Croft & Ingelfinger, 1969). A comparable effect in different gastric mucosal tissues was obtained by Hersey (1974) after application of theophylline or cyclic AMP. In these experiments a $Q(O_2)$ (mmol H^+/mmol O_2) of about 3 was calculated. These data fit very well in the results of Kowalewski & Kolodej (1972) and of Russell & Kowalewski (1973), obtained after stimulation of isolated canine stomachs with histamine or with the cholinergic drug bethanechol. They calculated a $Q(O_2)$ of about 2.

From thermodynamic computations of concentrations of the main inorganic ions H^+, K^+, Na^+ and Cl^- in gastric juice, in comparison with concentrations of these ions in blood plasma, Russell & Kowalewski (1973) calculated a molar free energy ($\Delta G = nRT \ln c_2/c_1$) of about 80.7 kJ/mol in terms of O_2 consumed. The free energy derived from oxidation of glucose to CO_2 and H_2O was calculated as -527 kJ/mol O_2. Based on these data, a ratio of the free energy used for the secretion of gastric juice to the free energy liberated of about 0.15:1 was determined. This ratio can be seen as an approximation of the overall efficiency of the gastric secretory process.

When the gastric glands are not secreting actively, neither ATP, nor the availability of endogenous substrates are regarded as rate-limiting factors controlling gastric secretion of acid (Hersey, 1974). Intracellular biochemical control mechanisms have been proposed to be primarily responsible for the potentiation of both oxidative metabolism and acid secretion. The potent stimulation of acid secretion by secretagogues, such as histamine, pentagastrin, theophylline and the cholinergic drug bethanechol, has been assumed to be mediated by their action on important intracellular control sites, resulting in a substrate mobilization (Ohkura & Hattori, 1975). Most of these secretagogues have been shown to increase intracellular cyclic AMP levels (Section 2.3.7), which agent has been mentioned as an activator of substrate mobilizing enzymes as glycogen phosphorylase and lipase (Sutherland & Robison, 1966; Nigon & Harris, 1968; Corbin et al., 1970).

In isolated frog gastric mucosa, respiratory quotient (RQ) values decreased to about 0.75 after theophylline stimulation, indicating that a highly reduced substrate such as fatty acids, was providing the bulk of the substrate (Hersey, 1974). In other experiments, exogenously added water-soluble salts of short- and medium-chain fatty acids stimulated both oxidative metabolism and acid secretion of frog gastric mucosa (Alonso et al., 1967). Isolated rat gastric mucosa was shown not to be stimulated by fatty acids. In rat gastric mucosa with glycogen as the main substrate for acid secretion, substrate mobilization is realized after activation of glycogen phosphorylase. In these tissues, glucose was proposed to be oxidized via the hexose monophosphate shunt pathway (Sernka & Harris, 1972; Sernka, 1975). All the enzymes of this hexose monophosphate shunt pathway reside in the cytoplasm, resulting in an extramitochondrial production of NADPH. Therefore these authors suggested that acid secretion may be achieved without involvement of mitochondrial respiratory chain components.

The positive relation between acid secretion and oxidative metabolism, however, implies that the respiratory chain plays an important role in the acid secretory mechanism. The terminal respiratory chain inhibitor CN^- was found to produce a rapid and complete inhibition of acid secretion, simultaneously with a reduction of the respiratory chain cytochromes (Hersey, 1974). Another inhibitor of acid secretion, amytal, was investigated by Sachs et al. (1967). They indicated that this agent acts between NADH and flavoprotein in the respiratory chain sequence and demonstrated a close kinetic relationship to exist between inhibition of secretion and flavoprotein oxidation. Hersey & Jobsis (1969) showed that the intact gastric mucosal cells contain a normal sequence of respiratory chain components, as determined by direct measurement of the absorption spectra. They concluded that the gastric mucosa does not contain absorbing components aside from the normal respiratory chain components present in the mitochondria. Direct evidence has also been provided against the existence of individual extramitochondrial cytochromes by Hersey et al. (1975). They localized cytochrome oxidase cytochemically, based upon the oxidative polymerization of 3,3'-diaminobenzidine to an osmiophilic reaction product, which was found in frog gastric mucosa to be exclusively restricted to the mitochondria. No reaction product was found within the reticular tubules or at the apical plasma membrane, and therefore they concluded that models of secretion based upon the existence of extramitochondrial respiratory components such as that of Sernka & Harris (1972) presented previously, have to be ruled out.

After stimulation of acid secretion a further reduction of the respiratory intermediates is obtained. At a low mucosal pH level, however, oxidation of respiratory chain components may occur. Hersey (1974) obtained at a pH of the solution bathing the mucosa lower than 2.5 substantial respiratory chain redox changes. At this low pH level, when acid secretion may cease as was documented in Section 2.3.4, a further reduction of flavoprotein and an oxidation of the cytochromes was noticed. This result was suggested to indicate a specific coupling site between the respiratory and the acid secretory mechanism, different from those of the oxidative phosphorylation sites, and at the level where the respiratory chain components change from hydrogen plus electron carriers to electron carriers. Hersey proposed that through this coupling site part of the respiratory activity might be regulated by the gastric acid secretory mechanism.

It has not yet been established unequivocally whether the respiratory energy transport of the hydrogen ion secretion mechanism is mediated by an ATPase system or not. One of the arguments in favor of ATP as an intermediate in energy transport is the inhibition of acid secretion after administration of 2,4-dinitrophenol, an uncoupling agent, which prevents ATP production without inhibition of respiratory activity (Sachs et al., 1968). Moreover, Forte et al. (1965) demonstrated that after reoxygenation of anoxic frog mucosa, acid secretion returned when intracellular ATP concentration was restored.

The complexity of the proposed models for hydrogen ion secretion, however, increased when rhodanide was found to inhibit acid secretion, without affecting intracellular ATP-levels or chloride secretion. This finding, and the proposed coupling site between acid secretory and respiratory activity after acidification of the mucosa, cited above, are regarded as arguments against an ATP-mediated energy transport mechanism for acid secretion. If ATP is not involved, however, it is difficult to decide in what way energy be-

comes available for the transport of hydrogen ions into the intracellular canaliculi. A mitochondrial membrane bound redox mechanism was proposed by Hersey et al. (1975), which transfers hydrogen ions into the intracellular reticular tubules without intermixing with the cytoplasm. A possible explanation of such a mechanism was given by Ito (1961) from morphological studies. In actively secreting parietal cells, he noticed a change in organization of the intracellular reticular tubules. Therefore he proposed the hydrogen ions to be secreted into the tubular lumen, since the mitochondria were found in close proximity to the intracellular tubular system. Such a mechanism would agree with the large vacuoles present in the actively secreting parietal cells as distended parts of the intracellular tubular system, as proposed by Davenport (1971). It is also in line with the results of Forte et al. (1976), who noticed in isolated piglet gastric mucosa, stimulated with histamine, an elaboration of the apical and canalicular cell membrane simultaneously with a depletion of the intracellular tubular system, indicating that at the onset of acid secretion apical surface elaboration is achieved by fusions of tubules with the canalicular membrane.

Recently, however, new arguments have been provided in favor of an ATPase mediated energy transport mechanism to be involved in the hydrogen ion secretion mechanism. Sachs et al. (1972) isolated from dog gastric mucosa a rhodanide-inhibitable, HCO_3^--stimulated, Mg^{2+}-dependent ATPase, different from the Na^+ transport linked and ouabain-inhibitable (Mg^{2+},Na^+,K^+)-ATPase. Subcutaneous injections of the cholinergic drug carbachol, of pentagastrin or of histamine stimulated acid secretion and increased the (Mg^{2+}, HCO_3^-)-ATPase activity in the mitochondrial fraction of rat gastric mucosa (Narumi & Kanno, 1973; Ohkura & Hattori, 1975). Administration of these gastric secretory stimulants was also found to stimulate mitochondrial carbonic anhydrase activity. From these results, it was concluded that this mitochondrial ATPase and carbonic anhydrase are involved in the acid secretory process.

Narumi & Kanno (1973) demonstrated in addition that subcutaneous treatment with the carbonic anhydrase inhibitor acetazolamide inhibited (Mg^{2+}, HCO_3^-)-ATPase activity in the mitochondrial fraction, which also suggests that carbonic anhydrase is functionally linked to this ATPase. This possibly explains the essential role of carbonic anhydrase in gastric secretion, which was suggested by Teorell (1951). Besides carbonic anhydrase is supposed to play an essential neutralizing role at a step, which is subsequent to hydroxyl formation. Hersey & High (1971) observed an alkaline pH shift in frog gastric mucosa after inhibition of acid secretion with acetazolamide. However, also under normal conditions the interior of the parietal cell in canine gastric mucosa was shown to be more alkaline, when compared with muscular cells, despite carbonic anhydrase activity was not inhibited. Croft & Ingelfinger (1969) estimated in dog parietal cells an intracellular pH of about 7.3.

2.3.9 Secretion of pepsinogen

As in gastric juice of monogastric animals, pepsin is an integral component of the juice secreted in the ruminant abomasum. The precursor of pepsin, pepsinogen, is secreted by the chief or peptic cells of the abomasal fundic glands. In different species, including sheep, pepsinogen has also been identified in the antral glands. Hanley et al. (1966)

isolated pepsinogen from both fundic and antral mucosa of humans, pigs and rabbits. Grossman & Marks (1960) in dogs and McLeay & Titchen (1975) in sheep demonstrated pepsin activity in juice collected from innervated antral pouches. In man, Samloff & Liebman (1973) demonstrated pepsinogen to be even present in duodenal mucosa by use of an immunofluorescence technique. In mammals, the pepsinogen secreting fundic chief cells were found to be distinct of the acid secreting parietal cells, in that they are principally cells synthesizing a protein product, which is stored in granules for rapid secretion later, but pepsinogen may also be produced and secreted continuously (Hirschowitz, 1967).

In the chief cells the precursor and inactive form of pepsin, pepsinogen, is synthesized. Pepsinogen is a protein with a molecular weight up to 42500. Under acid conditions, pH lower than 6.0, pepsinogen is converted to pepsin. In this conversion, the N-terminal part of the pepsinogen molecule is split off, resulting in the formation of pepsin with a molecular weight of about 35000. Among the cleavage products a pepsin inhibitor is present, which is readily digested by pepsin under acid conditions.

Not only between, but also within species, different pepsinogens and thus different pepsins have been identified (Seyffers et al., 1963; Hanley et al., 1966; Seyffers & Tkatch, 1970). In order of decreasing electrophoretic mobility to the anode at pH 8.5 or after different fractionation procedures, different pepsinogens were distinguished, their relative proportions differing between species, such as man, dog, pig and rabbit, and between extracts originating from fundus or antrum. After activation, these pepsinogens were shown to yield pepsins, functionally different, regarding optimal pH and substrate specificity. In bovine abomasal mucosa, the existence of more than one pepsinogen has been demonstrated as well (Chow & Kassell, 1968; Antonini & Ribadeau Dumas, 1971).

Bovine and porcine pepsinogens were shown to have comparable molecular weights, although several amino acid residues were found to be different. In both pepsinogen types, a large number of acidic and a small number of basic amino acid residues was present, resulting in an isoelectric point of about pH 1, a high stability in strongly acid solutions and a maximal catalytic activity in moderately acid solutions (pH 1.5-2.5). Pepsin is an endopeptidase, attacking peptide linkages in the interior of the polypeptide chains, some preference existing at amino acid residues containing aromatic side chains.

Pepsinogen secretion can be increased by vagal stimulation. In dogs, a marked rise in pepsin output in response to intake of a meal was noticed in an innervated pouch, but not in a denervated gastric pouch (Schofield & Chir, 1957). Pepsinogen secretion is also stimulated after direct electrical stimulation of the vagal trunks, or indirectly by insulin, 2-deoxy-D-glucose or sham feeding, while after denervation pepsinogen secretion is significantly depressed, as was reviewed by Hirschowitz (1967). Malagelada et al. (1976) found in humans a double peak pepsin response after eating. The first peak of pepsin secretion preceded the maximal acid output and was possibly mediated by a cephalic stimulation of pepsinogen secretion. In dog denervated gastric pouches, pepsinogen secretion was stimulated by intravenous infusion of the cholinergic drug methacholine (Dutt & Magee, 1972).

In dogs, distension of the gastric fundus was found to stimulate pepsinogen secretion through a vagally mediated reflex mechanism (Grossman, 1962). Intramural reflexes are involved as well in the regulation of pepsinogen secretion. In dog denervated fundic pouches, Johnson (1972) demonstrated that pepsinogen secretion was stimulated after topical

application of hydrochloric acid. This pepsinogen response was blocked by intravenous infusion of atropine and therefore an intramural cholinergic reflex mechanism was concluded to be involved. In later experiments, the same worker found that pouch acidification potentiated the pepsinogen response to intravenous infusion of histamine and secretin but not to intravenous infusion of the cholinergic drug urecholine (Johnson, 1973).

The experimental results on stimulation of pepsinogen secretion after histamine are rather ambiguous. Linde (1953) reported that pepsin output in dogs decreased after application of histamine. In other experiments, the effect of histamine on pepsinogen secretion seemed to depend upon the vagal innervation of the stomach. In dog denervated gastric pouches, histamine stimulated pepsinogen secretion more actively than in vagally innervated gastric pouches (Hirschowitz, 1957). Such an antagonism between histamine and cholinergic stimulation was also demonstrated by Gibson et al. (1974) in dogs. Whereas the histamine H_2-receptor antagonist metiamide was reported to inhibit gastric secretion of acid in response to cholinergic stimuli, such as urecholine and 2-deoxy-D-glucose, metiamide was found to potentiate pepsinogen secretion in response to these cholinergic stimuli. Ley et al. (1969) and Kowalewski & Kolodej (1973) concluded that in dogs histamine can be regarded as a weak stimulant in comparison with cholinergic stimulation. In rats and humans, however, Samloff (1971) concluded that the histamine dose required for maximal pepsinogen output is close to that for maximal acid output.

Gastrin generally exhibits a weakly stimulative effect on pepsinogen secretion. In man, maximally stimulated pepsinogen secretion after gastrin was demonstrated to be of the same order as after stimulation with histamine (Makhlouf et al., 1967, 1968). In dog denervated gastric pouches, Dutt & Magee (1972) found no consistent rise in pepsinogen output by endogenously released gastrin or by intravenously infused pentagastrin. Johnson (1973) also concluded that pentagastrin does not stimulate pepsinogen secretion in the dog.

Nakajima & Magee (1970) reported that dog denervated pouch pepsinogen output was significantly increased after duodenal acidification to pH 3. In these experiments, intravenous infusion of secretin caused a similar response in pepsinogen secretion, whereas administration of cholecystokinin markedly inhibited pepsinogen secretion. In dog innervated fundic pouches, secretin potentiated and cholecystokinin inhibited pepsin output in response to a meal (Sjodin, 1972). In dogs, a stimulated pepsinogen secretion after duodenal acidification was also noticed by Saik et al. (1969) and in isolated canine stomachs, secretin reduced the output of hydrochloric acid maintained by a continuous intra-arterial pentagastrin infusion, but increased pepsinogen output (Kowalewski & Kolodej, 1974). Comparable effects of secretin and cholecystokinin on pepsinogen secretion have been shown in man (Berstad & Petersen, 1972; Petersen & Berstad, 1973).

In dogs, acidification of the duodenal bulb, however, was found to inhibit pepsin output of innervated fundic pouches, without a concomitant effect on pancreatic enzyme or bicarbonate output, indicating that secretin and cholecystokinin were not involved. A neural or bulbogastrone mediated pathway was proposed (Nilsson, 1975).

In sheep, the importance of vagal stimulation of pepsinogen secretion by the abomasum was shown after insulin-induced hypoglycaemia-mediated vagal excitation. When the rumino-reticulum was emptied a similar effect was noticed, although the pepsin response was smaller than before emptying since insulin-induced hypoglycaemia also induces an increased

flow of digesta to the abomasum by a concurrent reticular hypermotility. Abomasal pepsin output of sheep was also found to be affected by injection of the cholinergic drug carbachol (Hill, 1965).

In sheep innervated abomasal fundic pouches, an increased pepsin output was noticed when sheep were teased with food or fed, the response after feeding depending upon amount and quality of food. This increased pepsin output was proposed to indicate a vagally mediated stimulation, acting directly on the pepsinogen secreting cells. Since this response was not modified after antral resection it was concluded that gastrin does not affect abomasal secretion of pepsinogen, a theory fitting very well with gastrin being a weak stimulant of pepsinogen secretion (McLeay & Titchen, 1970, 1974). In later experiments, however, in contrast with the findings of Dutt & Magee (1972) and of Johnson (1973) in dogs, intravenous infusion of pentagastrin was found to stimulate pepsinogen secretion in sheep. The same effect was noticed after subcutaneous administration of histamine or of cholinergic stimuli, such as carbachol and insulin (McLeay & Titchen, 1975).

2.3.10 Secretion of mucus

Hydrochloric acid at the concentration present in gastric juice is lethal to cells and should be capable to damage the gastric mucosa. Under these acid conditions also pepsin could take part in attacking the stomach wall. Protection of the gastric mucosal surface cells against these agressive agents is supposed to be realized by the gastric mucosal barrier (Davenport, 1972), defined as that property of the gastric mucosa which impedes diffusion of acid and pepsin from the gastric lumen to the mucosa. Specific agents, such as bile salts, lysolecithin and acetylsalicylic acid are capable to damage the barrier, probably by destroying the mucus synthesizing cells after accumulation of these substances in the gastric mucosa.

Once the barrier is damaged, acid can diffuse rapidly into the mucosa, and consequently, the release of histamine from mucosal stores and the activity of histidine decarboxylase were found to be greatly increased. Histamine was indicated (Section 2.3.6) as a potent stimulator of acid secretion, but a considerable part of the hydrogen ions secreted under these conditions will diffuse back from the gastric lumen into the mucosa. Besides, histamine is known to cause vasodilatation and a subsequent increased mucosal blood flow. Mucosal capillaries may be destroyed when mucosal hydrogen ion influx rate is high, and so a damaged mucosal barrier may even lead to mucosal bleeding.

Local damage of the gastric mucosal barrier probably occurs permanently in healthy subjects. However, production of mucus by the mucous epithelial cells was shown to be increased after local irritation of the gastric mucosa (Menguy et al., 1969). Moreover, mature epithelial cells continuously desquamate from the mucosal surface, being replaced by new cells. Davenport (1972a) proposed a complete renewal of the surface epithelial cells every three days.

Waldron-Edward & Skoryna (1970) showed that human gastric mucus is an adhesive 'gum' just after secretion. It forms a continuous coating over the surface epithelial cells and swells to a gel as it imbibes water. At the concentration level, when gel structure is still intact, the mucus layer is probably not only capable to exclude higher molecular

weight proteins such as pepsin, but is also supposed to prevent a rapid back-diffusion of hydrogen ions from the gastric lumen into the mucosa. When more water is absorbed by the mucus layer, however, these protecting capacities are lost, the mucus macro-molecules are desintegrated by pepsin and the degradation products are liberated. This desintegrating process occurs continously at the surface of the mucus layer.

Gastric mucosubstances exhibit different biological activities. Glass (1962) demonstrated anticoagulants, Castle's intrinsic factor, and immunologically active substances to be present in human gastric mucus. In man, mucus glycoprotein configuration was found to be different between individuals and to depend upon subject blood group specifity (Waldron-Edward & Skoryna, 1970; Oates et al., 1974).

The molecular weight of mucus glycoproteins is thought to be higher than 2×10^6. Evidence is available that the basic unit of the glycoproteins consists of a polypeptide chain with a relatively high concentration of serine and threonine. The serine and threonine residues are involved in O-glycosidic linkages to a large number of oligosaccharide chains of variable length, the linking sugar being 2-acetamido-2-deoxy-D-galactose (N-acetyl-galactosamine). The oligosaccharide chains were found to contain not only N-acetyl-galactosamine, but also N-acetyl-glucosamine (2-acetamido-2-deoxy-D-glucose), galactose and fucose, and a single sialic acid residue (N-acetyl-neuraminic acid) as the terminal monosaccharide derivative.

In dogs, Ley et al. (1969) demonstrated that gastric mucus secretion was increased by cholinergic stimulation, but inhibited by histamine. Splanchnic nerve stimulation in man was shown to induce secretion of gastric mucus as well (Davenport, 1971). In dogs, intravenous administration of the cholinergic drug urecholine was shown to induce extrusion of mucus by fundic mucous cells. After this excretory phase, an increased synthesis of mucus was observed. Administration of pentagastrin, gastrin and histamine caused an increased synthesis of mucus, as studied in biopsies of the gastric mucosa, which was not followed by an increased secretion of mucus (Gerard, 1968; Gerard et al., 1968). Secretin in physiological amounts was shown to stimulate gastric secretion of mucus in cats (Vagne et al., 1970) and in humans (Andrē et al., 1972). In cats, an increased secretion of mucus was obtained also after administration of pentagastrin or cholecystokinin and after duodenal acidification (Vagne & Perret, 1976).

Gastric secretion of mucus is studied in general by determination of mucus substances in gastric juice. High concentrations of mucus substances in gastric juice may provide information on secretion of mucus, but they need not necessarily coincide with a well constituted mucus layer on the gastric mucosa.

2.4 BLOOD FLOW IN THE GASTRIC MUCOSA

The ruminant abomasum and the simple stomach in non-ruminant mammals derive their blood supply from four different arteries (Section 2.2). Smaller arteries pierce the muscular coat and form an arterial plexus, which gives off small branches to the muscular layers and to the mucosa. In the mucosa transition occurs from arterioles to capillaries, which unite to form a superficial venous plexus around the mucosal glands. The veins then form a more deeply placed venous plexus in the submucosa before going out through the

muscularis. In addition, from the mucosal arteries arterio-venous shunts arise, which drain into veins of the submucosal venous plexus, allowing the blood to bypass the mucosal capillary bed (Davenport, 1971). Consequently, the amount of blood perfusing the mucosal capillaries is not only determined by total blood flow through the stomach wall, but also by the mode of opening of the arterio-venous shunts, as regulated by the shunt wall smooth muscular fibres. As summarized by Jacobson (1968), mucosal blood perfusion is probably affected by local vasodilator metabolites, produced by the active glandular cells of the mucosa. Besides, mucosal blood flow is supposed to be regulated neurally and hormonally. Sympathetic stimulation inhibits, whereas cholinergic stimulation increases mucosal blood perfusion. Both histamine and gastrin stimulate, but secretin inhibits blood flow in the gastric mucosa (Davenport, 1971; Sanders, 1976).

Blood, passing the mucosal capillaries, provides the mucosal glands with nutrients and since gastric secretion is an active process requiring energy and thus substrates, not only total blood flow through the gastric wall, bu.. also distribution of total gastric blood flow in mucosal and non-mucosal blood flow has been frequently proposed to be related to gastric secretory activity. In dogs, intravenous infusion of histamine resulted in an increased total blood flow to both gastric fundus and antrum, as measured by electromagnetic flowmeters (Rudick et al., 1965). With a ^{42}K clearance technique, Delaney & Grim (1965) showed a comparable effect in the dog after histamine infusion, without a change in distribution to the different tissues of the gastric wall. Domanig et al. (1966) found a transient rise in total gastric blood flow after intravenous or intra-arterial histamine infusion in the dog. Gastric blood flow, however, fell to prestimulative control levels during active gastric secretion. Jacobson et al. (1966) concluded a relationship to be absent between total gastric blood flow and gastric secretory activity in dogs after histamine infusion.

Total stomach blood flow need not be representative for blood flow in the gastric mucosa. Therefore different techniques have been developed to study the mucosal part of total stomach blood flow, such as clearance of isotopes, heat clearance and distribution of radioactive microspheres. Another clearance technique frequently used, is based on the difference in acidity between gastric contents and blood plasma. Weakly ionized bases such as aminopyrine and aniline are not dissociated at the plasma pH level and thus freely permeable to lipoidal membranes. At the acidity level of gastric contents ionization occurs. The ions performed are no longer permeable to the gastric mucosa and are trapped in the gastric lumen. From theoretical assumptions, Jacobson et al. (1967) expected gastric juice aminopyrine concentration to be about 10000-fold of plasma concentration. In general, however, concentration ratios up to 100:1 were detected. That these ratios found were far much lower than the theoretical equilibrium ratio, was concluded to indicate that the rate at which aminopyrine is delivered to the membrane for transport is determined by the gastric mucosal circulation. In consequence, the clearance of aminopyrine through the gastric mucosal membrane can be used for a reliable approximation of blood flow in the gastric mucosa.

Blood flow in the gastric mucosa of dogs, as measured by the aminopyrine clearance technique, was shown to be elevated after histamine infusion (Jacobson et al., 1966; Jacobson & Chang, 1969; Rudick et al., 1971). The ratio of gastric juice aminopyrine con-

centration to plasma aminopyrine concentration, and thus the ratio of blood flow in the gastric mucosa to gastric secretory rate, however, was found to decrease when gastric secretory activity increased. This suggests that gastric secretory activity and blood flow in the gastric mucosa are not related linearly, and that gastric secretory activity is not limited by relatively lower gastric mucosal perfusion rates. Besides, blood flow in the gastric mucosa was higher after histamine than after gastrin infusion at comparable gastric secretory rates (Jacobson & Chang, 1969). The greater mucosal blood flow, observed during histamine stimulation, was suggested to be due to more marked vasoactive properties of histamine in comparison with gastrin, inducing blood flow in the gastric mucosa in excess of that required to support secretion.

In dogs, cessation of intra-arterial histamine infusion was followed by a sharp decrease in total gastric blood flow to or below the prestimulative control level. Gastric secretion, however, continued to be elevated for a period of about 20 min, resulting in a decreased gastric venous oxygen saturation. From this evidence, it was concluded that blood flow in the gastric mucosa is not rate-limiting under normal conditions and that an increased energy requirement may be satisfied either by an increase in blood flow in the gastric mucosa or by an increase in oxygen and substrate extraction (Domanig et al., 1966).

Using a heat clearance technique, Bell & Shelley (1968) suggested nevertheless that in dogs a close relationship between mucosal blood flow and acid secretion exists. Although, however, mucosal blood flow was found to rise with an increased gastric secretion of acid after intravenous histamine or pentagastrin infusion, these two events did not always occur simultaneously. Moreupon, submaximal acid secretion was shown to increase further, when the stimulation dose of histamine or pentagastrin was increased without a concomitant additional rise in mucosal blood flow.

When blood flow in the gastric mucosa is inhibited seriously, however, gastric secretory activity may be limited by the decreased mucosal blood flow. Rudick et al. (1971) demonstrated in dogs that a decreased mucosal blood flow, after infusion of vasopressin, was accompanied intimately by a decreased gastric acid secretory activity.

So far mainly studies on blood flow in the gastric mucosa in relation to gastric secretory activity after pharmacological stimuli, were reviewed. From this evidence, however, it is difficult to decide whether or not a causal relationship exists between changes in blood flow in the gastric mucosa and gastric secretion of acid, since several drugs were found to affect both gastric secretion of acid and blood flow in the gastric mucosa, possibly through separate pathways. Relatively little is known about gastric mucosal blood perfusion in relation to gastric secretion of acid under physiological conditions. Bochenek et al. (1971) found that gastric mucosal circulation paralleled gastric secretion of acid in feeding trials with dogs, as measured with the aminopyrine clearance technique. During the time intervals immediately after feeding, the increase in mucosal blood flow preceded the increase in gastric secretion of acid. It was concluded that a mechanism for increasing mucosal blood flow may exist, which is independent of gastric secretion, or that the increased mucosal blood flow may result from an increased gastric mucosal metabolic activity, which preceded observable increases in gastric secretion. Besides, ratios between aminopyrine concentrations in gastric juice and blood plasma were elevated during periods of duodenally fat-induced inhibition of gastric secretion of acid. This suggests that the

primary effect is on gastric secretion and that this effect is not necessarily mediated by a limited blood flow in the gastric mucosa.

The final conclusion, whether or not blood flow in the gastric mucosa and gastric secretion of acid are causally related, has therefore still to be drawn. Several quantitative deviations from parallelism of gastric secretion of acid and blood flow in the gastric mucosa suggest, in fact, the existence of physiological mechanisms, which regulate secretory activity and blood flow in the gastric mucosa independently.

2.5 CONTRACTILE ACTIVITY OF THE ABOMASUM

2.5.1 Introduction

It has been discussed (Section 2.3.3) that abomasal activity in ruminants is more regular in time than gastric activity in monogastric animals, concerning both secretory and motor activity. Under normal conditions the abomasum will never be empty, it secretes continuously and abomasal contents are pushed forward into the duodenum in a rather regular pattern due to a more or less continuous abomasal peristaltic activity.

Principally motor activity of the abomasum does not seem to be different from the motility of the simple stomach and therefore information achieved from experiments with monogastric animals will be referred to as well.

2.5.2 Electrical activity of smooth muscle

The muscularis mucosae and the main muscle coats of the gastro-intestinal tract are composed of smooth muscular fibres. In general, the main muscle coats are arranged in an inner circular and an outer longitudinal layer. In the non-ruminant simple stomach, Christensen (1971) distinguished three muscle layers, the incomplete longitudinal layer lieing mainly in two bands along the two curvatures, the circular layer investing all but the cardiac region, and the inmost oblique layer, also incomplete, but present in the fundic part and extending distally on the lateral surfaces in two broad sheets, fusing with the circular layer at the onset of the antral part. Of the ruminant abomasum such a detailed description of the arrangement of the main muscle coats is not available. In the abomasal antral part, the normal arrangement of an inner circular and an outer longitudinal muscle layer has been established.

In striated skeletal muscles, contraction of fibres is caused by interaction between the myosin and actin filaments. Contraction is induced by a release of calcium ions from the cisternae of the sarcoplasmic reticulum into the sarcoplasm. In smooth muscular fibres, the existence of thick myosin filaments in association with thin actin filaments was demonstrated recently and contractions are also supposed to be triggered by an increase in intracellular concentration of calcium ions released by the sarcoplasmic reticulum (Somlyo et al., 1973).

Among smooth muscular cells a close electrical coupling was found. From electron microscopy, intracellular bridges, called nexuses, were described. These nexuses are not cytoplasmatic connections, but they are tight junctions between cell membranes, and are

supposed to perform low-resistance electrical shunts between cells, necessary for the propagation of smooth muscular electrical activity (Christensen, 1971).

Contraction of smooth muscle cells follows electrical changes at their membrane, electrical changes depending on electrolyte distribution between cell contents and extracellular fluid. The resting membrane potential is mainly dependent on intracellular potassium ion concentration. Cytoplasm is 40-60 mV negative to the extracellular fluid associated with a net outward current of potassium ions, which diffuse down the concentration gradient across the cell membrane extracellularly, since intracellular potassium ion concentration is kept high by an active pump mechanism. In general, however, this transmembrane potential of the gastro-intestinal smooth muscular cells is not stable, even when no contractile activity is observed. Membrane permeability was shown to fluctuate in a regular pattern coincident with transmembrane potential changes after an increased net inward current flow of mainly sodium ions. This basic pattern of electrical activity in smooth muscle is known as the slow wave electrical activity.

Depending on type and orientation of recording, the configuration of the slow wave may differ. Transmembrane recordings obtained with intracellular micro-electrodes, or with pressure or suction electrodes, showed slow waves of a simple sinusoidal form in rat and guinea pig stomach (Boev, 1972). Each slow wave cycle consisted of a rapid depolarization of approximately 10 mV, a plateau and a gradual repolarization, which was terminated by the depolarization of the next slow wave. In the dog inactive antrum, transmembrane recordings show a different type of slow wave, consisting of a fast spike, which declines exponentially, called the slow wave first potential (Daniel, 1966).

After extracellular recording the slow wave shows a pattern like the first derivative of the transmembrane-recorded slow wave. This type of slow wave configuration was present in the extracellularly recorded abomasal electromyogram of cattle (Ruckebush & Kay, 1971), of sheep (Ruckebush & Laplace, 1967; Ruckebush, 1970) and of the preruminant calf (Bell & Grivel, 1975).

Gastric slow waves are presumably generated in the longitudinal muscle layer. In contrast to the small intestine, slow wave frequency in the stomach is the same in all regions from which slow waves can be recorded. This indicates a single frequency level to exist in the stomach. In dogs, transverse or oblique transection of the muscle layers in the antral region resulted in a decreased slow wave frequency distal to the cut. Therefore gastric slow waves were suggested to originate from a gastric pacemaker located in the greater curvature near the proximal part of the gastric fundus (Weber & Kohatsu, 1970). The existence and localization of such a pacemaker accounts for the fact that gastric slow waves normally propagate in a distal direction. They are supposed to be conducted by the longitudinal muscle layer and to spread electrotonically to the underlieing circular layer, since they were not recorded from isolated circular muscle (Bortoff, 1972).

It has been shown that the frequency level of the gastric slow wave is rather constant. Minor modulations concerning frequency, amplitude, and propagation velocity were demonstrated after neural or hormonal excitation or inhibition (Daniel, 1965; Cooke et al., 1972). In the antral part of the stomach, slow wave activity is always present, but opinions differ on the existence of slow waves in the gastric fundus. In the sheep abomasal fundus, Ruckebush (1970) concluded slow wave activity to be absent. According to other

experimental results, however, slow wave activity was accepted to be present in the fundus of sheep (Laplace, 1970) and of calves (Bell & Grivel, 1975), but not in the normal regular pattern.

Differences in frequency between species have been noticed. In humans, a slow wave frequency of 3 cycles/min (Couturier et al., 1971), in dogs of about 5 cycles/min (Daniel, 1965; Cooke et al., 1972), in sheep of 7 cycles/min (Laplace, 1970; Ruckebush, 1970), in cattle of about 5 cycles/min (Ruckebush & Kay, 1971), and in calves of 4-5 cycles/min (Bell & Grivel, 1975) were reported.

Gastric slow waves are thought to be generated in a pacemaker centre, they spread distally and propagation velocity is supposed to increase towards the pylorus. In dogs, Daniel (1965) noticed an increase of about 0.6 cm/sec proximally to about 3 cm/sec in the distal part of the antrum.

It has been suggested that antral slow waves may play a role in coordinating smooth muscle contractile activity at the duodenal junction during gastric emptying (Bortoff & Davis, 1968; Duthie et al., 1971; Bedi & Code, 1972; Bell & Grivel, 1975). Antral slow waves were proposed to propagate across the gastro-duodenal junction along longitudinal muscle bundles passing from antrum to duodenum, or through intramural neural connections, spreading electrotonically into duodenal muscle where they periodically augment the depolarization of the duodenal slow waves.

Slow waves are not believed to trigger smooth muscular contractile activity directly. They are involved in the control of rhythmicity in gastro-intestinal muscle, by virtue of the fact that they alternately increase and decrease the probability of spike discharge and consequently contractile activity. Slow waves spread from the longitudinal layer electrotonically to the circular layer and affect the excitability of circular muscle in accordance with that of longitudinal muscle. Spike discharges are fast depolarizations of smooth muscular cells, and are thought to be initiated by an increased intracellular calcium concentration (Davenport, 1971; Somlyo et al., 1973). Their configuration may be different. In the dog active antrum, the slow wave first potential change is followed by a second potential change built up by fused fast spike potentials (Daniel, 1966). Probability of fast spike potential changes is highest when transmembrane potential is lowest and therefore in the case of the sinusoidal slow wave pattern the burst of fast spike potentials can be noticed only on the plateau of depolarization of the slow wave.

Smooth muscular contractile activity is preceded by a variable number of spike discharges, as triggered by the slow wave rhythmicity. A simple spike discharge does not necessarily induce contractile activity. When frequency of spike discharge per slow wave cycle is higher and when more smooth muscle cells are involved in the fast depolarization process, smooth muscular contraction will be more intensive. Both frequency of spike discharges and whether or not spike discharges occur, in phase with slow wave rhythmicity, are affected neurally and hormonally, as is discussed in Section 2.5.3.

2.5.3 Neural and hormonal control of contractile activity

Gastro-intestinal smooth muscular contractile activity is affected both parasympathetically and orthosympathetically. As was discussed in Section 2.2, two main intramural nerve

plexuses are present in the gastro-intestinal wall, the myenteric plexus located in the interspace between the longitudinal and circular muscular layer, and the submucosal plexus. The parasympathetic fibres are preganglionic and cholinergic, and synapse with the ganglions of the myenteric and submucosal plexus. Stimulation of intramural postganglionic cholinergic fibres leads to a release of acetylcholine, which increases excitability of smooth muscle cells by an increase in membrane permeability, and thus by a decrease of the transmembrane potential (Davenport, 1971). Whether the postsynaptic adrenergic sympathetic fibres synapse with plexus ganglions or with smooth muscle cells directly or with both is not yet quite understood. Daniel (1968) proposed sympathetic neural elements to innervate the myenteric plexus only, inhibiting smooth muscular electrical and mechanical events by diminishing the parasympathetic response. Intravenous or topical application of catecholamines reduces slow wave amplitude as well as slow wave propagation velocity, and thus contraction activity and contraction propagation velocity. In goats, the inhibiting effect of catecholamines on antral motility was shown by Ehrlein (1970).

Evidence has also been presented for the existence of a non-adrenergic inhibition of gastro-intestinal motility. The vagus nerves are known to contain fibres whose stimulation causes inhibition of gastric motility (Daniel, 1969). This inhibition was not prevented after administration of adrenergic blocking agents, such as phentolamine and propranolol. Bülbring & Gershon (1967) presented evidence in guinea pigs and mice that this vagal inhibitory transmitter is serotonine (5-hydroxy-tryptamine). According to the experimental results of Anuras et al. (1974), this type of neural control mechanism should be especially important in the regulation of pyloric sphincter function. In isolated pyloric circular muscle strips of opossums, cats, dogs and humans, electrical stimulation caused relaxation, which was not antagonized by adrenergic or cholinergic blocking agents. Such a relaxation was not found in circular muscle strips isolated from antrum or duodenum, which contracted upon electrical stimulation or showed no response.

The intramural nerve fibres are not only effectory functional, but also sensory. In the regulation of gastro-intestinal motility two main neural pathways are involved, reflex mechanisms whose afferent and efferent fibres are located in sympathetic and parasympathetic nerves, and reflexes not involving higher neural centers, but operating through the intramural plexuses. Based on functional relationships different gastro-intestinal receptor types have been classified. In the cat gastric and in the sheep abomasal fundic and antral region, the existence of slowly adapting tension receptors was described by Iggo (1955) and Harding & Leek (1973), stimulation leading to an increase of frequency of discharge of particular vagal afferent fibres. In later experiments, rapidly adapting mucosal receptors were detected in sheep abomasal fundus and antrum, and in the duodenum, responding to tactile stimulation and acid solutions (Leek & Harding, 1975). In the cat, activation of antral tension receptors was found to inhibit gastric motility neurally through a spinal reflex mechanism, mediated by splanchnic adrenergic fibres, and to cause a vagally mediated reflex relaxation of the fundus (Abrahamsson, 1974). In dogs, duodenal distension was demonstrated to lead to a temporary slowing of the antral rhythm and a corresponding reduction in frequency of antral peristaltic activity. This neural mechanism was not blocked by pyloric section, but was completely abolished after sympathectomy and bilateral vagotomy (Daniel & Wiebe, 1966). In goats, inhibition of antral motor activity after duo-

denal distension or intraduodenal introduction of hydrochloric acid or of short- and long-chain fatty acids was described by Ehrlein & Hill (1970). A similar inhibiting effect on gastric antral motor activity was noticed after introduction of hypertonic solutions in the proximal part of the duodenum in humans (Hunt, 1959) and in preruminant calves (Bell & Razig, 1973), but whether or not excitation of the postulated osmo-receptors leads to a hormonally or neurally mediated signal is not quite clear. That this connection is not intramural in the preruminant calf, was evidenced by Bell & Grivel (1975), who found that transection of the duodenum near to the pylorus did not abolish the inhibiting response.

In the regulation of gastric motility also gastro-intestinal hormones play an important role. Stimulation of gastric motor activity after gastric distension in pigs was partly effectuated by a stimulated release of gastrin, as reported by Stadaas et al. (1974), gastrin acting probably through a stimulated release of acetylcholine, as was postulated by Vizi et al. (1973) in the guinea pig ileum. In the unweaned calf, however, inhibition of abomasal fundic and antral motility was demonstrated after intravenous infusion of pentagastrin, although the antral slow wave rhythm persisted (Bell et al., 1975).

Inhibition of gastric motor activity after duodenal infusion of acid, of amino acids or of fatty acids is supposed to be mediated partly through hormonal pathways after release of secretin or cholecystokinin and possibly of other peptide hormones such as enterogastrone or bulbogastrone (Section 2.3.5). In man, extracts of duodenal mucosa were shown to inhibit gastric motility (Johnson et al., 1966).

Also the pressure of the pyloric sphincter may be affected hormonally. Opossum pylorus pressure was shown to be augmented by cholecystokinin and secretin, a response which was antagonized after administration of gastrin (Fisher et al., 1973).

2.5.4 Mechanism of evacuation

Gastric emptying is not simply regulated by the stomach itself. The effector systems of gastric smooth muscles are under multifactorial control by facilitatory and inhibitory mechanisms activated from receptors located mainly in the small intestine. Hunt (1959) proposed that these control mechanisms are necessary in order to keep volume and composition of the duodenal contents within certain limits.

Gastric emptying is probably not only dependent on gastric motor activity, but also on pressure gradient between gastric lumen and duodenum and on pyloric contractile activity. Normally the gastric antrum is regarded as the main propelling organ. Together with the propagation of the gastric slow wave (Section 2.5.2), antral peristaltic waves pass distally with an increasing propagation velocity towards the pylorus. A portion of the fundic contents is supposed to be squeezed into the antrum and propelled distally. Depending on pyloric contractile activity it should be determined whether the antral contents, pushed forward by the antral peristaltic contraction, are pumped into the duodenum or partly rejected into the gastric fundus (Davenport, 1971).

In practice, however, the relationship between gastric outflow rate and gastric motor activity has been shown to be more complex. Bell & Grivel (1975) suggested that the abomasal fundus of the preruminant calf is more than a simple storage vessel and plays an important role in the pulsatile evacuation of the abomasal contents. Abomasal antral con-

tractions were supposed to be reinforced by both rhythmic and tonic fundic contractions and abomasal outflow was occasionally even noticed during periods of antral inactivity.

In dogs, gastric emptying of slightly hypertonic solutions of NaCl and $NaHCO_3$ was retarded after intravenous infusion of pentagastrin, although the frequency of the antral slow wave rhythm was increased (Dozois & Kelly, 1971). These findings were confirmed by Cooke et al. (1972) in dogs. In these experiments, intravenous administration of pentagastrin significantly delayed gastric emptying while an increase was noticed in the slow wave frequency in antrum and duodenum and also of the frequency and intensity of the antral contractile activity. In man, gastric emptying decreased proportionally to an intravenous infusion of porcine gastrin (Hunt & Ramsbottom, 1967). In the unweaned calf, the same effect on abomasal emptying was noticed after intravenous infusion of pentagastrin, in this case accompanied, however, by an inhibition of abomasal fundic and antral contractile activity (Bell et al., 1975). In an isolated rat stomach-duodenum preparation, Armitage & Dean (1963) demonstrated that gastric emptying may occur when antral peristaltic activity was less intensive.

Intraduodenal instillation of an isotonic NaCl solution in dogs was shown to stimulate gastric emptying (Dozois & Kelly, 1971). Simultaneous recordings of the electromyogram, however, showed a decreased frequency and propagation velocity of the antral slow waves. Stemper & Cooke (1975) demonstrated that in dogs a direct relationship between antral contractile activity and gastric emptying rate does not always exist, when measured simultaneously. Although in their experiments in general the rate of gastric emptying increased with frequency and intensity of antral contractions, gastric emptying was occasionally noticed when no antral contractile activity was detectable, and during maximal antral contractile activity gastric emptying was occasionally absent. They concluded that although the antrum has a significant role in gastric emptying, other factors may modify the process. In this context, Weisbrodt et al. (1969) suggested that in dogs the delay in gastric emptying of a fat meal arises from incoordination between antral and duodenal contractile activity. They found that when emptying was rapid, antral activity was increased and duodenal activity was decreased. When gastric emptying was delayed the opposite effect occurred.

In rabbits, Ehrlein (1976) showed that the relation between gastric emptying and gastric antral and pyloric motility differed with consistency of digesta. Injection of saline into the stomach did not affect gastric peristalsis substantially but increased gastric emptying. When mashed potatoes with variable viscosity were introduced into the stomach, both gastric motility and emptying were distinctly increased. It was concluded that after feeding or injecting into the stomach of meals with a low viscosity, intragastric pressure upon contraction is lower than after introduction into the stomach of more viscous meals. With increasing viscosity a greater resistance to flow is exerted, intragastric pressure is more increased upon contraction, the tension receptors of the gastric wall are more actively excitated, resulting in a more substantial relaxation of the gastric fundus. Whereas gastric emptying of liquid meals could be induced by the gastric fundus, emptying of more viscous meals did not happen without peristaltic contraction activity of the antral part. In this context, it was suggested that in ruminants, abomasal emptying is not determined predominantly by abomasal antral contractile activity, but that fundic contractile activity is involved as well, since in general viscosity of abomasal contents is

low.

Further information on gastric emptying rate did become available with the 'test meal' method. In such experiments, the recovery of a liquid meal is determined by emptying the stomach via an oesophageal tube a particular period after instillation of the meal into the stomach. Based on this method, gastric emptying was found to depend upon volume of the gastric contents. In general, gastric emptying has been found to be exponential in form, although in some cases a linear relationship was shown between gastric emptying rate and the square root of gastric volume (Hopkins, 1966). In the preruminant calf, Bell & Razig (1973) demonstrated that abomasal emptying is exponential in character whether large or small volumes of fluid are instilled into the abomasum.

Gastric emptying rate in man, after introduction of solutions of inorganic or organic substances into the stomach, depends upon the osmotic pressure of the solution and on the nature of solute. Gastric emptying was increased by hypotonic solutions of NaCl, Na_2SO_4, $NaHCO_3$, and urea, whereas hypertonic solutions of the same solutes delayed gastric emptying. Of other substances, such as HCl, H_2SO_4, $(NH_4)_2SO_4$, KCl, $CaCl_2$, sorbitol and glucose, both hypotonic and hypertonic solutions slowed gastric emptying (Hunt & Pathak, 1960). These findings were explained on the assumption of an osmo-receptor mechanism, inhibiting gastric emptying. These receptors were postulated to be present in the small intestine, since introduction of these solutes into the proximal part of the duodenum exhibited the same response. The majority of the solutes is assumed not to penetrate this osmo-receptor, but sodium ions and urea are thought to be subject to facilitated transport across the osmo-receptor membrane. A signal from these osmo-receptors, slowing gastric emptying, is thought to be elicited upon deflation of the osmo-receptor cell, when a net flux of water from the osmo-sensitive compartment is induced by the osmotic action of the solutes. Bell & Razig (1973, 1973a) concluded that in the preruminant calf the same receptors are effective in controlling abomasal emptying.

In man, slowing of gastric emptying produced by disaccharides was generally consistent with the effect occurring after hydrolysis of disaccharides. It was suggested that excitation of the osmo-receptors occurs after hydrolysis of disaccharides by disaccharidases, attached to the brush border of the small intestinal epithelial cells, and consequently the osmo-receptors were supposed to be located between the brush border microvilli (Elias et al., 1968). In dogs, disaccharides and dipeptides exhibited a potency, which was almost twice of their respective constituent monosaccharides or amino acids at equimolar concentrations (Cooke et al., 1976).

Intraduodenal infusion of triglycerides in rats caused a potent inhibition of gastric evacuation activity, the inhibitory effect being lost after diversion of bile or of both bile and pancreatic juice (Morgan, 1963). It was suggested that inhibition of gastric emptying arises from free fatty acids. In man, salts of acetic acid up to decanoic acid showed to be relatively ineffective in slowing gastric emptying. Salts of higher fatty acids, especially of myristic acid, were far more effective, probably due to their higher fat solubility (Hunt & Knox, 1968).

In man, perfusion of the jejunum with hypertonic solutions did not affect gastric emptying and therefore it was concluded that in man the osmo-receptors are present in the duodenum but not in the jejunum (Meeroff et al., 1975). Bell & Grivel (1975) found that in

the preruminant calf intraduodenal infusion of an isotonic $NaHCO_3$ solution stimulated abomasal emptying. After transection of the pylorus the same effect was noticed and therefore an intramural reflex mechanism was ruled out.

In dogs, perfusion of the proximal duodenum indicated that receptors responding to acid are present directly distally to the pylorus, but not receptors activated by monosaccharides or fatty acids (Cooke, 1975). It was suggested that inhibition of gastric emptying by acid in the proximal duodenum is partly mediated by neural mechanisms, because of the rapidity (1 min) of onset of inhibition of emptying. In dogs, introduction into the duodenum of isotonic or hypertonic solutions of mono- and disaccharides, of amino acids or of fatty acids did not increase plasma secretin concentration (Boden et al., 1975).

Also amino acids have been shown to delay gastric emptying. In man, inhibition of gastric emptying was related to the molar concentration of the amino acid in the test meal (Cooke & Moulang, 1972). In dogs, only tryptophan showed an inhibitory effect (Stephens et al., 1975), which was antagonized by methionine but not by lysine (Cooke et al., 1976; Stephens et al., 1976). Phenylalanine, a potent cholecystokinin releaser, however, did not slow gastric emptying and therefore it was suggested that tryptophan does not act only through a release of cholecystokinin.

Although it is not quite well established, excitation of small intestinal receptors to act through hormonal or neural pathways, enterohormones released by the small intestinal mucosa are known to delay gastric emptying. In man, a delayed gastric emptying was shown after intravenous administration of secretin and cholecystokinin at doses submaximal for pancreatic secretion (Chey et al., 1970; Vagne & André, 1971).

3 Abomasal secretion of acid in relation to ration composition

3.1 INTRODUCTION

Abomasal and gastric secretion of acid results from the interplay of neural and hormonal activities, induced in advance of, during, and after food intake (Sections 2.3.2-2.3.5). Excitation of neural and hormonal control mechanisms was indicated to depend upon amount and composition of digesta in different gastro-intestinal compartments. Therefore it is to be expected that abomasal secretion of acid in sheep is affected by ration composition.

In dogs, a relationship between gastric secretion of acid and diet composition was demonstrated by Saint Hilaire et al. (1960). On the basis of the amounts of acid secreted by innervated pouches of the gastric fundus, a close relationship was found between gastric secretion of acid and protein content of the diet ($r = 0.97$). Since in general, proteins are more potent buffers than carbohydrates and fats, a close relationship was also demonstrated between gastric secretion of acid and diet buffering capacity ($r = 0.81$). In man, Rune (1973) found a lower postprandial duodenal pH after intake of a high-protein meal than after a low-protein meal. In dogs, the physical structure of the diet was found to affect gastric acid secretory activity. When equal amounts were supplied of the same diet, chopped or blenderized, the highest acid outputs of vagally denervated fundic pouches and the highest serum gastrin levels were observed after feeding on the blenderized meal (Ashby & Himal, 1975).

In ruminants, abomasal secretion of acid in relation to ration composition was indicated to depend on the events taking place in the forestomachs. The almost continuous passage of digesta in the abomasum was described to exhibit a continuous stimulation of abomasal secretion of acid (Section 2.3.3). Although digesta composition is substantially modified as a result of the fermentation process in the forestomachs, amount and composition of digesta entering the abomasum depend partly on amount and composition of the ration. Hogan & Weston (1967) found that feeding sheep on a higher-protein (13.8 g N per day) or on a lower-protein (5.5 g N per day) diet resulted in an abomasal outflow of digesta of 5.0 and 6.8 litre/day, an abomasal outflow of organic matter of 156 and 204 g/day, and an amount of nitrogen leaving the abomasum of 9.5 and 8.1 g/day, respectively. When sheep were offered ryegrass, harvested at different stages of maturity, digesta flow from the abomasum was relatively high after feeding early harvested ryegrass (Weston & Hogan, 1968). In dairy cows, a high duodenal passage rate of digesta was found when the ration consisted of fresh mown grass, in comparison with a ration consisting of hay and concentrates (van 't Klooster & Rogers, 1969; van 't Klooster et al., 1972).

It is to be expected that differences in composition and quantity of digesta passing the abomasum induce secretion of variable amounts of hydrochloric acid. On this matter, however, evidence in ruminants is hardly available. On the basis of acid secretion by inner-

vated abomasal fundic pouches, both secretory rate and acidity of abomasal juice increased when the ration was changed from a mixture of lucerne and wheaten hay to lucerne hay, or when dry matter intake was raised (McLeay & Titchen, 1973, 1974).

The pH of digesta entering the abomasum approximates neutrality (Section 2.3.3). Therefore in our experiments the amount of hydrochloric acid secreted in the abomasum was estimated by titration of the proximal duodenal contents to pH 7. This method takes no account of back-diffusion of hydrogen ions from abomasal contents into the blood stream. Davenport (1967a), using unstimulated, vagally denervated fundic pouches in dogs, found H^+ losses of 3-14% after 30 min. Also a minor neutralization of acid digesta, leaving the abomasum, may be caused by the alkaline juice, secreted by the Brunner glands, situated proximally to the duodenal re-entrant cannulas. This effect seems to be of little importance, since in sheep no differences in acidity were found between digesta collected from an antral cannula or from a cannula inserted into the proximal duodenum (Weston, 1976). Since hydrogen ions are secreted in the abomasum together with chloride ions, the amount of chloride leaving the abomasum, eventually corrected for the amount of chloride leaving the omasum, may deliver an additional parameter for abomasal acid secretory activity.

In order to test the dependence of abomasal secretion of acid in sheep on ration composition, three feeding trials were carried out.

3.2 METHODS

Animals. Texel wethers, weighing 45-60 kg, were fitted with hard plastic re-entrant cannulas into the proximal part of the duodenum, a few centimeters behind the pylorus. An operation technique similar of that of Hogan & Phillipson (1960) was used (Fig. 1). In some of the

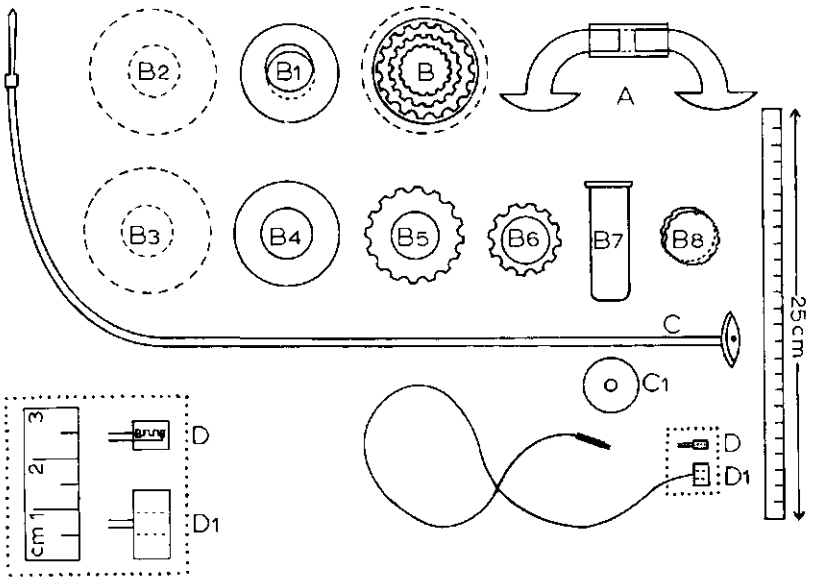

Fig. 1. A: Re-entrant cannulas; B: Rumen cannula, B_1-B_8: Dismantled; C: Abomasal infusion tube, C_1: Serosal ring; D: Strain gauge at two scales, D_1: Fixated on a metal sheet and incorporated in silicone.

sheep, a hard plastic rumen cannula of inner diameter 25 mm was inserted into the dorsal rumen sac, about 10 cm distal to the last rib. Surgery was performed several weeks before the start of the experiments. Sheep were kept individually in metabolism cages, and care was taken to start the experiments after the sheep had been well trained.

Rations. The rations used were partly derived from own resources. The grass used in Feeding Trial 1 was harvested in October and the grass used in Feeding Trial 3 in May, and kept at -20 ^{o}C. Hay was harvested in June. Of the components of the semisynthetic diets, hereafter called 'semisynthetics', soya protein (promine D) was derived from Central Soya Chemurgy (Chicago), cellulose (AKU-Floc) from AKZO Plastics BV (Zeist), and maize starch from 'De Byenkorf' BV (Koog a/d Zaan).

In Feeding Trial 1, three rations of different composition (Table 1) were offered to three sheep, randomized over the sheep according to a 3 x 3 Latin square design. Rations, offered to the sheep, were spread over the day in 6 equal portions at 6:30 h (1x), 10:30 h (1x), 14:30 h (1x), 18:30 h (1x), and 22:30 h (2x). Sheep received the different rations about two weeks before collection of duodenal digesta was started.

In Feeding Trial 2, rations consisted of a fixed amount of ryegrass straw and variable amounts of a semisynthetic diet. With the semisynthetics assigned in Feeding Trial 1 (Table 1), the effect of four different ration compounds, i.e. soya protein, $KHCO_3$, maize starch and cellulose, on abomasal secretion of acid was studied. These four ration compounds were added in all possible combinations to a fixed basic ration, as demonstrated in Table 2. Rations were offered to the sheep in 2 equal portions at 6:30 and 18:30 h. The 16 different rations were given to four sheep, randomized over the sheep according to a 2^4 factorial design, repeated twice, with the 4-factor interactive component confounded with the effect due to differences between sheep (Table 3). The trial was divided in 8 experimental periods of 2 weeks. Sheep received the particular rations 10 days before duodenal

Table 1. Amount and composition of rations in Feeding Trial 1.
For abbreviations see Methods (Section 3.2).

		Ration		
		A hay	B grass	C ryegrass straw and semisynthetics
Hay	g/day	850		
Grass	g/day		2700	
Ryegrass straw	g/day			480
Minerals	g/day	6	6	8
Cellulose	g/day			120
Sucrose	g/day			40
Soya oil	g/day			16
Soya protein	g/day			60
Maize starch	g/day			76
DM intake	g/day	752	735	712
CP intake	g/day	130	146	79
NDR intake	g/day	271	232	282
K intake	mmol/day	598	412	220
Na intake	mmol/day	134	129	118
Cl intake	mmol/day	376	161	225

Table 2. Amount and composition of rations in Feeding Trial 2. To a fixed basic ration, consisting of 500 g ryegrass straw, 80 g cellulose, 50 g sucrose, 20 g soya oil and 10 g minerals, none, one or more of the four following variable ration components were added in amounts as given: 1: 100 g cellulose, 2: 200 g soya protein, 3: 90 g $KHCO_3$, and 4: 100 g maize starch. For abbreviations see Methods (Section 3.2). Ration composition is coded binary.

Binary code	Intake				
	DM g/day	CP g/day	K mmol/day	Na mmol/day	Cl mmol/day
0000	589	28	198	59	243
0001	682	28	198	59	243
0010	778	207	204	67	247
0011	871	207	204	67	247
0100	678	28	1068	77	243
0101	771	28	1068	77	243
0110	867	207	1074	85	247
0111	960	207	1074	85	247
1000	676	28	198	59	243
1001	769	28	198	59	243
1010	865	207	204	67	247
1011	958	207	204	67	247
1100	765	28	1068	77	243
1101	858	28	1068	77	243
1110	954	207	1074	85	247
1111	1047	207	1074	85	247

Table 3. Experimental scheme of Feeding Trial 2. Rations are coded binary.

Experimental period	Sheep			
	1	2	3	4
1	1001	0010	1110	1010
2	0110	1101	0001	0101
3	0011	1000	1011	1111
4	1100	0111	0100	0000
5	0000	0100	0111	1100
6	1111	1011	1000	0011
7	0101	0001	1101	0110
8	1010	1110	0010	1001

sampling was started.

In Feeding Trial 3, three rations of different composition (Table 4) were given to three sheep, randomized over the sheep according to a 3 x 3 Latin square design. Rations were offered to the sheep in 4 equal portions at 7:00, 9:00, 17:00 and 19:00 h. Sheep received the different rations about two weeks before collection of duodenal digesta was started.

Experimental procedure. At sampling days, the duodenal re-entrant cannulas were disconnected about half an hour before sampling of duodenal digesta was started. Digesta were allowed to flow through a tube, connected with the proximal cannula, into a vial kept at body temperature in a waterbath (39 °C). During this half hour period, duodenal digesta were return-

Table 4. Amount and composition of rations in Feeding Trial 3. For abbreviations see Methods (Section 3.2).

		Ration		
		A grass	B hay and grass	C hay and semisynthetics
Grass	g/day	5000	2500	
Hay	g/day		500	500
Cellulose	g/day			87
Sucrose	g/day			75
Soya oil	g/day			25
Soya protein	g/day			130
Maize starch	g/day			150
$KHCO_3$	g/day			33
DM intake	g/day	900	907	921
CP intake	g/day	218	159	167
NDR intake	g/day	155	234	213
K intake	mmol/day	585	435	447
Na intake	mmol/day	161	139	137
Cl intake	mmol/day	121	151	100

ed as frequent as possible into the duodenum through the distal cannula, in order to adapt the sheep to the experimental procedure. During the experiments, vial contents were weighed, sampled and returned after each 10-min period.

In Feeding Trial 1, per sheep and per ration, duodenal digesta were sampled for a 12-h period (9:00-21:00 h) on three consecutive days. After each 10-min period, a proportional sample (15%) was removed. Samples were replaced by donor digesta, collected from the same sheep one week in advance and stored at -20 °C. Samples were pooled hourly and kept at -20 °C.

The same procedure was followed in Feeding Trial 2, except that duodenal digesta were sampled from 6:30-18:30 h. In this trial, after each 10-min period, a proportional sample (10%) was removed and not replaced by donor digesta. Samples were pooled daily and kept at -20 °C. Fluid samples from the ventral rumen sac were taken on the day following the three 'duodenal' sampling days at 8:00, 11:00, 14:00 and 17:00 h.

In Feeding Trial 3, per ration and per sheep, duodenal digesta were sampled for a 12-h period (7:00-19:00 h) on five consecutive days. Ten percent samples were removed from duodenal digesta and replaced by donor digesta collected one week in advance from the same sheep and kept at -20 °C. Samples were pooled per 3-h period and stored at -20 °C.

Analyses of rations. Samples were dried to constant weight at 101 °C for the estimation of dry matter (DM). Nitrogen was estimated by the Kjeldahl method using HgO and K_2SO_4 as catalysts (Bouman, 1949), crude protein (CP) being calculated as N x 6.25. Crude fibre was estimated as the neutral detergent residue (NDR) (Gaillard & Nijkamp, 1968). For the Cl analyses, samples were boiled in water for 10 min and centrifuged (Christ UJ3 centrifuge, 2000 g). Cl was estimated in the supernatant fraction according to the method of Volhard (Vogel, 1961). Samples were ashed (3 h at 500 °C); K and Na were estimated in the ash fraction, after solution in concentrated HCl and after proper dilution, with the Kipp HD45 flamephotometer.

Analyses of digesta. Duodenal passage rates of digesta (PRD) were measured during the experiments. The average per hour was calculated for each 12-h experimental period. In duodenal samples, dry matter (DM) and crude protein (CP) were determined as described under rations. After measuring the pH, total acid concentration (TA) of duodenal samples was estimated by titration to pH 7 with NaOH 0.1 mol/litre (Radiometer Copenhagen, Type TTT1^{c}). Duodenal and ruminal chloride concentrations (Cl) were determined in the supernatant solution (Christ UJ3 centrifuge, 2000 g), according to the method of Volhard (Vogel, 1961). Duodenal pepsin activity was determined in the same supernatant samples against porcine pepsin (Merck, Darmstadt; 35000 U/g) as a standard. Supernatant samples were incubated with 2.0% hemoglobin (pH 1.7, 20 min, 25 ^{o}C). Incubation was stopped by precipitation of the protein with trichloracetic acid. Samples were centrifuged (Christ UJ3 centrifuge, 2000 g) and in the supernatant solutions extinctions were read at 660 nm (Beckman B spectrophotometer), exactly 4 min after addition of the reagent of Folin and Ciocalteu (Baars, 1962). Duodenal passage rates of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), of chloride (PRCl), and of pepsin (PRPE) were calculated by multiplying the concentrations by the individual duodenal passage rates of digesta. PRCl and PRPE were corrected for dry matter content of digesta. Volatile fatty acids in rumen samples of Feeding Trial 2 were analysed gaschromatographically (Becker, Unigraph F, Type 2035; 1 m glass column (4 mm), filled with Chromosorb W AW-DMCS (60-80 mesh) with 20% Tween 80; carrier gas N_2, saturated with formic acid). Polyethylene glycol was determined in ruminal and duodenal supernatant solutions (Christ UJ3 centrifuge, 2000 g) by Hydén's method (1955), except that the turbidity was read exactly 10 min after addition of trichloracetic acid (Beckman B spectrophotometer).

Statistics. Data of Feeding Trials 1 and 3 were analysed statistically according to the analysis of variance for a Latin square design. Of Feeding Trial 2, the analysis of variance was used, appropriate for a 2^4 factorial design (Snedecor & Cochran, 1962). Statistical comparisons of duodenal data between rations were made using the two-tailed Student t test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Standard deviations are indicated by SD, unless otherwise mentioned.

3.3 RESULTS OF FEEDING TRIAL 1

Duodenal passage rates of digesta, as affected by ration composition and feeding time, were plotted in Figure 2. Each column represents the mean of 9 sampling periods. Standard deviations are indicated by the vertical bars. Although differences in duodenal passage rates of digesta between hours were not significant in general, some regular pattern in relation to feeding time for the different rations could be noticed. Especially when the semisynthetic ration (C) was used, the highest duodenal passage rate of digesta occurred in the last hour before the sheep were fed.

In Table 5, total acid and chloride concentrations are shown, as affected by ration composition and feeding time. Composition of duodenal digesta did not show very substantial fluctuations over the experimental periods, probably due to the frequent feeding regime.

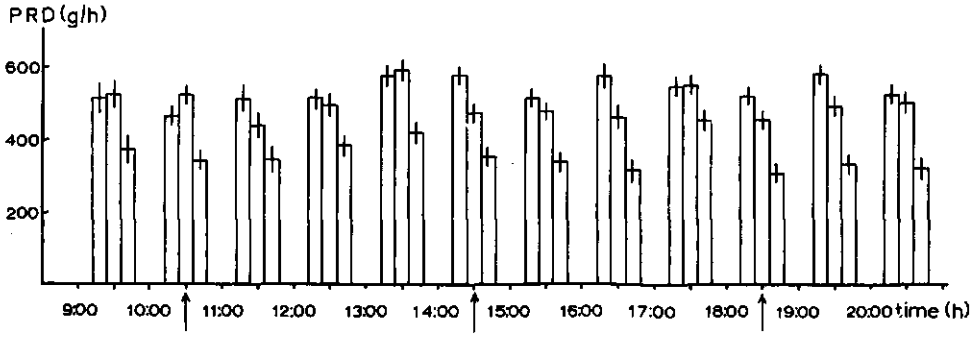

Fig.2. Duodenal passage rate of digesta (PRD), as affected by ration composition and feeding time with the rations of Feeding Trial 1 (Table 1). Times of feeding are indicated by the arrows. Of each set of columns the left represents Ration A, the middle Ration B, and the right one Ration C. Standard deviations are indicated by the vertical bars (± 1 SD).

Table 5. Total acid (TA) and chloride (Cl) content (mmol/kg) of duodenal digesta in Feeding Trial 1, as influenced by ration composition (Table 1) and feeding time. Standard deviations (SD): 0.9 and 0.8, respectively.

Time period h	Ration A		B		C	
	TA	Cl	TA	Cl	TA	Cl
1	45.4	111.8	53.3	121.6	41.4	103.4
2	43.9	109.2	51.8	121.5	40.8	103.5
3	42.6	110.6	48.2	119.1	38.8	103.2
4	42.9	110.2	50.0	118.6	42.4	106.4
5	44.3	112.0	52.3	118.0	42.1	107.1
6	43.4	109.6	49.3	115.6	42.3	105.1
7	42.7	110.8	49.0	118.7	39.4	105.0
8	43.6	112.4	50.2	119.3	40.4	106.4
9	43.0	112.0	52.7	119.4	42.9	105.0
10	41.1	109.0	51.1	117.8	42.2	104.8
11	43.0	109.5	49.5	119.4	40.2	107.7
12	42.7	112.9	50.2	120.2	39.8	107.1

In Table 6, amount and composition of duodenal digesta are given, as influenced by ration composition (Table 1). Each figure was calculated from the hourly average per 12-h sampling period. Not only amount of digesta passing in the proximal duodenum depended upon ration composition, but also composition of duodenal digesta, as demonstrated in Table 6 for the duodenal pH and in Table 5 for total acid and chloride concentration. After the semisynthetic ration (C), dry matter content was highest, but crude protein content lowest, as was expected since crude protein content of the semisynthetic ration was lowest. Duodenal pH was lowest with Rations B and C, respectively. These pH figures as such are not indicative for abomasal secretory activity of acid, since abomasal and thus proximal duodenal pH is also affected by buffering capacity of abomasal digesta. This could also be concluded from the data on duodenal passage rate of total acid (PRTA) and of chloride (PRCl). For example, with Ration C, duodenal pH was significantly lower, when compared

Table 6. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), of chloride (PRC1), and of pepsin (PRPE), and the pH of duodenal contents with the rations of Feeding Trial 1 (Table 1). Significant differences (two-tailed Student t_{20}, for critical levels see Section 3.2) are indicated by * (A-B), ▽ (B-C), and † (A-C).

		Ration			SD
		A hay	B grass	C ryegrass straw and semisynthetics	
PRD	g/h	534.2^{**}	$497.6^{\nabla\nabla\nabla}$	$357.2^{\dagger\dagger\dagger}$	6.0
PRDM	g/h	17.87^{***}	$19.61^{\nabla\nabla}$	$18.38^{\dagger\dagger\dagger}$	0.23
PRTA	mmol/h	22.60^{***}	$24.96^{\nabla\nabla\nabla}$	$14.69^{\dagger}$	0.24
pH		3.03^{**}	$2.93^{\nabla\nabla\nabla}$	$2.95^{\dagger\dagger\dagger}$	0.02
PRCP	g/h	4.80^{**}	$5.24^{\nabla\nabla\nabla}$	$2.89^{\dagger\dagger\dagger}$	0.07
PRC1	mmol/h	56.46^{**}	$56.70^{\nabla\nabla\nabla}$	$35.43^{\dagger\dagger\dagger}$	0.75
PRPE	mg/h	1.61^{**}	$1.35^{\nabla\nabla\nabla}$	$2.17^{\dagger\dagger\dagger}$	0.05

Table 7. Correlation coefficients between duodenal passage rate of total acid (PRTA), of chloride (PRC1), and of crude protein (PRCP) in Feeding Trial 1, before and after correction for the effect of duodenal passage rate of digesta (PRD) (r not corrected and r_{PRD} corrected for PRD). Significances (*) of the partial correlation coefficients (r_{PRD}) were determined by the two-tailed Student t_{24} test, calculated according to the formula: $t_{n-3} = r \times SQRT(n-3)/SQRT(1-r^2)$. For critical levels see Methods (Section 3.2).

	PRTA	PRC1		PRCP	
	r	r	r_{PRD}	r	r_{PRD}
PRD	0.786	0.962	-	0.835	-
PRTA	-	0.911	0.919^{***}	0.962	0.900^{***}
PRC1	-	-	-	0.931	0.851^{***}

with Ration A, but PRTA and PRC1 were higher with Ration A. The effect of ration composition on duodenal pepsin activity is discussed in Chapter 6.

Since acid in the abomasum is secreted as hydrochloric acid, a close relationship between duodenal passage rate of total acid and of chloride is to be expected. Besides, abomasal secretion of acid is assumed to be stimulated by specific agents, such as protein breakdown products (Sections 2.3.4 and 2.3.5). Proteins exhibit a high buffering capacity and since buffering capacity of abomasal contents helps to regulate abomasal secretion of acid, i.e. by virtue of the fact that the release of gastrin is facilitated or inhibited according to the pH of abomasal contents, proteins of abomasal digesta are expected to affect abomasal secretion of acid directly through protein breakdown products and indirectly through their buffering capacity. These proposed relationships, between abomasal secretion of hydrogen and chloride ions and between abomasal secretion of acid and protein content of abomasal digesta, were tested by calculation of the correlation coefficients between duodenal passage rate of total acid, of chloride, and of crude protein. Since these three parameters were all closely related to duodenal passage rate of digesta, also the partial correlation coefficients, corrected for duodenal passage rate of digesta, were

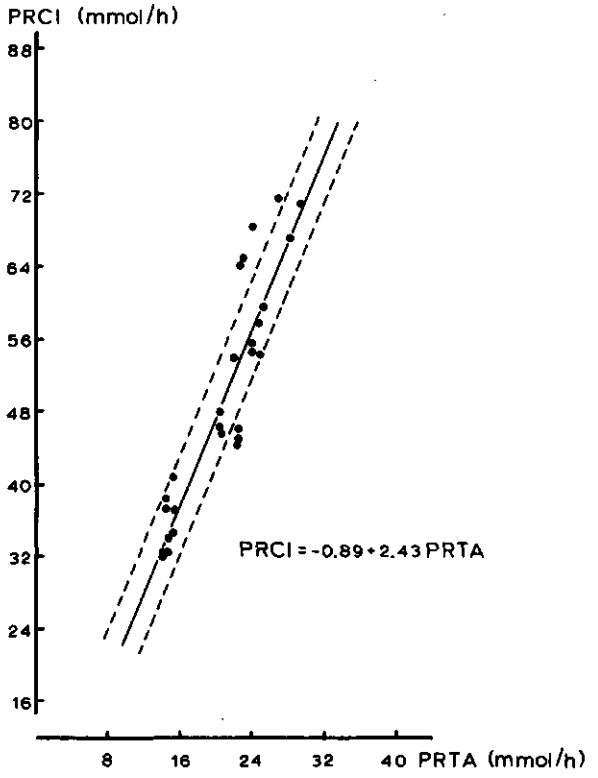

Fig. 3. Relation between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) of Feeding Trial 1. The standard deviation is indicated by the broken lines (± 1 SD).

calculated, as shown in Table 7. Between all parameters, the correlation was highly significant.

Per sampling period of 12 h, the hourly average of duodenal passage rate of chloride was plotted against that of duodenal passage rate of total acid (Fig. 3). The calculated regression coefficient (2.43, SD 0.22) was significantly different ($P < 0.001$) from 1. A value of 1 was to be expected, since hydrogen and chloride ions are assumed to be secreted by the parietal cells of the abomasal fundic glands in equimolar amounts.

3.4 RESULTS OF FEEDING TRIAL 2

When dry matter intake per animal per day is kept constant, diminishing one ration component implies the increase of one or more of the other components, and in fact the combined effect of these ration components is studied. Therefore in this feeding trial, the relationship between ration composition and abomasal secretory activity of acid was studied by adding four different ration compounds in all possible combinations to a fixed basic ration. From the results of Feeding Trial 1 (Section 3.3), proteins were concluded to be important in affecting abomasal secretion of acid. Proteins were supposed to stimulate abomasal secretion of acid not only through their buffering capacity. For this reason, soya

protein and an inorganic buffering compound ($KHCO_3$) were chosen as a variable ration compound. Carbohydrates in ruminant rations, such as starch and cellulose, are easily attacked during forestomach fermentation, resulting in a production of volatile fatty acids, which have been suggested to stimulate abomasal secretion of acid (Hill, 1960; Ash, 1961), and therefore maize starch and cellulose were chosen as the other two variable ration compounds (Table 2).

Duodenal passage rates of digesta of Feeding Trial 2, as influenced by ration composition and feeding time, were plotted in Figure 4. In this figure, each column height was computed as the hourly duodenal passage rate of digesta after addition of a particular variable ration component to the fixed basic ration at 100% level and the other three components at 50% level. For example, the first of each set of columns represents all rations containing cellulose (100%), irrespective of the addition of the other three ration components (Table 2). Therefore the duodenal passage rates of digesta in the first columns were calculated at a 50% level of soya protein, of $KHCO_3$ and of maize starch. Standard deviations are indicated by the vertical bars. Despite, sheep were fed less frequently in comparison with the feeding regime of Feeding Trial 1, a consistent pattern in duodenal passage rate of digesta, as influenced by feeding time, was hardly detectable.

In Table 8, the single and dual factor (2-factor interactions) effects of the variable ration components on amount and composition of digesta passing through the proximal duodenum are given. The regression coefficients in Table 8 are independent and indicate half of the total effect, since the x vectors used for the multiple regression analysis were composed of 1 and -1. All regression coefficients were calculated from the respective hourly averages per sampling period of 12 h. The constant values of the calculated multiple regression equations are indicated in this table as the y axis intercept. As could be concluded from the effect of the variable ration components on duodenal passage rate of total acid and of chloride, soya protein and $KHCO_3$ were the strongest stimulators of abomasal secretion of acid. The increase in buffering capacity of digesta entering the abomasum after addition of soya protein or $KHCO_3$ to the basic ration, however, exceeded the stimu-

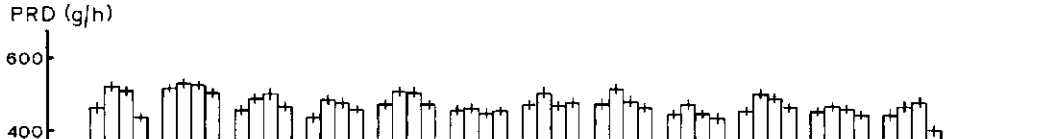

Fig. 4. Duodenal passage rate of digesta (PRD), as affected by ration composition and feeding time with the rations of Feeding Trial 2 (Table 2). Times of feeding are indicated by the arrows. Of each set of columns the first represents rations containing cellulose, the second rations containing soya protein, the third rations containing $KHCO_3$, and the fourth one rations containing maize starch. Standard deviations are indicated by the vertical bars ($\pm$ 1 SD).

Table 8. Single and dual factor effects of the variable components of the rations in Feeding Trial 2 (Table 2) on duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), of chloride (PRCl), and of pepsin (PRPE), and on the pH of duodenal contents. For significances (two-tailed Student t_{77}) see Methods (Section 3.2). Single and dual factor effects are coded binary.

	PRD g/h	PRDM g/h	PRTA mmol/h	pH	PRCP g/h	PRCl mmol/h	PRPE mg/h
	y axis intercept						
	437.0	20.47	20.82	2.89	3.76	48.98	9.58
	regression coefficients						
0001	22.7***	1.25***	0.80***	0.01	0.17***	2.25***	0.21
0010	55.1***	2.12***	3.63***	0.06***	1.45***	7.30***	-2.47***
0100	42.5***	0.66***	0.77***	0.08***	0.16***	5.24***	-0.23
1000	20.1***	1.05***	0.53*	0.02	0.14**	2.12***	1.79***
0011	-16.0**	-0.65***	-0.41	-0.01	-0.04	-1.51**	-1.60***
0101	- 4.2	-0.33	0.06	-0.01	-0.02	-0.45	-0.38
1001	7.4*	0.31	0.10*	0.01	-0.02	0.37*	0.98**
0110	11.4*	0.05	0.56**	0.01	-0.08	1.34*	0.22
1010	-21.8***	-0.60**	-0.63**	0.01	0.06	-2.69***	-2.08***
1100	-11.9*	-0.11	-0.38	0.01	-0.04	-1.43*	-0.72*
SD	5.0	0.18	0.23	0.01	0.04	0.56	0.30

lative effect of those two variable ration components on abomasal secretion of acid, since the resulting proximal duodenal and thus abomasal pH was increased.

All ration compounds were not acting only through their effect on composition of digesta entering the abomasum, since after addition of the variable ration compounds to the basic ration in all cases duodenal passage rates of digesta and of dry matter were increased. From the increases in the duodenal passage rate of dry matter, when multiplied by 2 x 24, conclusions can be drawn regarding the digestion of the organic variable ration components, when compared with the amounts of dry matter ingested (Table 2). Besides, the three non-protein variable components induced an increase in duodenal passage rate of crude protein, probably by interference with microbial protein synthesis in the forestomachs, but possibly also by their effect on passage rate of digesta through and on retention time of digesta in the forestomachs and thus on the amount of protein degraded by fermentation in the forestomachs. In general, the dual factor effects, the interactive effects of two variable ration components, were of minor importance. When present, however, they were in general not attributable to their effect on digesta composition, but to an increased or decreased passage rate of digesta. The effect of the variable ration components on duodenal pepsin activity is discussed in Chapter 6.

The effect of the variable ration compounds on volatile fatty acid concentrations, determined in samples removed from the ventral rumen sac, is shown in Table 9. The regression coefficients were calculated on the basis of the mean volatile fatty acid concentrations per sampling day. In this table, regression coefficients and y axis intercepts may be read as in Table 8. Significant increases in acetic acid, propionic acid and butyric acid concentrations were found after addition of soya protein to the basic ration, probably

Table 9. Single and dual factor effects of the variable components of the rations in Feeding Trial 2 (Table 2) on ruminal concentration of acetic acid (HAc), propionic acid (HPr), and butyric acid (HBu). For significances (two-tailed Student t_{13}) see Methods (Section 3.2). Single and dual factor effects are coded binary.

	HAc mmol/kg	HPr mmol/kg	HBu mmol/kg
	y axis intercept		
	47.00	18.78	7.16
	regression coefficients		
0001	0.89	1.23	0.07
0010	4.58**	2.18**	0.98**
0100	1.81	0.13	-0.10
1000	1.03	-0.36	1.00***
0011	0.75	1.31	-0.17
0101	0.64	0.21	0.20
1001	-2.18	-0.93	-0.77**
0110	0.45	0.11	-0.07
1010	1.00	0.93	0.12
1100	-0.20	0.26	0.26
SD	1.19	0.63	0.24

caused by a stimulating effect of soya protein on forestomach microbial activity. An increase in butyric acid concentration was also noticed after addition of maize starch, whereas addition of cellulose and maize starch caused a significant interactive decrease in butyric acid concentration.

Volatile fatty acids have been proposed to stimulate abomasal secretion of acid (Hill, 1960; Ash, 1961). Therefore the relationship between duodenal passage rate of total acid and of chloride, and ruminal volatile fatty acid concentrations was calculated. Addition of soya protein to the basic ration increased significantly ruminal volatile fatty acid concentrations. It also caused an increase in duodenal passage rate of crude protein, which was closely related to abomasal secretory activity of acid (Table 12). For this reason, the correlation coefficients between ruminal volatile fatty acid concentrations and duodenal passage rate of total acid and of chloride were corrected for the common correlative effect of both variable pools with duodenal passage rate of crude protein, as shown in Table 10. No consistent relationship between ruminal volatily fatty acid concentrations and abomasal secretory activity of acid could be demonstrated.

In rumen fluid samples, chloride concentrations were determined also. If one assumes the following:

1. Chloride concentration differs not substantially between reticulum and ventral rumen sac.
2. Chloride concentration is approximately doubled in the omasum (von Engelhardt & Giesecke, 1972; von Engelhardt & Hauffe, 1975).
3. Chloride concentration of abomasal juice is 155 mmol/litre (Section 2.3.8).
4. The amount of chloride entering the abomasum equals the amount of digesta entering the abomasum times the double ruminal chloride concentration, or equals the duodenal passage rate of chloride minus the abomasal secretory rate of chloride.

Table 10. Correlation coefficients between ruminal volatile fatty acid concentrations, acetic acid (HAc), propionic acid (HPr), and butyric acid (HBu), and duodenal passage rate of total acid (PRTA) and of chloride (PRCl) in Feeding Trial 2, before and after correction for the effect of duodenal passage rate of crude protein (PRCP) (r not corrected and r_{PRCP} corrected for PRCP; 1: rations without $KHCO_3$, 2: rations with $KHCO_3$, and 3: all rations). Significances (*) of the partial correlation coefficients (r_{PRCP}) were determined by the two-tailed Student t_{45} (1,2) and t_{93} (3) test, calculated according to the formula: $t_{n-3} = r \times SQRT(n-3)/SQRT(1-r^2)$. For critical levels see Methods (Section 3.2).

		PRCP	PRTA		PRCl	
		r	r	r_{PRCP}	r	r_{PRCP}
PRCP	1	-	0.824	-	0.737	-
	2	-	0.916	-	0.847	-
	3	-	0.863	-	0.747	-
HAc	1	0.660	0.536	-0.019	0.519	0.062
	2	0.662	0.618	0.038	0.535	-0.065
	3	0.651	0.602	0.104	0.568	0.162
HPr	1	0.534	0.432	-0.016	0.311	-0.144
	2	0.511	0.418	-0.143	0.288	-0.317*
	3	0.522	0.421	-0.068	0.280	-0.193
HBu	1	0.551	0.512	0.123	0.478	0.126
	2	0.591	0.597	0.174	0.574	0.171
	3	0.556	0.549	0.165	0.459	0.079

5. The secretory rate of abomasal juice equals the duodenal passage rate of digesta minus the amount of digesta entering the abomasum, or equals the abomasal secretory rate of chloride divided by 155.
Then S = (PRCl - 2 x RCoCl x PRD)/(155 - 2 x RCoCl), where RCoCl is ruminal chloride concentration, PRD is duodenal passage rate of digesta, PRCl is duodenal passage rate of chloride, and S is secretory rate of abomasal juice.

On the assumptions of a hydrogen ion concentration in abomasal juice of 80 mmol/litre and a chloride ion concentration in abomasal juice of 155 mmol/litre (Section 2.3.8), the theoretical amounts of hydrogen and chloride ions secreted were also calculated. In Table 11, the statistically analysed effects of the variable ration compounds on secretory rates of abomasal juice, of hydrogen and of chloride ions are given. In this table, regression coefficients and y axis intercepts may be read as in Table 8. The effects of the variable ration components on the calculated amounts of hydrogen and chloride ions secreted by the abomasum corresponded well with the effects on duodenal passage rate of total acid and of chloride (Table 8), except when $KHCO_3$ was added to the basic ration. After addition of $KHCO_3$, the calculated amount of hydrogen ions secreted was much higher than the increase in duodenal passage rate of total acid. This difference was to be expected, since bicarbonate concentration of digesta entering the abomasum will be high when $KHCO_3$ is added to the basic ration. Under the acid abomasal conditions, bicarbonate ions recombine with hydrogen ions, resulting in a decreased duodenal passage rate of total acid, which was also indicated by the ratio between the effects of $KHCO_3$ on duodenal passage rate of total acid and of chloride (Table 8).

As in Feeding Trial 1, the relationship between duodenal passage rate of total acid

Table 11. Single and dual factor effects of the variable components of the rations in Feeding Trial 2 (Table 2) on abomasal secretory rate of abomasal juice, of hydrogen ions and of chloride ions. For significances (two-tailed Student t_{77}) see Methods (Section 3.2). Single and dual factor effects are coded binary.

	Abomasal secretory rate		
	abomasal juice g/h	hydrogen mmol/h	chloride mmol/h
	y axis intercept		
	288.4	23.07	44.70
	regression coefficients		
0001	14.2^{***}	1.14^{***}	2.21^{***}
0010	40.6^{***}	3.25^{***}	6.30^{***}
0100	29.6^{***}	2.36^{***}	4.58^{***}
1000	15.3^{***}	1.22^{***}	2.37^{***}
0011	-8.1^{*}	-0.65^{*}	-1.25^{*}
0101	-3.2	-0.26	-0.49
1001	3.1	0.25	0.48
0110	6.8^{*}	0.55^{*}	1.06^{*}
1010	-15.3^{***}	-1.22^{***}	-2.37^{***}
1100	-9.0^{**}	-0.72^{**}	-1.40^{**}
SD	3.2	0.26	0.50

Table 12. Correlation coefficients between duodenal passage rate of total acid (PRTA), of chloride (PRCl), and of crude protein (PRCP) in Feeding Trial 2, before and after correction for the effect of duodenal passage rate of digesta (PRD) (r not corrected and r_{PRD} corrected for PRD; 1: rations without $KHCO_3$, 2: rations with $KHCO_3$, and 3: all rations). Significances (*) of the partial correlation coefficients (r_{PRD}) were determined by the two-tailed Student t_{45} (1,2) and t_{93} (3) test, calculated according to the formula: $t_{n-3} = r \times SQRT(n-3)/SQRT(1-r^2)$. For critical levels see Methods (Section 3.2).

		PRTA	PRCl		PRCP	
		r	r	r_{PRD}	r	r_{PRD}
PRD	1	0.863	0.965	-	0.682	-
	2	0.942	0.985	-	0.802	-
	3	0.885	0.981	-	0.708	-
PRTA	1	-	0.904	0.538^{***}	0.824	0.637^{***}
	2	-	0.967	0.672^{***}	0.916	0.799^{***}
	3	-	0.906	0.418^{***}	0.863	0.718^{***}
PRCl	1	-	-	-	0.737	0.410^{**}
	2	-	-	-	0.847	0.552^{***}
	3	-	-	-	0.747	0.382^{***}

and of chloride was studied in this experiment. Since this relationship was affected by the addition of $KHCO_3$ to the basic ration, calculations were made on rations with and without $KHCO_3$ and on all rations. In Figure 5, duodenal passage rate of chloride was plotted against that of total acid. The calculated regression coefficients, 2.10 (SD 0.15) for rations not containing $KHCO_3$, 2.15 (SD 0.08) for rations containing $KHCO_3$, and 2.25 (SD

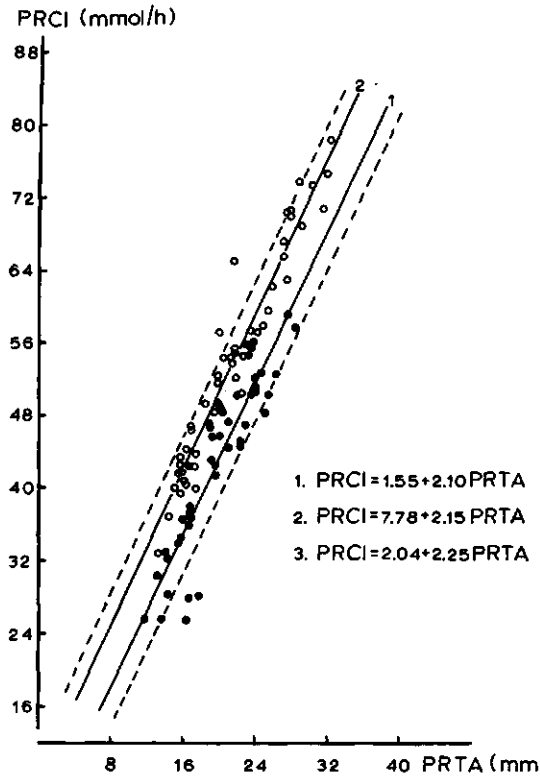

Fig. 5. Relation between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) of Feeding Trial 2 (1 and •: rations without $KHCO_3$, 2 and ○: rations with $KHCO_3$, and 3: all rations). Standard deviations are indicated by the broken lines (1: −1 SD and 2: +1 SD).

0.11) for all rations, were significantly different ($P < 0.001$) from 1. A value of 1 was to be expected, since hydrogen and chloride ions are assumed to be secreted by the abomasal fundic gland parietal cells in equimolar amounts. In Table 12, the partial correlation coefficients between duodenal passage rate of total acid and of chloride, corrected for the effect of duodenal passage rate of digesta, are given. In the same way, the relationship between duodenal passage rate of crude protein with duodenal total acid and chloride passage rates was calculated (Table 12). Between all parameters, a highly significant relationship existed, which was not affected by addition of $KHCO_3$ to the rations.

3.5 RESULTS OF FEEDING TRIAL 3

The grass ration in Feeding Trial 1 (Table 1) did not result in a higher duodenal passage rate of digesta, when compared with the hay ration (Table 6). This grass was mown, however, in the autumn. In experiments with dairy cows (van 't Klooster & Rogers, 1969; van 't Klooster et al., 1972), extremely high duodenal flow rates were observed with young grass. Because of the expected link between duodenal passage rate of digesta and abomasal secretion rate, it was thought of interest to test the effect of grass, harvested early in

forestomach fermentation process was obviously lower with Ration C, possibly caused by a higher passage rate of digesta and thus by a lower ruminal digesta retention time. The stimulative effect on abomasal secretion of acid of Rations B and C seemed, however, not to be different, since no significant differences existed in duodenal passage rates of total acid and of chloride with Rations B and C, respectively.

As in the preceding feeding trials, the relationships were calculated between duodenal passage rate of total acid and of chloride and between duodenal passage rate of crude protein and abomasal secretory activity of acid, as estimated from duodenal passage rate of total acid and of chloride. In Figure 7, hourly averages per sampling period of 12 h of duodenal passage rate of chloride were plotted against those of total acid. The resulting regression coefficient (1.58, SD 0.22) was slightly different from 1 ($P < 0.05$), which value was to be expected since hydrogen and chloride ions are assumed to be secreted by the parietal cells of the abomasal fundic tubular glands in equimolar amounts. In Table 15, the partial correlation coefficients between duodenal passage rate of crude protein, of total acid and of chloride are given, as corrected for duodenal passage rate of digesta. Between all parameters a highly significant relationship was found, which was hardly affected by the $KHCO_3$ containing semisynthetics of Ration C.

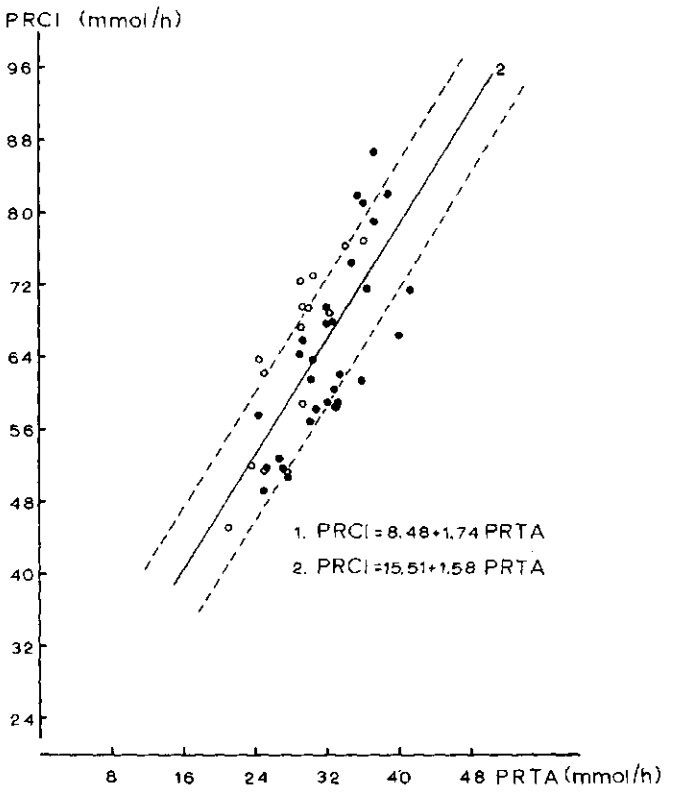

Fig. 7. Relation between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) of Feeding Trial 3 (1: excluding and 2: including the $KHCO_3$ containing Ration C; •: rations without $KHCO_3$ and ∘: ration with $KHCO_3$). The standard deviation is indicated by the broken lines (± 1 SD).

Table 15. Correlation coefficients between duodenal passage rate of total acid (PRTA), of chloride (PRCl), and of crude protein (PRCP) in Feeding Trial 3, before and after correction for the effect of duodenal passage rate of digesta (PRD) (r not corrected and r_{PRD} corrected for PRD; 1: excluding, and 2: including the $KHCO_3$ containing Ration C). Significances (*) of the partial correlation coefficients (r_{PRD}) were determined by the two-tailed Student t_{27} (1) and t_{42} (2) test, calculated according to the formula: $t_{n-3} = r \times SQRT(n-3)/SQRT(1-r^2)$. For critical levels see Methods (Section 3.2).

		PRTA	PRCl		PRCP	
		r	r	r_{PRD}	r	r_{PRD}
PRD	1	0.645	0.970	-	0.666	-
	2	0.576	0.944	-	0.641	-
PRTA	1	-	0.760	0.721^{***}	0.893	0.812^{***}
	2	-	0.734	0.708^{***}	0.879	0.813^{***}
PRCl	1	-	-	-	0.783	0.755^{***}
	2	-	-	-	0.776	0.677^{***}

3.6 DISCUSSION

In some of the present trials, a dependence of the duodenal flow pattern was noticed on feeding time. In Feeding Trial 3, highest duodenal flow rates were generally found the last hour before sheep were fed (Fig. 6). Although fluctuations in amounts of digesta passing the duodenum were high, as indicated by the standard deviations of the duodenal flow rates, statistically significant differences between the duodenal flow rates in the hours preceding and following the times of feeding could be calculated. In Feeding Trial 1, when sheep were fed every 4-h period, duodenal passage rate of digesta tended to show a comparable flow pattern. In Feeding Trial 2, however, no consistent duodenal flow pattern in relation to feeding time could be detected. The diurnal pattern in duodenal digesta passage rate in relation to feeding time found in Feeding Trials 1 and 3 agrees with the results of Phillips & Dyck (1964). In sheep, fed on oat straw and concentrates once daily, a monophasic diurnal pattern was observed with the highest duodenal passage rates of digesta just before and during feeding and the lowest rates 6-12 h after feeding.

In other papers, however, different duodenal flow patterns were described. Phillipson (1952) found that sheep feeding on hay or concentrates showed a decreased duodenal passage rate of digesta over the succeeding interval of about 15 min, after which the duodenal flow rate returned to its level before feeding when the sheep got hay, or exceeded it when they got concentrates. Ash (1961a) demonstrated a close relationship between amount of digesta leaving the abomasum and amount of acid secreted by an innervated fundic pouch in sheep. The highest abomasal secretory rates were observed the first 2 h after sheep were fed on chopped dried grass and concentrates twice daily. Hill (1960) found an increased abomasal secretory rate the first hour after the start of feeding on a meal of chopped hay, reducing gradually the next 3-4 h to the level before feeding. Thompson & Lamming (1972) found in sheep, fed on barley straw and concentrates once daily, the highest duodenal flow rates 4-12 h after feeding. Differences between passage rates of duodenal digesta and of dry

matter were highest when the barley straw was ground, in comparison with long or chopped barley straw. When sheep were fed on concentrates twice daily, Knight et al. (1972) observed the highest abomasal inflow rates of dry matter 2-3 h after feeding, as measured by the polyethylene glycol dilution technique in samples removed from the abomasum. In our trials, sheep were not fed ad libitum. The highest duodenal flow rates before the sheep were fed could be the result of an increased passage rate of digesta caused by an increased motor activity of the reticulum when sheep were expecting food, as found by McLeay & Titchen (1970), when sheep were teased with food or fed.

Composition of duodenal digesta was found to be hardly variable when sheep were fed more frequently. In Feeding Trial 1, minor increases in contents of total acid and of chloride in duodenal digesta were noticed in general in the hour before sheep were fed (Table 5). In Feeding Trial 3, when sheep were fed less frequently, a more variable duodenal flow pattern was noticed, but composition of duodenal digesta did not fluctuate substantially either (Table 13). When sheep were fed on grass or on hay and grass, the lowest contents of dry matter were found the first hours after feeding; when fed on hay and semisynthetics, however, there was no relationship between duodenal content of dry matter and feeding time. In Feeding Trial 3, duodenal content of chloride tended to be highest the first hours after feeding, probably through chloride in the ration (Table 13).

In all feeding trials, abomasal secretion of acid, as estimated from the amounts of total acid and of chloride leaving the abomasum, depended upon ration composition (Tables 6, 8 and 14). A close relationship was noticed between abomasal secretory activity of acid and the amount of crude protein passing through the abomasum (Tables 7, 12 and 15). The amount of protein entering the abomasum depends on the amount of protein present in the ration and on the conditions affecting protein degradation and synthesis by forestomach fermentation. In Feeding Trial 2, the amount of crude protein leaving the abomasum was affected by non-protein ration compounds as well (Table 8). In Feeding Trials 1 and 3, the amount of crude protein passing through the proximal duodenum depended not only on the amount of crude protein ingested. Expressed as a percentage of the amount of crude protein ingested and assuming that the average duodenal passage rate of crude protein per sampling period of 12 h is representative for the whole day, amounts of crude protein leaving the abomasum of 88.6, 86.1, 87.8, 78.4, 83.3 and 85.2% could be calculated with Rations A, B and C of Feeding Trials 1 and 3, respectively (Tables 6 and 14). So protein content of the ration and ration components, affecting forestomach fermentation, can be important in stimulating abomasal secretion of acid. Proteins probably act on abomasal secretion of acid through their buffering capacity, since addition of an inorganic buffering compound ($KHCO_3$) in Feeding Trial 2 caused a stimulated abomasal secretion of acid as well, but probably also through stimulation of the release of gastrin, as is discussed in Chapter 5.

In man, Rune (1973) found a lower postprandial duodenal pH after intake of a high-protein meal, when compared with a low-protein meal. In the present experiments, this effect of protein content of the ration on duodenal pH was not observed. For example, with the rations of Feeding Trial 2, the duodenal pH was significantly increased after addition of soya protein to the fixed basic ration. These different observations can be explained probably by the fact that in humans the meal is retained in the stomach for a longer period. When the high-protein diet was supplied, about 3 h postprandially a lower duodenal

pH was still observed. From the dilution rate of polyethylene glycol in abomasal contents in sheep fed on hay and concentrates, as measured in the duodenum against time after intra-abomasal injection of the marker, in own experiments an average abomasal digesta retention time of about 45 min was determined. This possibly indicates that proteins present in abomasal digesta are not able to induce abomasal secretion of acid in excess of their buffering capacity during this relatively short period.

Frequently, ration composition has been suggested to affect abomasal secretion of acid through volatile fatty acids synthesized in the rumen (Hill, 1960; Ash, 1961). In Feeding Trial 2, however, no relationship could be demonstrated between rumen volatile fatty acid concentrations and abomasal secretion of acid (Table 10).

Physical structure of the diet is supposed to affect abomasal secretion of acid indirectly, by influencing the events taking place in the forestomachs. When more roughages are supplied, time spent ruminating increases, digesta retention time in the forestomachs increases, but roughage material is less readily attacked by rumen micro-organisms (Stevens & Sellers, 1968). In Feeding Trial 3, Rations B and C were more or less of the same chemical composition, but differed physically. In stimulating abomasal secretion of acid, no differences could be detected, as estimated from the amounts of total acid and of chloride leaving the abomasum (Table 14).

In all feeding trials, a close relationship could be demonstrated between the amounts of total acid and of chloride passing in the proximal duodenum (Tables 7, 12 and 15). Chloride concentration of abomasal juice is accepted to be higher than hydrogen concentration (Section 2.3.8), since part of the chloride is secreted as neutral chloride, reciprocally linear to hydrogen ion concentration (Hill, 1965, 1968). At lower abomasal secretory rates, hydrogen ion concentration was found to be low, whereas stimulation of abomasal secretion resulted in an increased hydrogen ion concentration in abomasal juice. In comparison with hydrogen ion concentration, abomasal juice chloride concentration is less variable (Section 2.3.8). Therefore, but also as a result of ration chloride, the ratio between abomasal chloride and hydrogen ion concentration will be higher than 1:1, but will decrease with increased secretory rates of abomasal juice. The regression coefficients in Figures 3, 5 and 7 are indeed all higher than 1. At a lower average secretion rate (Figures 3 and 5), regression coefficients even exceeded 2, but at a higher average secretion rate (Fig. 7), a regression coefficient lower than 2 was found, indicating that at a higher abomasal secretory rate the ratio between chloride and hydrogen ions secreted decreases.

The concentrations of chloride and hydrogen ions in pure abomasal juice, as used to calculate the secretory rates of abomasal juice in Feeding Trial 2 are averages of the data given by Ash (1959) and Hill (1960, 1965, 1968); for chloride and hydrogen ions 155 and 80 mmol/litre, respectively. On the assumptions made in Section 3.4, we may conclude that the calculated data on chloride and hydrogen ion secretion (Table 11) fit well with the data on total acid, except when $KHCO_3$ was added to the basic ration, and on chloride secretion, as determined in the proximal duodenum (Table 8). The tentative estimate of chloride entering the abomasum from chloride concentration in rumen fluid, made in the calculation of the secretory rates of abomasal juice, is of minor importance for the calculated figures, since rumen chloride concentration is about 10% of abomasal chloride concentration. If the correction for the amounts of chloride entering the abomasum should

have been doubled in the calculation of the amounts of juice secreted by the abomasum in Feeding Trial 2, about 10% lower abomasal secretory rates of chloride would have been calculated. On the assumption that a linear extrapolation of the regression lines presented in Figures 3, 5 and 7 is correct, the conclusion that the amount of chloride entering the abomasum is of little importance, proportionally to the amounts of chloride leaving the abomasum, could also be drawn from the respective y axis intercepts in these figures. In Feeding Trial 3 (Fig. 7), this amount seemed a little higher, regarding the y axis intercept. This intercept, however, was decreased when the data on the $KHCO_3$ containing semisynthetic ration were excluded. As was documented previously, the ratio between duodenal passage rate of chloride and of total acid is expected to decrease at higher abomasal secretory rates, resulting in a non-linear relationship between amounts of chloride and of total acid leaving the abomasum. In such regression equations, the y axis intercepts should be even lower than those obtained after linear extrapolation of the linear regression lines mentioned.

The average calculated secretory rate of abomasal juice in Feeding Trial 2 (Table 11), of about 65% of the average amount of digesta leaving the abomasum (Table 8), seems to be high, although Masson & Phillipson (1952) postulated a comparable ratio between abomasal juice and abomasal digesta in sheep. In 13 separate experiments (control experiments Chapter 4), from the dilution rate of polyethylene glycol, infused into the rumen, an average reticular outflow of fluid of 565 g/h (SD 32) could be calculated. On the assumption of a water absorption in the omasum of 15% (Section 2.3.3), an average amount of fluid of about 480 g/h would enter the abomasum, and as the average duodenal passage rate of digesta was found to be 637 g/h (SD 64), an abomasal juice secretion of about 150 g/h could be calculated. This figure, proportionally to the amounts of digesta leaving the abomasum, is far much lower, when compared with the calculated amounts of abomasal juice secreted in Feeding Trial 2. In these experiments, however, the ratio between the amount of digesta leaving the abomasum and the calculated amount of digesta leaving the reticulum was rather small. Ratios up to 1.7:1 were mentioned by Hogan & Weston (1969), which implicate an abomasal secretory rate of about 50% of the amount of digesta leaving the abomasum, on the assumption of an omasal absorption rate of water of 15%.

Such an abomasal secretory rate could also be calculated from the amounts of chloride leaving the abomasum in Feeding Trials 1 and 3. When in these feeding trials duodenal passage rates of chloride were decreased (Tables 6 and 14) by 20%, as a correction for the amounts of chloride entering the abomasum, correction factors exceeding those used in Feeding Trial 2, from an abomasal juice chloride concentration of 155 mmol/litre, secretory rates of abomasal juice, expressed as a percentage of the respective duodenal passage rates of digesta, of about 55, 59, 51, 57, 54 and 52% were calculated, for Rations A, B and C of Feeding Trials 1 and 3, respectively. These percentages and the secretory rates of abomasal juice calculated in Feeding Trial 2 are much higher than the secretory rates of abomasal juice, calculated from the comparison between the amounts of digesta leaving the reticulum and abomasum. A ratio between digesta flow from abomasum and reticulum of 1.7:1 fits well with such high secretory rates of abomasal juice, but in general, lower ratios have been mentioned (Weston & Hogan, 1968; Hogan et al., 1969).

The discrepancy in the results on abomasal secretory rate, calculated according to

both methods mentioned, is difficult to explain on the assumption of higher absorption rates of water from the omasum. A ratio between digesta flow from abomasum and reticulum of 1.3:1, as mentioned by Weston & Hogan (1968), should need a proportion of water absorbed in the omasum of about 35% at a secretory rate of abomasal juice of 50% of the amount of digesta leaving the abomasum. When secretory rate of abomasal juice was calculated from the amount of chloride leaving the abomasum, corrected for the amount of chloride entering the abomasum, chloride concentrations of abomasal juice far much higher than 155 mmol/litre are not justified either, since there are no indications that abomasal juice is secreted at a hypertonic concentration and since the chloride ion is the main anion in abomasal juice.

As is shown in the intra-abomasal infusion experiments (Chapter 4), duodenal and thus abomasal contents are slightly hypotonic. This difference in osmotic pressure between abomasal contents and blood plasma might force a net movement of water from the abomasal cavity into the blood, which might explain the discrepancy noticed when calculated the secretory rate of abomasal juice, either by comparing the digesta flow rates from abomasum and reticulum, or by dividing the amount of chloride leaving the abomasum, corrected for abomasal inflow rate of chloride, by chloride concentration of abomasal juice, since the abomasal mucosa has been shown to be highly permeable to water. Von Engelhardt et al. (1968) found a clearance rate of water in the goat abomasum as high as 500 g/h.

4 Abomasal secretion of acid as affected by intra-abomasal infusions

4.1 INTRODUCTION

In the preceding experiments (Chapter 3), proteins and buffering components of the ration were found to stimulate abomasal secretion of acid. Although concentrations of volatile fatty acids in the rumen were shown to be not significantly related to abomasal secretion of acid in these experiments, volatile fatty acids were found to stimulate abomasal secretion of acid in the emptied abomasum or in abomasal pouches (Hill, 1960; Ash, 1961; McLeay & Titchen, 1974). This suggests the possibility that abomasal secretion of acid is affected by ration composition partly indirectly after fermentation of the ration components in the forestomachs.

In order to study the direct effect of abomasal digesta composition on abomasal secretion of acid, proteins, inorganic buffers, amino acids and volatile fatty acids were infused into the abomasum through an abomasal infusion tube inserted into the abomasal fundus.

4.2 METHODS

Animals. The abomasal infusion experiments were carried out with Texel wethers, weighing 45-60 kg. As with the feeding trials (Section 3.2), re-entrant cannulas were fitted into the proximal part of the duodenum. In the same sheep, a silicone tube (7 x 3 mm), as shown in Figure 1, was inserted into the abomasal fundus. The end of the tube was kept in the abomasum by a silicone ring, made of silicone paste (Possehl Eisen und Stahl, Lübeck) reinforced with a plastic gauze, and sutured on the tube. A ring of artificial tissue (polyvinylalcohol sponge (Ramer Chemical and Co, Camberley), covered with silicone paste, was sutured on the abomasal serosal surface in order to prevent leakage of abomasal contents into the abdominal cavity. In some of the sheep, also a rumen cannula was placed into the dorsal rumen sac (Section 3.2). Surgery was performed several weeks before the start of the experiments, which were started after the sheep had been well trained. During the experiments, sheep were kept individually in metabolism cages.

Rations. For these intra-abomasal infusion experiments rations were kept constant. They consisted of 600 g hay and 300 g concentrates per day, offered to the sheep spread over the day in 6 equal portions at 4:00, 8:00, 12:00, 16:00, 20:00 and 24:00 h. Care was taken that per series of experiments hay and concentrates were used of the same batch. Net energy and protein content of these ration components were about 420 g SE (Starch Equivalent)/kg and 105 g DCP (Digestible Crude Protein)/kg for hay, and 650 g SE/kg and 140 g DCP/kg for concentrates, respectively.

Experimental procedure. Intra-abomasal infusions were carried out continuously, starting about 40 h in advance of the sampling period. At sampling days, the duodenal passage rate of digesta was measured as described in Section 3.2. Duodenal digesta were sampled for 8-h periods, from 8:00 to 16:00 h. Proportional samples of 10% were removed and not replaced by donor digesta. Per sampling period, samples were pooled and stored at -20 ^{o}C.

Abomasal infusates. In the first series of infusion experiments, a 10% soya protein suspension (promine D, Central Soya Chemurgy, Chicago) in saline was infused continuously into the abomasum of three sheep at two rates (Table 16), and was compared with a control (no intra-abomasal infusion), according to a 3 x 3 Latin square design, repeated twice (Section 4.3).

In the second series of infusion experiments, 5% suspensions of soya protein (α-protein), casein, soya protein (promine D), and of gluten in saline, a 2.5% solution of gelatin in saline, and a phosphate buffer 0.13 mol/litre pH 7 were infused continuously into the abomasum of three sheep. α-Protein, casein, gluten and gelatin were derived from Nutritional Biochemical Corporation (ICN, Cleveland). To the suspensions of α-protein, casein and gluten, 0.25% agar gel was added in order to prevent precipitation during infusion. Gelatin and the phosphate buffer, α-protein and casein, and promine D and gluten were infused into the abomasum (Table 16), and were compared with a control (no intra-abomasal infusion), according to three 3 x 3 Latin square designs, randomized over each other. The whole experimental scheme was repeated three times (Section 4.4).

In the third series of infusion experiments, potassium bicarbonate buffers of 0.15, 0.30, 0.45 and 0.60 molar concentrations were infused continuously into the abomasum of three sheep (Table 16). The solutions of $KHCO_3$ of 0.15 and 0.60 mol/litre, and of 0.30 and 0.45 mol/litre were infused into the abomasum, and were compared with a control (no intra-abomasal infusion), according to two 3 x 3 Latin square designs, randomized over each other. The whole experimental scheme was repeated three times (Section 4.5).

In the fourth series of infusion experiments, solutions 0.10 mol/litre of α-alanine, β-alanine, aspartic acid, arginine, glycine and methionine in $KHCO_3$ 0.15 mol/litre were infused continuously into the abomasum of three sheep (Table 16). In comparison with control infusion of $KHCO_3$ 0.15 mol/litre, intra-abomasal infusion of α-alanine and β-alanine, of aspartic acid and arginine, and of glycine and methionine were carried out according to three 3 x 3 Latin square designs, randomized over each other. The whole experimental scheme was repeated four times (Section 4.6).

In the fifth series of infusion experiments, 10% suspensions of soya protein (promine D) and of soya protein partly hydrolysed in vitro (promine DH), in saline were infused into the abomasum of three sheep (Table 16) in comparison with a control (no intra-abomasal infusion), according to a 3 x 3 Latin square design, repeated four times. The hydrolysis in vitro of the 10% soya protein suspension was carried out by addition of 0.04 g porcine pepsin (Merck, Darmstadt; 35000 U/g) per gram soya protein and incubation for 48 h (pH 2, 40 ^{o}C). The peptic hydrolysis was stopped by adding saturated NaOH till a pH of 7 was reached (Section 4.7).

In the sixth series of infusion experiments, solutions 0.5% of acetic acid, propionic acid, butyric acid and L-lactic acid in $KHCO_3$ 0.15 mol/litre were infused continuously

Table 16. Infusion rate (IR), osmotic pressure (OPI) and infusion rate of buffering capacity (IRBC), of dry matter (IRDM), of crude protein (IRCP), and of chloride (IRCl) of the various intra-abomasal infusates. Absent data indicate not determined or not detectable. Code refers to the respective sections, where the effects of the intra-abomasal infusions on amount and composition of duodenal digesta are described. Substance concentrations are indicated by M (mol/litre). Per section, standard deviations (SD) are indicated between the parentheses.

Code	Infusate	IR g/h	IRBC mmol/h	IRDM g/h	IRCP g/h	IRCl mmol/h	OPI mosm/kg
4.3	Promine D	25.9(0.5)	3.12(0.08)	2.66(0.05)	2.33(0.04)	3.58(0.07)	-
	Promine D	57.2	6.91	5.89	5.15	7.90	-
4.4	α-Protein	78.9(1.0)	2.59(0.06)	4.39(0.05)	3.31(0.04)	10.90(0.14)	-
	Promine D	73.2	5.09	4.01	3.26	10.04	-
	Casein	72.3	2.09	4.01	3.09	9.60	-
	Gelatin	156.9	2.16	5.01	4.03	23.04	-
	Gluten	71.8	1.33	4.21	2.86	10.14	-
	P buffer	73.1	6.30	1.55	-	-	-
4.5	0.15 M $KHCO_3$	70.8(0.8)	10.94(0.24)	-	-	-	-
	0.30 M $KHCO_3$	75.2	23.38	-	-	-	-
	0.45 M $KHCO_3$	74.6	34.71	-	-	-	-
	0.60 M $KHCO_3$	75.7	45.86	-	-	-	-
4.6	0.15 M $KHCO_3$	83.9(1.1)	12.96(0.22)	-	- (0.01)	-	256.7(1.7)
	α-Alanine	82.5	14.31	-	0.71	-	340.7
	β-Alanine	82.2	18.30	-	0.66	-	338.0
	Aspartic acid	82.1	12.40	-	0.71	-	266.2
	Arginine	84.5	22.50	-	2.92	-	300.5
	Glycine	83.9	14.65	-	0.71	-	337.5
	Methionine	82.6	13.93	-	0.70	-	343.2
4.7	Promine D	66.7(0.6)	9.98(0.17)	6.52(0.06)	5.61(0.05)	7.22(0.15)	358.0(3.7)
	Promine DH	68.4	14.50	7.10	5.93	8.26	476.2
4.8	0.15 M $KHCO_3$	77.2(1.0)	10.09(0.16)	-	-	-	251.7(2.0)
	Acetic acid	77.6	11.75	-	-	-	281.4
	Propionic acid	75.8	11.08	-	-	-	264.2
	Butyric acid	73.8	11.42	-	-	-	283.0
	L-lactic acid	77.8	10.65	-	-	-	270.0

into the abomasum of three sheep. The abomasal infusates were titrated to pH 7 with concentrated sulfuric acid in order to minimize differences in buffering capacity between infusates. Acetic acid and L-lactic acid, and propionic acid and butyric acid were infused into the abomasum (Table 16) in comparison with a control ($KHCO_3$ 0.15 mol/litre), according to two randomized 3 x 3 Latin squares. The whole experimental scheme was repeated three times (Section 4.8).

Analyses of abomasal infusates. Acidity of abomasal contents approximates pH 3 and therefore buffering capacity (BC) of the abomasal infusates, or the additional amount of hydrochloric acid to be secreted by the abomasum in order to maintain the pH of abomasal contents, was estimated by titration to pH 3 with HCl 0.1 mol/litre (Radiometer Copenhagen, Type TTT1^c). Samples were dried to constant weight at 101 ^oC for the estimation of dry matter (DM). Nitrogen was estimated by the Kjeldahl method using HgO and K_2SO_4 as catalysts (Bouman, 1949), crude protein (CP) being calculated as N x 6.25. Analyses of chloride (Cl) of the suspensions or solutions in saline infused into the abomasum were performed in the supernatant solution (Christ UJ3 centrifuge, 2000 g), according to the method of Volhard (Vogel, 1961). Osmotic pressure (OPI) of the abomasal infusates was estimated in the liquid

phase (MSE 65 ultracentrifuge, 70000 g) with the Knauer milliosmometer (depression of freezing point). Volatile fatty acids were determined gaschromatographically (Hewlett Packard 5750 G; 1.5 m glas column (2.7 mm), filled with 20% neopentylglycolsuccinate and 2% phosphoric acid on 60-80 mesh firebrick; carrier gas N_2). L-lactic acid was estimated enzymatically. Samples were incubated for 10 min at 25 ^{o}C in a Boehringer (Mannheim) test solution, containing a glycylglycin 220 mmol/litre glutamate 36 mmol/litre buffer, pH 10.0, NAD 3.43 mmol/litre, glutamate pyruvate transaminase 0.146 mg/ml and L-lactate dehydrogenase 0.073 mg/ml. Extinctions were read at 340 nm with the Beckman-DU spectrophotometer.

Infusion rates of in vitro buffering capacity (IRBC), of dry matter (IRDM), of crude protein (IRCP), of chloride (IRCl) and of organic acids were calculated by multiplying the respective concentrations by the individual infusion rates (IR), IRCl being corrected for dry matter content of the suspensions. For all intra-abomasal infusion experiments IR, IRBC, IRDM, IRCP, IRCl and OPI of the different intra-abomasal infusates are summarized in Table 16.

Analyses of digesta. Duodenal passage rates of digesta (PRD) were measured during the experiments. The average per hour was calculated for each experimental period of 8 h. Duodenal passage rates of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), of chloride (PRCl), and of pepsin (PRPE) were computed as described in Section 3.2. The duodenal pH was measured before total acid concentration was determined. Polyethylene glycol concentrations in ruminal and duodenal samples were estimated by the method of Hydén (Section 3.2). Duodenal osmotic pressures (OPD) and duodenal volatile fatty and L-lactic acids concentrations were determined as described under abomasal infusates.

Corrections applied. As described in Section 3.1, abomasal acid concentration was determined by estimation of total acid concentration of the proximal duodenal contents by titration to pH 7, on the assumption that the pH of digesta entering the abomasum approximates neutrality (Section 2.3.3). Where the pH of the abomasal infusates deviated from 7, the buffering capacity to pH 7 of the abomasal infusates was determined by titration to pH 7. Multiplication of this buffering capacity with the respective infusion rates delivered the correction factor for the duodenal passage rate of total acid (PRTA), negative when the infusates had a pH lower than 7 and positive when this pH was higher than 7.

When solutions are infused into the abomasum, containing $KHCO_3$, bicarbonate ions entering the acid abomasal contents will recombine with hydrogen ions resulting in a formation of water and carbondioxide, causing irreversible loss of hydrogen ions from abomasal contents and thus a decreased duodenal total acid concentration. Due to this carbondioxide formation, ions disappear from abomasal contents and thus abomasal and duodenal osmotic pressure will decrease (Sections 4.5, 4.6 and 4.8). In order to correct for this loss of hydrogen ions, the theoretical duodenal osmotic pressure was calculated, assuming that no recombination between hydrogen and bicarbonate ions had occurred. This theoretical duodenal osmotic pressure (OPT) for each experimental period was computed according to the formula: OPT = (IR x OPI + (PRD - IR) x OPR)/PRD. In this formula OPT represents the theoretical duodenal, OPI the infusate, and OPR the reference duodenal osmotic pressures. In those series of experiments without intra-abomasal infusions in the control experiments,

the average control duodenal osmotic pressure was used as the reference duodenal osmotic pressure (Section 4.5). In the other series of experiments (Sections 4.6 and 4.8), when $KHCO_3$ 0.15 mol/litre was infused into the abomasum in the control experiments, a reference duodenal osmotic pressure of 250 mosm/kg was postulated. IR and PRD were used previously in this section. From these computed osmotic pressures (OPT) and the duodenal osmotic pressures measured (OPD), duodenal passage rates of total acid were increased by half of the milliosmoles lost, 0.001 x 0.5 x PRD x (OPT - OPD), half since the decrease of the abomasal and duodenal osmotic pressures is caused by equimolar amounts of hydrogen and bicarbonate ions. The reliability of this method is discussed in Section 4.7.

All protein suspensions or solutions infused into the abomasum were made up in saline. In these abomasal infusates chloride concentrations were determined, which delivered the amounts of chloride infused (IRCl) when multiplied by the respective infusion rates (IR) and corrected for dry matter content. As in the feeding trials, duodenal passage rate of chloride (PRCl) was used as an additional estimate for abomasal secretory activity of acid and therefore duodenal passage rates of chloride were decreased by the amounts of chloride infused.

Statistics. Duodenal data of the intra-abomasal infusion experiments were analysed statistically according to the analysis of variance for a Latin square design (Snedecor & Cochran, 1962). In those experiments, intra-abomasal infusions were carried out according to more than one Latin square design, data were analysed statistically according to the separate Latin square designs, and standardized on the average of the different control values. As the overall standard deviation, per parameter the average of the standard deviations, obtained from the separate Latin square statistical analyses, was calculated.

Although intra-abomasal infusions of $KHCO_3$ buffers of various concentrations (Section 4.5) were carried out according to the statistical design described, problems with a duodenal cannula of one of the sheep forced us to calculate the duodenal data on the experimental results of two sheep. In this case, per intra-abomasal infusate duodenal data were compared with control data of the same sheep nearest by in time, and independent of the difference between sheep. Per parameter, the standard deviation was computed as the average of the standard deviations of the separate statistical analyses.

Statistical comparisons of the duodenal data after the various intra-abomasal infusions versus the respective control duodenal data were made using the two-tailed Student t test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Standard deviations are indicated by SD, unless otherwise mentioned.

4.3 INFUSION OF SOYA PROTEIN

In order to test the validity of the intra-abomasal infusion technique in studying abomasal secretion of acid, the intra-abomasal infusion experiments were started with a 10% soya protein (promine D) suspension in saline, infused into the abomasum at two infusion rates (Table 16).

In these and in the next experiments, the effect of the intra-abomasal infusions on abomasal secretion of acid was studied by comparing the duodenal passage rates of total

acid (PRTA) and of chloride (PRCl), both values eventually corrected as described in Section 4.2, with the amounts of total acid and chloride leaving the abomasum in the respective control experiments. This method can only be valid when entry rate of digesta from the omasum into the abomasum is not affected by these continuous intra-abomasal infusions. In the present experiments, this hypothesis was tested by comparing the increases in the duodenal passage rates of dry matter (PRDM) and of crude protein (PRCP), after intra-abomasal infusion of the soya protein suspension, with the amounts of dry matter and of crude protein infused, respectively. As indicated in Table 17, those increases in the amounts of dry matter and crude protein leaving the abomasum, 2.89 g/h (SD 1.80) and 2.38 g/h (SD 0.47) for the lower infusion level, and 6.53 g/h (SD 1.80) and 5.42 g/h (SD 0.47) for the higher infusion level, respectively, did not differ significantly from the respective infusion rates of dry matter (IRDM) and of crude protein (IRCP), as indicated in Table 16. The number of observations was rather small, and in consequence the coefficient of variation of the data given in Table 17 is rather high, but regarding the close agreement between the increases in the average duodenal passage rates of dry matter and of crude protein and the amounts of dry matter and crude protein infused, it strongly suggests that abomasal entry rate of digesta was not affected by the intra-abomasal infusions, carried out continuously. Further support for this conclusion could be derived from studies on duodenal polyethylene glycol (PEG) recoveries, carried out simultaneously with the intra-abomasal infusions, in two of the sheep. PEG was infused continuously into the rumen through the rumen cannula inserted into the dorsal rumen sac. In case reticulo-ruminal outflow of digesta and thus abomasal entry rate of digesta should be influenced by the intra-abomasal infusions, an effect on both rumen PEG concentration (samples removed from the ventral rumen sac) corrected for the PEG infusion rate, and duodenal PEG recovery, calculated as the amount of PEG passing in the duodenum divided by the infusion rate of PEG, was to be expected. For the control experiments, and after intra-abomasal infusion of soya protein at the lower and higher level, ruminal PEG concentrations (mg/ml) of 1.50, 1.47 and 1.52 (SD 0.02) and duodenal PEG recoveries (%) of 93.9, 92.0 and 95.3 (SD 4.8) were found, respectively, indicating that both parameters were not significantly affected by intra-abomasal infusion of soya protein.

On the other hand, the duodenal passage rate of digesta (PRD) after intra-abomasal infusion of soya protein was to be expected to be increased with the rate of infusion (IR) plus the calculated amount of abomasal juice secreted in excess. As can be seen from

Table 17. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), of chloride (PRCl), and of pepsin (PRPE), and the pH of duodenal contents, as affected by continuous intra-abomasal infusion of a 10% soya protein suspension in saline at infusion rates (IR) of 25.9 g/h (1) and 57.2 g/h (2). For significances (two-tailed Student t_{11}) see Methods (Section 4.2).

	PRD g/h	PRDM g/h	PRTA mmol/h	pH	PRCP g/h	PRCl mmol/h	PRPE mg/h
Control	658.5	25.70	31.50	3.07**	5.85***	62.16	41.94
IR_1	676.1	28.59**	33.45*	3.24**	8.23***	63.20	35.82*
IR_2	738.1	32.23**	38.97*	3.26**	11.27***	68.74	29.28*
SD	28.0	1.04	1.43	0.03	0.27	2.84	2.61

the data of Table 17, the standard deviation of duodenal passage rate of digesta was relatively high, when compared with the rates of infusion. Probably for that reason the duodenal flow rates after intra-abomasal infusion of soya protein were not significantly different from the duodenal flow rates measured in the control experiments.

After intra-abomasal infusion of soya protein at both infusion rates, duodenal passage rates of total acid (PRTA) and of chloride (PRCl) were increased, although not or hardly significantly regarding the high coefficients of variation of the individual values. After both infusion rates, the increases in duodenal passage rate of total acid and of chloride were of the same order. The increases in the amount of total acid leaving the abomasum at both infusion levels and thus the additional amount of hydrochloric acid secreted by the abomasum equalled the infusion rate of buffering capacity (IRBC, Table 16). Nevertheless, the pH of abomasal and duodenal contents was significantly increased, indicating that buffering capacity of the soya protein may be changed after infusion into the abomasum by a partly peptic hydrolysis. The effect of intra-abomasal infusion of soya protein on duodenal pepsin activity (PRPE) is discussed in Chapter 6.

4.4 INFUSION OF DIFFERENT PROTEINS AND OF A PHOSPHATE BUFFER

An increased protein content of the ration was found to stimulate abomasal secretion of acid. Besides, the amount of crude protein passing through the abomasum was found to be closely related to abomasal acid secretory activity (Chapter 3). In Section 4.3, intra-abomasal infusion of a 10% soya protein suspension in saline at a rate of 57.2 g/h was described to stimulate abomasal secretion of acid.

Proteins might act non-specifically on behalf of their buffering capacity, affecting indirectly the release of gastrin, but probably also more specifically by stimulating the release of gastrin. In Section 2.3.5, differences were described in gastrin releasing potency between amino acids, and consequently amino acid composition of the different proteins might be involved as well. The objective of the following experiments was to determine, whether the stimulative effect of proteins on abomasal secretion of acid is determined only by their buffering capacity and if not whether their amino acid composition is of importance as well. Therefore five different proteins, in comparison with a phosphate buffer, were infused continuously into the abomasum (Table 16). In Table 18, the proportional amino acid composition of the different proteins is given.

In Table 19, amount and composition of duodenal digesta is given as affected by intra-abomasal infusion of the different proteins and of the phosphate buffer. The respective increases in duodenal passage rate of dry matter (PRDM) and crude protein (PRCP) after the various intra-abomasal infusions did not differ significantly from the respective amounts of dry matter (IRDM) and crude protein (IRCP) infused into the abomasum (Table 16), indicating that also after these intra-abomasal infusions abomasal digesta entry rate was not inhibited. After infusion of promine D, of gluten and of the phosphate buffer, which were found to be the most active stimulators of abomasal secretion of acid, the increases in the duodenal passage rates of digesta (PRD) tended to be greater than the respective infusion rates (IR, Table 16), which indicated that due to the stimulated abomasal secretory activity of acid an additional amount of abomasal juice was secreted. These

Table 18. Amino acid composition of the different proteins infused into the abomasum (Section 4.4), expressed as g per 100 g amino acids determined.

	α-Protein	Promine D	Casein	Gelatin	Gluten
Lys	6.06	6.63	8.31	4.08	1.86
His	2.70	2.35	2.77	0.79	2.04
Arg	7.82	8.05	3.63	7.91	3.67
Asp	11.56	11.54	6.45	5.42	3.14
Thr	3.69	3.50	4.01	1.77	2.56
Ser	5.59	5.54	5.71	3.51	5.02
Glu	19.98	20.34	20.65	9.98	36.90
Pro	5.39	5.80	10.30	13.01	12.13
OH-Pro	-	-	-	12.29	-
Gly	3.99	3.88	1.82	22.75	3.37
Ala	4.03	3.74	2.91	8.55	2.58
Cys	0.65	1.35	0.42	0.09	2.38
Val	5.25	4.95	6.50	2.47	4.06
Meth	1.33	1.26	2.96	0.84	1.73
Ileu	4.87	4.84	4.94	1.24	3.62
Leu	7.80	7.70	8.39	2.72	6.50
Tyr	3.88	3.45	5.30	0.74	3.47
Phe	5.40	5.10	4.93	1.85	4.97

Table 19. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), of chloride (PRC1), and of pepsin (PRPE), and the pH of duodenal contents, as affected by continuous intra-abomasal infusion of different proteins and of a phosphate buffer. For composition of the intra-abomasal infusates and for significances (two-tailed Student t_{20}) see Methods (Section 4.2).

	PRD g/h	PRDM g/h	PRTA mmol/h	pH	PRCP g/h	PRC1 mmol/h	PRPE mg/h
Control	575.3	23.06	30.38	2.96	6.43	62.46	31.67
α-Protein	653.1*	28.49**	35.54*	3.09	10.37***	65.81	18.26***
Promine D	711.6***	28.82**	39.68***	2.99	9.63***	70.41*	25.83*
Casein	648.7*	29.21***	33.74	3.13*	9.87***	65.95	12.98***
Gelatin	761.4***	28.71**	33.21	3.06	11.05***	67.82	45.10***
Gluten	714.4***	30.40***	37.58***	2.93	9.53***	70.65*	24.63*
P buffer	709.0***	24.45	37.15**	3.16**	6.61	69.42	35.50
SD	19.2	0.88	1.07	0.04	0.28	1.99	1.45

differences were not significant, however. Probably, the high coefficients of variation of the increases in duodenal passage rate of digesta can be set responsible for that.

Abomasal secretion of acid was stimulated most actively by intra-abomasal infusion of promine D of gluten and of the phosphate buffer, as could be concluded from the increases in duodenal passage rate of total acid (PRTA) and of chloride (PRC1). In Figure 8, duodenal passage rate of total acid was plotted against buffering capacity (IRBC, Table 16) infused. The broken line in this figure represents equality between the increases in the amounts of total acid leaving the abomasum and buffering capacity of the respective infusates. As shown in this figure, the proteins tended to stimulate abomasal secretion of acid in excess of IRBC. The stimulative effect on acid secretion in excess of IRBC was significant after intra-abomasal infusion of promine D ($P < 0.05$) and gluten ($P < 0.01$), but not after

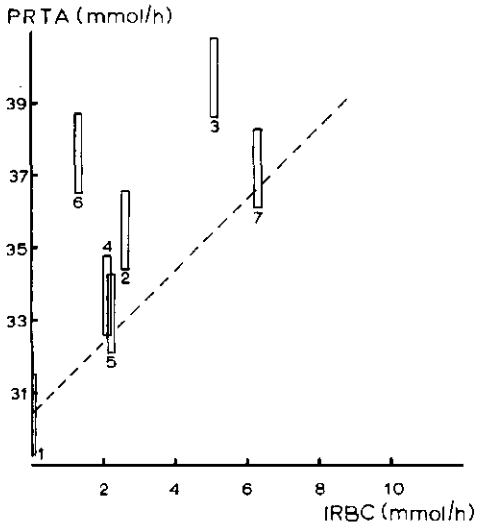

Fig. 8.

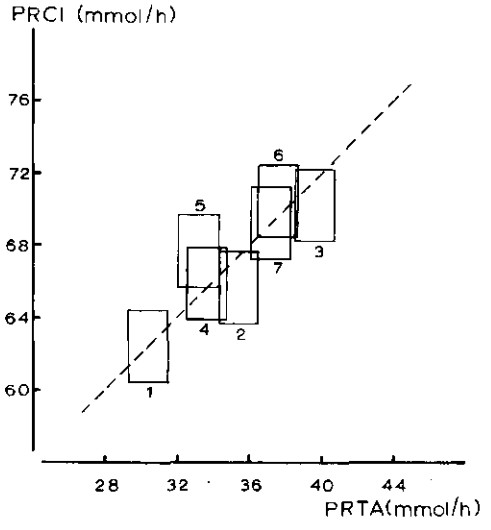

Fig. 9.

Relation between duodenal passage rate of total acid (PRTA) and buffering capacity infused into the abomasum (IRBC) (Fig. 8), and between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) (Fig. 9) of the control experiments (1) and after intra-abomasal infusion of α-protein (2), promine D (3), casein (4), gelatin (5), gluten (6), and of a phosphate buffer (7). For composition of the intra-abomasal infusates see Methods (Section 4.2). Each column represents the average value ± 1 SD in both directions.

infusion of α-protein, casein and gelatin. Nevertheless, after intra-abomasal infusion of α-protein and gelatin, the duodenal pH tended to increase and was significantly increased after casein infusion. After intra-abomasal infusion of promine D and gluten, acid secretion was stimulated in excess of IRBC, but the duodenal pH seemed not to be affected, although after gluten the duodenal pH tended to decrease. These results suggest that buffering capacity of the protein infusates is probably changed after infusion into the abomasum due to a partial peptic hydrolysis.

The increases in duodenal passage rate of chloride after the various intra-abomasal infusions were hardly significant in general, probably caused by the high coefficients of variation of those increases. Nevertheless, when compared PRCl with PRTA, the highest increases in PRCl were noticed together with the highest increases in PRTA. In Figure 9, PRCl was plotted against PRTA, the broken line indicating duodenal total acid and chloride passage rates to increase in equimolar amounts. In all cases the increases in PRTA and PRCl did not deviate significantly from equimolarity.

The effect of infusion of different proteins and of a phosphate buffer on duodenal pepsin activity (PRPE) is discussed in Chapter 6.

4.5 INFUSION OF $KHCO_3$ BUFFERS OF VARIOUS CONCENTRATIONS

Stimulation of abomasal secretion of acid after intra-abomasal infusion of proteins was found not to be mediated only by their buffering capacity, whereas of a phosphate buffer a stimulative effect on acid secretion was found too (Section 4.4). Stimulation of acid

secretion after infusion of inorganic buffers is probably not mediated by specific stimulating agents. They are more likely to act through their effect on the pH of abomasal contents. Since at a higher pH of abomasal contents the release of gastrin is less inhibited, an indirectly stimulated gastrin release might result in an increased abomasal secretory activity (Chapter 5).

In order to confirm the stimulative effect of inorganic buffers on abomasal secretion of acid, $KHCO_3$ buffers of 0.15, 0.30, 0.45, and 0.60 molar concentrations were infused continuously into the abomasum (Table 16).

In Table 20, amount and composition of duodenal digesta is given as affected by intra-abomasal infusion of these $KHCO_3$ buffers. Continuous intra-abomasal infusion of these buffers did not influence abomasal digesta entry rate, as could be concluded from the data on duodenal passage rate of dry matter (PRDM) and of crude protein (PRCP). As indicated by the duodenal passage rates of total acid (PRTA), corrected as described in Section 4.2, and to a lesser extent by the duodenal passage rates of chloride (PRCl), infusion of these $KHCO_3$ buffers into the abomasum stimulated acid secretion. The stimulative effect was found to be less than the respective buffering capacities (IRBC, Table 16). The stimulative effect of the $KHCO_3$ buffers on acid secretion tended to induce an increase in duodenal passage rate of digesta (PRD), which was higher than the respective infusion rates (Table 16), probably caused by the additional amount of abomasal juice secreted. When more concentrated buffers were infused into the abomasum, acid secretion was more actively stimulated, but a corresponding increase in PRD was not noticed, however.

In Figure 10, the increases in duodenal passage rate of chloride (PRCl) were plotted against buffering capacity infused, illustrating that the stimulative effect on abomasal secretion of acid was less than IRBC, as could also be concluded from the respective increases in the pH of duodenal contents. This stimulative effect was proportionally less when a more concentrated $KHCO_3$ buffer was infused into the abomasum. The broken line in this figure represents equality between the stimulative effect on abomasal secretion of acid and buffering capacity of the infusates. In Figure 11, duodenal passage rate of chloride was plotted against that of total acid (PRTA), the broken line indicating duodenal total acid and chloride passage rates to increase in equimolar amounts. After all intra-abomasal infusates the respective increases in PRCl and PRTA did not deviate significantly from equimolarity.

Table 20. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), of chloride (PRCl), and of pepsin (PRPE), and the pH and osmotic pressure (OPD) of duodenal contents, as affected by continuous intra-abomasal infusion of $KHCO_3$ buffers of various concentrations in mol/litre (M). For significances (two-tailed Student t_9) see Methods (Section 4.2).

	PRD g/h	PRDM g/h	PRTA mmol/h	pH	PRCP g/h	PRCl mmol/h	PRPE mg/h	OPD mosm/kg
Control	596.6	21.71	28.00	3.07	6.14	73.31	12.92	253.5
0.15 M $KHCO_3$	718.0*	24.25	32.86***	3.41*	6.37	80.45	17.54	238.5**
0.30 M $KHCO_3$	735.8*	23.38	38.98***	3.55**	6.30	84.67	17.79	243.3*
0.45 M $KHCO_3$	734.8*	23.99	43.48***	3.61**	6.12	87.02*	17.88	254.5
0.60 M $KHCO_3$	728.3*	23.73	46.62***	3.72**	6.07	90.93*	18.40*	268.2**
SD	24.0	0.82	1.09	0.07	0.20	3.01	1.11	1.8

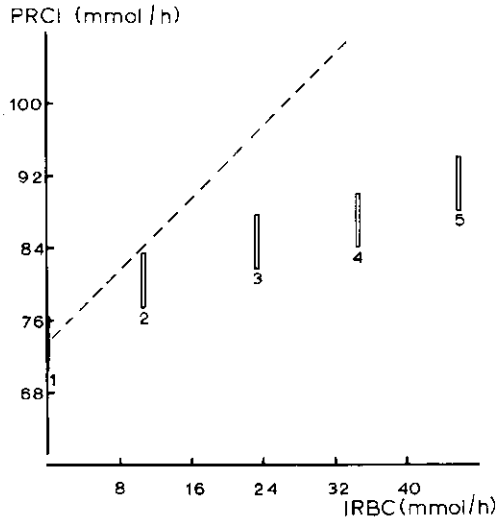

Fig. 10.

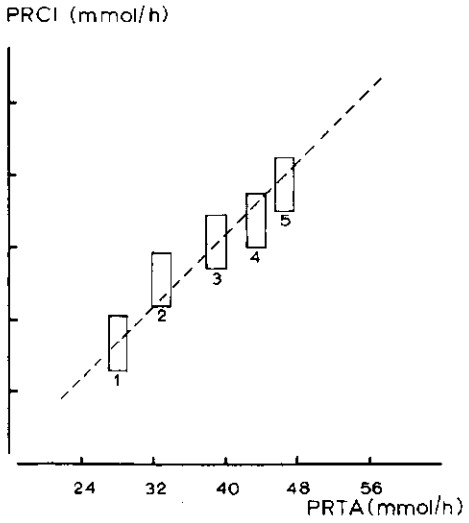

Fig. 11.

Relation between duodenal passage rate of chloride (PRCl) and buffering capacity infused into the abomasum (IRBC) (Fig. 10), and between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) (Fig. 11) of the control experiments (1) and after intra-abomasal infusion of $KHCO_3$ buffers of 0.15 (2), 0.30 (3), 0.45 (4) and 0.60 (5) molar concentrations. Each column represents the average value $\pm$ 1 SD in both directions.

As discussed previously, introduction of bicarbonate ions into the acid abomasal contents results in a formation of carbondioxide, and consequently in a decreased osmotic pressure (OPD) of abomasal and duodenal contents, as illustrated in Table 20 when $KHCO_3$ of 0.15 and 0.30 mol/litre were infused into the abomasum. With these effects of intra-abomasal $KHCO_3$ infusions on abomasal and duodenal osmotic pressure, duodenal passage rates of total acid were corrected as described under Methods (Section 4.2). Intra-abomasal infusion of these buffers was supposed to induce an additional secretion of hydrogen and chloride ions in equimolar amounts. That such a relationship between PRTA and PRCl was found (Fig. 11) supports the reliability of this method of correcting PRTA.

The effect of intra-abomasal infusion of these $KHCO_3$ buffers on duodenal pepsin activity (PRPE) is discussed in Chapter 6.

4.6 INFUSION OF AMINO ACIDS

As concluded previously, protein composition is supposed to act on abomasal secretion of acid, possibly through a selective stimulation of the release of gastrin, mainly by lower molecular protein fragments or even by particular amino acids. Although under normal conditions amino acid concentrations are not expected to be high in the abomasum, amino acid residues of the protein fragments might be involved in the regulation of gastrin release. Single amino acids, such as glycine and β-alanine, were described to stimulate the release of gastrin, whereas α-alanine was less potent (Section 2.3.5).

In order to study the effect of amino acids on abomasal secretion of acid, these three amino acids, α-alanine, β-alanine and glycine, were infused into the abomasum. Besides,

three other arbitrary chosen amino acids, i.e. methionine, aspartic acid and arginine, were used (Table 16).

In Table 21, amount and composition of duodenal digesta is given as influenced by intra-abomasal infusion of these amino acid solutions 0.10 mol/litre in $KHCO_3$ 0.15 mol/litre, in comparison with intra-abomasal infusion of $KHCO_3$ 0.15 mol/litre in the control experiments. As indicated by the duodenal passage rates of total acid (PRTA), corrected as described in Section 4.2, and by the duodenal passage rates of chloride (PRCl), the amino acids infused into the abomasum at these infusion rates (Table 16) were not found to stimulate acid secretion, except after infusion of α-alanine when PRTA and PRCl tended to increase. This effect of α-alanine on abomasal secretion of acid was possibly mediated by an increased digesta entry rate into the abomasum, since duodenal passage rate of dry matter (PRDM) was slightly increased and duodenal passage rate of crude protein (PRCP) tended to be increased in excess of the amount of crude protein infused (IRCP, Table 16). When methionine was infused into the abomasum, acid secretion was significantly inhibited, as indicated by the decreases in PRTA and PRCl. Inhibition of acid secretion seemed not to be achieved directly by methionine, regarding the pH of the duodenal contents, but indirectly by a decreased entry rate of digesta into the abomasum as could be concluded from the significantly decreased PRDM.

In Figure 12, duodenal passage rate of chloride (PRCl) was plotted against buffering capacity (IRBC) of the intra-abomasal infusates, the broken line indicating equality between the effect of the intra-abomasal infusates on PRCl and their buffering capacity. Of the amino acid solutions infused into the abomasum, IRBC of the solutions of β-alanine and arginine was highest, in comparison with IRBC of the control infusions of $KHCO_3$ 0.15 mol/litre (Table 16). The stimulative effect of both of these amino acid infusates, however, did not differ significantly from the stimulative effect of $KHCO_3$ 0.15 mol/litre on acid secretion, as was also indicated by the significantly increased pH of duodenal contents after infusion of β-alanine and arginine into the abomasum (Table 21). In Figure 13, PRCl was plotted against PRTA, the broken line indicating duodenal total acid and

Table 21. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), and of chloride (PRCl), and the pH and osmotic pressure (OPD) of duodenal contents, as affected by continuous intra-abomasal infusion of solutions 0.10 mol/litre of α-alanine, β-alanine, aspartic acid, arginine, glycine and methionine in $KHCO_3$ 0.15 mol/litre, in comparison with intra-abomasal infusion of $KHCO_3$ 0.15 mol/litre in the control experiments. For significances (two-tailed Student t_{29}) see Methods (Section 4.2).

	PRD g/h	PRDM g/h	PRTA mmol/h	pH	PRCP g/h	PRCl mmol/h	OPD mosm/kg
Control	632.4	18.69	34.69	3.15	4.67	66.30	224.4
α-Alanine	684.0	21.12*	37.67	3.17	5.90***	72.91	238.0***
β-Alanine	612.5	18.36	35.07	3.41*	5.18	67.41	238.6***
Aspartic acid	615.0	19.35	35.57	3.35	5.34*	63.26	237.9***
Arginine	576.9	17.67	32.79	3.81***	7.18***	66.64	243.8***
Glycine	615.2	19.06	35.11	3.30	5.34*	67.29	238.0***
Methionine	526.8**	14.84**	28.45*	3.00	4.30	56.83**	244.7***
SD	20.3	0.64	1.49	0.06	0.17	1.96	1.9

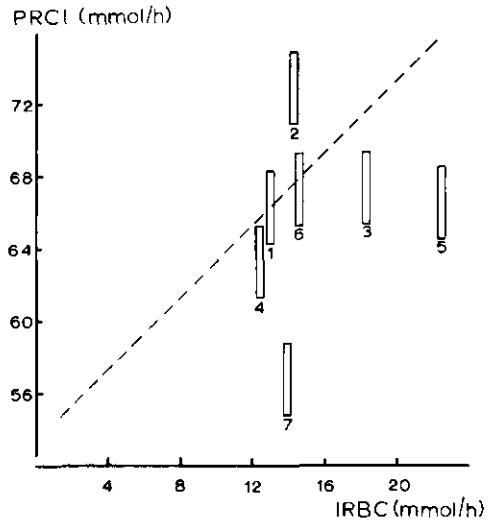

Fig. 12.

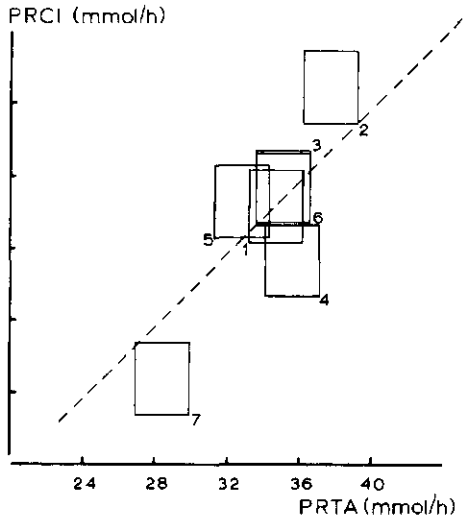

Fig. 13.

Relation between duodenal passage rate of chloride (PRCl) and buffering capacity infused into the abomasum (IRBC) (Fig. 12), and between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) (Fig. 13), after intra-abomasal infusion of $KHCO_3$ 0.15 mol/litre in the control experiments (1) and of solutions 0.10 mol/litre in $KHCO_3$ 0.15 mol/litre of α-alanine (2), β-alanine (3), aspartic acid (4), arginine (5), glycine (6) and methionine (7). Each column represents the average value ± 1 SD in both directions.

chloride passage rates to increase in equimolar amounts. When present, no significant differences between the respective increases in PRCl and PRTA could be noticed.

4.7 INFUSION OF PARTLY HYDROLYSED SOYA PROTEIN

Proteins were suggested to stimulate abomasal secretion of acid partly by selective stimulation of gastrin release by lower molecular protein fragments. A stimulative effect on acid secretion, however, was hardly noticed after intra-abomasal infusion of amino acids.

In order to test the hypothesis that mainly lower protein fragments are involved in the stimulation of gastrin release (Section 2.3.5), partly hydrolysed soya protein (promine DH), prepared as described under Methods (Section 4.2), was infused continuously into the abomasum. Abomasal secretion of acid after infusion of this soya protein was compared with the secretion resulting from infusion of a soya protein (promine D) suspension, not hydrolysed in advance, and from a control without intra-abomasal infusion.

The composition of the soya protein infusates was characterized as follows. Of the soya proteins, promine D and promine DH, the soluble fractions in Na_2CO_3-$NaHCO_3$ 0.01 mol/litre pH 9.0 were fractionated on a G-25 Sephadex column (100 x 2.6 cm). The solubilities of promine D and promine DH in this buffer were 14.0 and 73.4%, respectively. Fractionation of both soya protein infusates on the G-25 Sephadex column was carried out by elution with the same buffer at a rate of about 30 ml/h. Fractions of 5 ml were collected. In these fractions, concentrations of the peptides were estimated by addition of 0.5 ml of a nin-

hydrin solution (2% ninhydrin + 0.6% $SnCl_2$) to 1 ml of the eluate. After incubation at 95 °C for 7 min, the extinctions were read at 570 nm (Beckman B spectrophotometer). The elution patterns of the soluble fractions of both soya proteins are shown in Figure 14. On the assumption of a fractionation range for G-25 Sephadex up to a molecular weight of 5000, percentages of the peptides of the original, soluble as well as insoluble in the carbonate buffer, proteins with a molecular weight lower than 5000 of 4.6 and 40.9% were calculated for promine D and promine DH, respectively.

In Table 22, amount and composition of duodenal digesta is given as affected by intra-abomasal infusion of both soya proteins. After both intra-abomasal infusates, the increases in duodenal passage rate of dry matter (PRDM) and of crude protein (PRCP) did not differ significantly from the amounts of dry matter and crude protein infused, respectively (IRDM, IRCP; Table 16), indicating that entry rate of digesta into the abomasum was not substantially influenced by continuous intra-abomasal infusion of these soya protein preparations. After intra-abomasal infusion of promine D, the increase in duodenal passage rate of digesta (PRD) was not, but after promine DH this increase tended to be higher than the amount infused (IR, Table 16), probably caused by the abomasal juice secreted additionally.

The osmotic pressure of duodenal contents (OPD) was significantly increased after both intra-abomasal infusates, caused by the hypertonicity of the soya protein suspensions. In order to estimate the reliability of the corrections of duodenal passages rate of total acid (PRTA) when $KHCO_3$ was infused into the abomasum, as described under Methods (Section 4.2), from duodenal passage rate of digesta (PRD), the amount of the soya protein suspensions infused into the abomasum (IR), the osmotic pressure of these intra-abomasal infusates (OPI), and the average duodenal osmotic pressure of the control experiments (OPR),

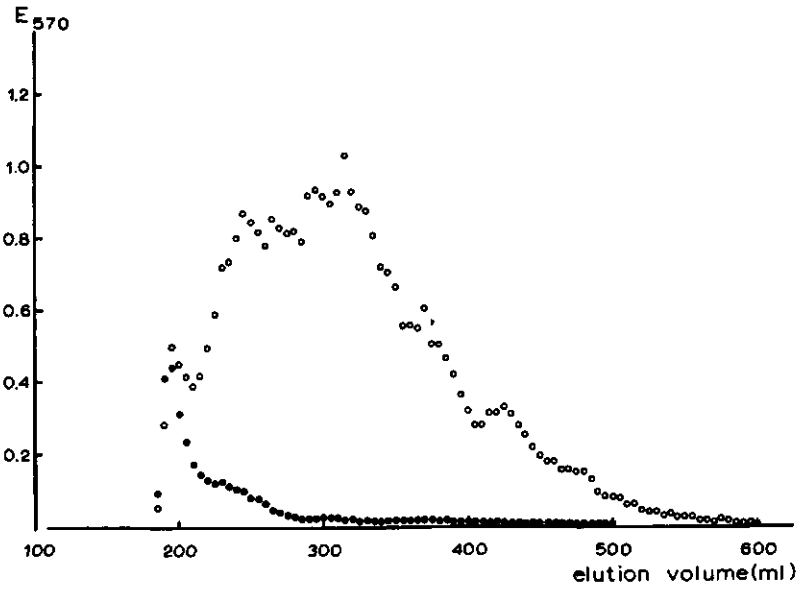

Fig. 14. Sephadex G-25 elution patterns of the soluble fractions of soya protein as such (promine D, •) and of soya protein partly hydrolysed (promine DH, ∘), eluated by Na_2CO_3-$NaHCO_3$ 0.01 mol/litre pH 9.0.

Table 22. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), and of chloride (PRC1), and the pH and osmotic pressure (OPD measured and OPT calculated as described in Section 4.2) of duodenal contents, as affected by continuous intra-abomasal infusion of 10% soya protein (promine D not and promine DH partly hydrolysed in advance) suspensions in saline. For significances (two-tailed Student t_{29}) see Methods (Section 4.2).

	PRD g/h	PRDM g/h	PRTA mmol/h	pH	PRCP g/h	PRC1 mmol/h	OPD mosm/kg	OPT mosm/kg
Control	565.2	19.02	29.43	2.90	5.30	62.79	240.8	240.8
Promine D	608.0	23.77***	34.03*	3.10***	10.11***	67.62	262.1***	254.4***
Promine DH	662.0**	24.53***	39.05***	3.12***	10.75***	74.46**	270.6***	265.3***
SD	18.3	0.72	1.03	0.03	0.25	1.99	1.5	1.1

the theoretical duodenal osmotic pressure (OPT) was calculated according to the formula given in Section 4.2. From the differences between the osmotic pressures determined (OPD) and the osmotic pressures calculated (OPT), correction factors for the duodenal passage rates of total acid (PRTA) would be calculated of 2.34 and 1.75 mmol/h for promine D and promine DH, respectively. These factors fit very well within the limits of confidence of the data on PRTA. Besides, these differences between OPD and OPT, when soya protein was infused into the abomasum, may result in an underestimation of the reliability of the correction method, since the abomasal and thus the determined duodenal osmotic pressures may be increased in excess by the partial peptic proteolysis occurring in the abomasum.

Both intra-abomasal infusates caused stimulation of abomasal secretion of acid, as estimated from the increases in duodenal passage rate of total acid (PRTA) and of chloride (PRC1), as corrected for the amount of chloride infused. This stimulation was less than the respective buffering capacities infused (IRBC, Table 16), as could also be concluded from the significant increases in the pH of duodenal digesta. When averaged the respective increases in PRTA and PRC1 per intra-abomasal infusate, ratios between the stimulative effects on acid secretion and the respective buffering capacities infused of 0.47:1 and 0.73:1 for promine D and promine DH could be calculated. The higher potency of promine DH in stimulating acid secretion in comparison with promine D, despite IRBC was higher after promine DH infusion, is also shown in Figure 15, where PRTA was plotted against IRBC. In Section 4.5, after infusion of $KHCO_3$ buffers of various concentrations into the abomasum, stimulation of abomasal secretion of acid was proportionally less, when buffering capacity of the infusates was higher. When the stimulative effect of promine D in Figure 15 was extrapolated linearly, which results in an overestimation of the expected secretory responses at higher buffering capacities, at an IRBC of 14.5 mmol/h (promine DH) an average abomasal acid secretory activity of 36.1 mmol/h was calculated. Nevertheless, the abomasal acid secretory activity measured after promine DH infusion, and the secretory activity calculated by linear extrapolation differed slightly ($P < 0.05$, one-tailed Student t_{29}).

In Figure 16, PRC1 was plotted against PRTA. After both intra-abomasal infusates, the increases in PRC1 did not differ significantly from those in PRTA.

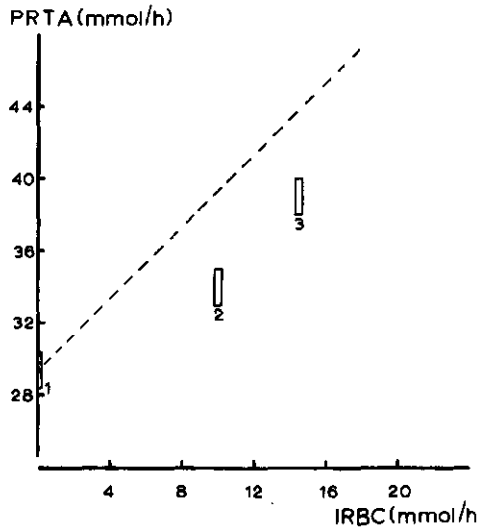

Fig. 15.

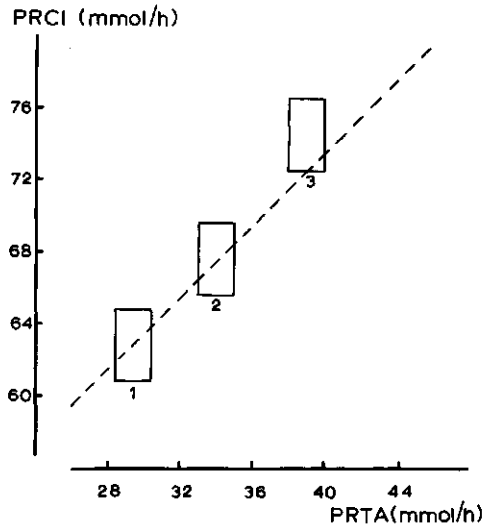

Fig. 16.

Relation between duodenal passage rate of total acid (PRTA) and buffering capacity infused into the abomasum (IRBC) (Fig. 15), and between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) (Fig. 16) of the control experiments (1) and after intra-abomasal infusion of 10% soya protein suspensions in saline, promine D (2) not and promine DH (3) partly hydrolysed in advance. Each column represents the average value ± 1 SD in both directions.

4.8 INFUSION OF VOLATILE FATTY ACIDS AND L-LACTIC ACID

Previously, abomasal secretion of acid was concluded to depend on ration composition (Chapter 3). In this context, a close relationship was demonstrated between acid secretion and protein content of the ration. Composition of digesta entering the abomasum is affected, however, not only by composition of the ration, but also by the events occurring during forestomach fermentation. In this regard, volatile fatty acids have been proposed to stimulate abomasal secretion of acid (Hill, 1960, Ash 1961). In Feeding Trial 2 (Section 3.4), however, no significant relationship could be detected between rumen volatile fatty acid concentrations and abomasal secretory activity of acid. Rumen fermentation may also result in a production of lactic acid under particular conditions, especially when the rumen microflora has to adapt to a change to a high concentrated ration. In order to decide whether or not volatile fatty acids stimulate abomasal secretion of acid, acetic, propionic and butyric acids were infused continuously into the abomasum. Besides, we studied the effect of the L-isomer of lactic acid on abomasal secretion of acid.

The effect of intra-abomasal infusion of these organic acids on amount and composition of duodenal digesta is given in Table 23. Proportionally to intra-abomasal infusion of $KHCO_3$ 0.15 mol/litre in the control experiments, intra-abomasal infusion of these organic acids tended to increase duodenal passage rate of digesta (PRD). The increase after butyric acid infusion was slightly significant, probably caused by the amount of abomasal juice secreted additionally. Neither duodenal passage rate of dry matter (PRDM) nor duodenal passage rate of crude protein (PRCP) was affected by the intra-abomasal infusions, indi-

Table 23. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), and of chloride (PRCl), and the pH and osmotic pressure (OPD), of duodenal contents, as affected by continuous intra-abomasal infusion of 0.5% solutions of acetic, propionic, butyric and L-lactic acids in $KHCO_3$ 0.15 mol/litre, in comparison with intra-abomasal infusion of $KHCO_3$ 0.15 mol/litre in the control experiments. For significances (two-tailed Student t_{20}) see Methods (Section 4.2).

	PRD g/h	PRDM g/h	PRTA mmol/h	pH	PRCP g/h	PRCl mmol/h	OPD mosm/kg
Control	574.8	18.48	30.00	3.11	4.90	61.10	229.9
Acetic acid	613.6	19.72	36.31**	3.06	5.01	66.21*	233.9
Propionic acid	619.7	19.35	34.95*	3.01	5.02	66.85*	234.1
Butyric acid	625.7*	19.17	34.77*	3.21	4.90	66.79*	229.7
L-lactic acid	621.2	19.32	33.05	3.22	4.99	65.17	231.6
SD	13.9	0.44	1.07	0.04	0.12	1.40	2.0

cating that entry rate of digesta into the abomasum was not significantly affected by these continuous intra-abomasal infusions.

From the increases in duodenal passage rate of total acid (PRTA) and of chloride (PRCl), it appeared that the volatile fatty acids in the amounts applied stimulated abomasal secretion of acid, although not actively. After intra-abomasal infusion of L-lactic acid, only a tendency was noticed PRTA and PRCl to increase. Stimulation of acid secretion by the organic acids, in comparison with the control experiments, however, did not result in a significantly decreased duodenal pH. In Figure 17, the increases in PRCl were plotted

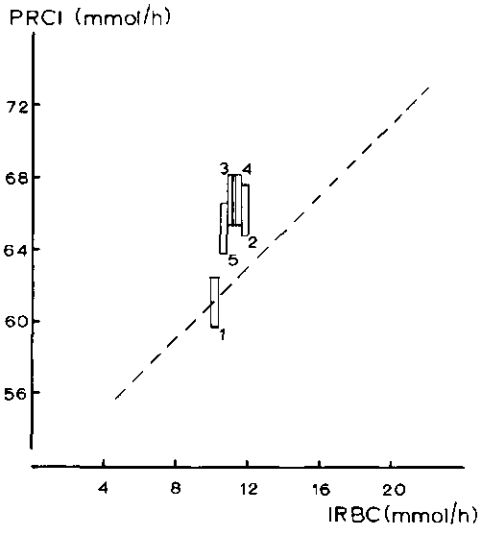

Fig. 17.

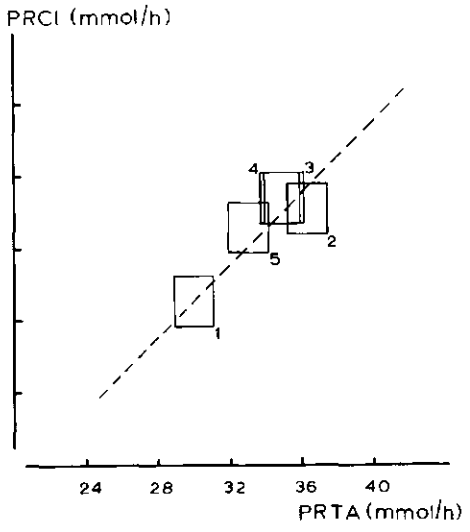

Fig. 18.

Relation between duodenal passage rate of chloride (PRCl) and buffering capacity infused into the abomasum (IRBC) (Fig. 17), and between duodenal passage rate of chloride (PRCl) and of total acid (PRTA) (Fig. 18), after intra-abomasal infusion of $KHCO_3$ 0.15 mol/litre in the control experiments (1) and of 0.5% solutions in $KHCO_3$ 0.15 mol/litre of acetic acid (2), propionic acid (3), butyric acid (4) and L-lactic acid (5). Each column represents the average value $\pm$ 1 SD in both directions.

Table 24. Duodenal passage rate (PR) of acetic acid (HAc), of propionic acid (HPr), of butyric acid (HBu), and of L-lactic acid (HLa), as affected by the amounts of the respective organic acids infused into the abomasum (IR). For significances (two-tailed Student t_{20}) see Methods (Section 4.2).

	IR mmol/h	PRHAc mmol/h	PRHPr mmol/h	PRHBu mmol/h	PRHLa mmol/h
Control	-	2.52	0.58	0.16	0.11
Acetic acid	6.40	5.10***	0.54	0.16	-
Propionic acid	4.90	2.93	1.94***	0.20	-
Butyric acid	4.15	2.95	0.66	1.02***	-
L-lactic acid	3.78	2.52	0.56	0.16	2.99***
SD	0.06	0.22	0.06	0.03	0.08

against buffering capacity infused (IRBC, Table 16). The broken line indicates equality between IRBC and the increases in PRC1. Proportionally to intra-abomasal infusion of $KHCO_3$ 0.15 mol/litre, the organic acids tended to stimulate acid secretion in excess of their buffering capacity. In Figure 18, PRC1 was plotted against PRTA. As can be seen from the reference broken line, the increases in PRC1 did not differ significantly from the increases in PRTA.

The amounts of volatile fatty acids and L-lactic acid infused into the abomasum were high in comparison with the amounts normally entering the abomasum. In Table 24, the amounts of these organic acids infused into the abomasum and their effects on the amounts leaving the abomasum are given. From the increases in duodenal passage rate of the respective organic acids in comparison with the respective amounts infused, absorption percentages from the abomasum were calculated of about 60, 72, 79 and 24% for acetic, propionic, butyric and L-lactic acids, respectively. These absorption percentages of volatile fatty acids in the abomasum, thus calculated, are substantially higher than the omasal volatile fatty acid absorption percentages, mentioned in Section 2.3.3.

4.9 DISCUSSION

In the present experiments with sheep in a steady-state situation, the effect of continuous intra-abomasal infusion of a particular substance on abomasal secretion of acid was estimated by comparing outflow of abomasal digesta in experiments, in which that substance was infused into the abomasum, with outflow of abomasal digesta when a control infusion or no intra-abomasal infusion was carried out.

That this intra-abomasal infusion technique is valid in studying abomasal secretion of acid could be deduced from the following findings. After intra-abomasal infusion of soya protein at two infusion rates, the increases in duodenal passage rate of dry matter (PRDM) and of crude protein (PRCP) did not differ significantly from the amounts of dry matter (IRDM) and of crude protein (IRCP) infused, respectively (Section 4.3). Moreover, in the same experiments, after a continuous polyethylene glycol (PEG) infusion into the rumen, neither ruminal PEG concentration nor duodenal PEG recovery were affected by these continuous intra-abomasal soya protein infusions. Both findings indicated that entry rate

of abomasal digesta was not affected by continuous intra-abomasal infusion of soya protein, and that the effect of these intra-abomasal infusions on abomasal secretion of acid may be estimated from outflow of abomasal digesta after intra-abomasal infusion in comparison with outflow of abomasal digesta in the control experiments. Also in the other intra-abomasal infusion experiments, the increases in PRDM and PRCP did not differ significantly from the amounts of dry matter and of crude protein infused, except when methionine (Section 4.6) was infused into the abomasum.

When continuous intra-abomasal infusions do not affect abomasal entry rate of digesta, duodenal passage rate of digesta (PRD) is expected to be increased with the infusion rate of the intra-abomasal infusate (IR) plus the amount of abomasal juice secreted in addition. When soya protein was infused into the abomasum at the lowest infusion rate (Section 4.3), the increase in PRD tended to be less than the infusion rate of the soya protein suspension (IR). At the highest infusion rate (57.2 g/h), however, this increase (79.6 g/h) did exceed IR. From the average of the increases in duodenal passage rate of total acid (PRTA) (7.47 mmol/h) and of chloride (PRCl) (6.58 mmol/h), from an acid concentration in acid abomasal juice of 155 mmol/litre, a volume of abomasal juice secreted in addition of 45.3 ml/h could be calculated, which tended to be higher than the difference between the increase in duodenal passage rate of digesta and the infusion rate of the soya protein suspension. After intra-abomasal infusion of different proteins and of a phosphate buffer (Section 4.4), the increases in PRD did not exceed the respective infusion rates of α-protein and casein. After promine D, gelatin, gluten and the phosphate buffer, from the average of the increases in PRTA and PRCl and from a concentration of acid in acid abomasal juice of 155 mmol/litre, volumes of abomasal juice secreted additionally of 55.6, 26.4, 49.6 and 44.3 ml/h could be calculated. The values were of the same order as the differences between the increases in PRD and the respective infusion rates of these intra-abomasal infusates. After intra-abomasal infusion of a $KHCO_3$ buffer 0.15 mol/litre (Section 4.5), PRD was increased in excess of IR. As could be deduced from the increases in PRTA and PRCl, more concentrated $KHCO_3$ buffers were stronger stimulators of abomasal secretion of acid, which was, however, not paralleled by an additional increase in PRD. Intra-abomasal infusion of β-alanine, aspartic acid, arginine and glycine did not affect acid secretion substantially. After infusion of these amino acids considerable effects on duodenal passage rate of digesta were not noticed either, except after arginine when PRD tended to decrease. After α-alanine, PRD tended to increase and after methionine a significant decrease was noticed (Section 4.6), but these responses were suggested to be caused indirectly by an increased, respectively decreased entry rate of abomasal digesta. When soya protein as such (promine D), or partly hydrolysed soya protein (promine DH), was infused into the abomasum (Section 4.7), after promine D infusion the increase in PRD (42.8 g/h) did not exceed IR (66.7 g/h). After promine DH, an additional increase in PRD of 28.4 g/h was noticed, which was less than an additional amount of abomasal juice secreted of about 68.7 ml/h, calculated from the average of the increases in PRTA and PRCl, and an acid concentration in acid abomasal juice of 155 mmol/litre. When volatile fatty acids and L-lactic acid were infused into the abomasum (Section 4.8), a slight stimulation of acid secretion was noticed, when compared with $KHCO_3$ 0.15 mol/litre infusion in the control experiments, coincident with tendencies of increased amounts of digesta leaving the abomasum. These increases in PRD did not de-

viate substantially from the amounts of acid abomasal juice secreted in addition, calculated as described before.

The coefficients of variation of those increases in PRD were high and therefore in general, on statistical grounds it was not possible to conclude if the PRD's measured differed significantly from the PRD's, calculated from the PRD's of the control experiments, the infusion rates of the intra-abomasal infusates and their stimulative effect on secretion of abomasal juice. Moreover, calculation of secretory rates of abomasal juice in the feeding trials (Chapter 3) from the amounts of chloride secreted in the abomasum yielded rates which exceeded those, computed from the differences in entry and emptying rates of abomasal digesta. Regarding this discrepancy, an efflux of water from the abomasal contents was suggested, which, under the present experimental conditions, was possibly affected by the continuous intra-abomasal infusions, resulting in lower duodenal passage rates of digesta than those expected. Therefore the conclusion to be drawn must be that the increase in duodenal passage rate of digesta after intra-abomasal infusion of a particular substance, corrected for the infusion rate of that substance, cannot be regarded as a reliable estimate of the stimulative effect of that particular substance on secretory activity of abomasal juice.

Since abomasal entry rate of digesta, however, was not affected in general by the continuous intra-abomasal infusions, the stimulative effect of these infusions on abomasal secretion of acid could be estimated from the increases in duodenal passage rate of total acid (PRTA) and of chloride (PRCl), within the limits of this method as discussed in Section 3.1. Moreover, in all intra-abomasal infusion experiments, but particularly in those experiments, in which soya protein was infused at two infusion rates (Section 4.3), and when $KHCO_3$ buffers were infused at different concentrations (Section 4.5), the abomasum was shown to secrete at less than its maximal rate under normal physiological conditions. After intra-abomasal infusion of the most concentrated $KHCO_3$ buffer, acid secretion was increased by about 66%, as calculated from the increase in PRTA. Although the relationship in these intra-abomasal infusion experiments between the increases in PRTA and PRCl and buffering capacity infused (IRBC) was non-linear (Fig. 10), even after the most concentrated $KHCO_3$ buffer the abomasum did not yet secrete at its maximal rate. From this evidence, the abomasum can be concluded to secrete certainly not at its maximal rate under normal physiological conditions, and hence regulation of abomasal secretion of acid can be studied by stimulation of abomasal secretion of acid after continuous intra-abomasal infusion of particular substances.

As estimated from the increases in PRTA and PRCl, abomasal secretion of acid was stimulated by continuous intra-abomasal infusion of proteins (Sections 4.3, 4.4 and 4.7). In what way stimulation of acid secretion by proteins is effectuated, was studied in Section 4.4. As demonstrated in Figure 8, abomasal secretion of acid was stimulated by promine D and gluten in excess of the buffering capacity of the protein preparations, infused into the abomasum at these infusion rates. After α-protein and casein significant differences between the stimulative effect on acid secretion and buffering capacity were not noticed. The duodenal pH, however, tended to be increased, which probably indicated that buffering capacity changes after intra-abomasal infusion, possibly due to a partial peptic hydrolysis. As promine D and gluten, however, were shown to stimulate abomasal secretion of acid in

excess of their in vitro buffering capacity, resulting in a duodenal pH, which hardly differed from the duodenal pH in the control experiments, we may conclude that stimulation of acid secretion by intra-abomasal protein infusions was probably not mediated only by protein buffering capacity, but also by other factors, such as protein amino acid composition. The selective effect of protein composition on the stimulation of abomasal secretion of acid is probably effectuated through a selective stimulation of the release of gastrin (Section 2.3.5). As shown in Table 18, amino acid composition of both soya proteins, α-protein and promine D, did not differ substantially. α-Protein, however, was crude crystalline, and promine D fine crystalline, and since the stimulating effect on acid secretion differed substantially, physical structure of the proteins entering the abomasum may be involved as well. As given in Table 16, the crude crystalline α-protein suspension had a lower buffering capacity (IRBC) than the fine crystalline promine D suspension and besides, the fine protein particles will be more readily degraded proteolytically, resulting in a higher production of lower molecular protein fragments and thus possibly in a stronger stimulation of the release of gastrin.

Buffering capacity of the intra-abomasal infusates was involved in the stimulative effect on abomasal secretion of acid. Intra-abomasal infusion of a phosphate buffer did result in an increased acid secretory activity, as estimated from the increases in PRTA and PRCl (Table 19). The same effect was noticed after intra-abomasal infusion of $KHCO_3$ buffers (Table 20). In comparison with the stimulatory effect of the phosphate buffer, intra-abomasal infusion of $KHCO_3$ buffers stimulated acid secretion less relative to IRBC. As shown in Figure 10, however, the stimulatory effect of the $KHCO_3$ buffers on abomasal secretion of acid was not proportional to IRBC. This probably explains why $KHCO_3$ buffers stimulated acid secretion proportionally less than the phosphate buffer, since the buffering capacity of the phosphate buffer was lower than with the $KHCO_3$ buffers (Table 16).

It is to be expected that inorganic buffers act on abomasal secretion of acid indirectly through their effect on the pH of abomasal contents, a higher pH being less inhibitory for the release of gastrin (Section 2.3.5) or a higher pH being less inhibitory for acid secretion by neurally mediated mechanisms (Leek & Harding, 1975; Konturek et al., 1976). The resulting abomasal juice secreted additionally was not equivalent to the respective buffering capacities infused. Probably, the stimulatory effect of the inorganic buffers was not enough to force the abomasal glands to bring the pH of the abomasal contents down to the control level. As can be seen from Figure 10, even after intra-abomasal infusion of $KHCO_3$ 0.60 mol/litre, when acid secretion was increased by about 66%, as calculated from the increase in PRTA, acid secretion did not seem to have reached its plateau. Extrapolation of the non-linear relation between acid secretion and IRBC indicated, however, a limitation of the abomasal secretory rate to exist, as is to be expected.

Amino acid composition of proteins was suggested to be involved in the stimulation of abomasal secretion of acid. From this supposition and from experimental results in non-ruminants (Section 2.3.5), amino acids were expected to stimulate acid secretion selectively. At the amounts applied in the present experiments, no stimulation of acid secretion could be noticed after infusion of β-alanine, glycine, aspartic acid and arginine (Table 21). Only after infusion of α-alanine, which has been stated to be a less potent gastrin releaser in dogs, acid secretion tended to be stimulated. Obviously, the effects of α-

alanine, β-alanine and glycine on acid secretion do not agree with the findings of Elwin (1974) in non-ruminants. In order to decide whether or not the regulation of the release of gastrin in ruminants is different from that in non-ruminants, a study on the direct relationship between intra-abomasal amino acid infusions and blood plasma gastrin level may be decisive (Chapter 5). The decrease in acid secretion after methionine infusion was most strikingly. In comparison with the control values, duodenal passage rate of digesta (PRD) and of dry matter (PRDM) were significantly decreased after intra-abomasal methionine infusion. The pH of duodenal contents after methionine infusion, however, tended to decrease, perhaps because methionine inhibits abomasal secretion of acid not in a direct way, but indirectly through a decreased abomasal entry rate of digesta. Methionine has been mentioned as an active releaser of cholecystokinin (Section 2.3.5), which hormone might be involved in the responses noticed after intra-abomasal methionine infusion.

The results of Elwin (1974), that among the various fractions of a protein hydrolysate the smaller molecular fractions were the more effective stimulators of gastric secretion, were confirmed in the present experiments. After intra-abomasal infusion of $KHCO_3$ buffers, it was concluded that acid secretion was stimulated proportionally less when buffering capacity of the infusate (IRBC) was higher. When infused, however, a soya protein suspension, partly hydrolysed in advance (promine DH), the stimulative effect on acid secretion in proportion to IRBC was higher, when compared with intra-abomasal infusion of soya protein as such (promine D), despite IRBC was higher when promine DH was infused (Table 22). These findings indicate that the smaller protein fragments are probably more active stimulators of acid secretion, possibly through a more potent stimulation of gastrin release.

Although in the feeding trials (Chapter 3), no relation could be demonstrated between abomasal secretion of acid and the forestomach fermentation metabolites, the volatile fatty acids, we studied the effect of volatile fatty acids and L-lactic acid on acid secretion after infusion into the abomasum. Proportionally to the amounts entering the abomasum under normal conditions, the amounts of volatile fatty acids and L-lactic acid applied were high. Regarding the effects of intra-abomasal infusion of these organic acids on the respective amounts leaving the abomasum, the amounts infused into the abomasum were for acetic, propionic, butyric and L-lactic acids about 1, 2, 5 and 26 times the physiological amounts entering the abomasum under the present experimental conditions. Even at these infusion rates acid secretion was not stimulated very substantially by the volatile fatty acids and L-lactic acid. This agrees with the results of Feeding Trial 2 (Table 10), in which no relation between ruminal volatile fatty acid concentrations and abomasal secretion of acid was found. It seems therefore that the physiological role of these organic acids in stimulating abomasal secretion of acid is of limited importance, except of their involvement in the buffering capacity of digesta entering the abomasum.

As demonstrated in Figures 9, 11, 13, 16 and 18, the increases in duodenal passage rate of total acid (PRTA) and of chloride (PRCl) after the various intra-abomasal infusions did not differ significantly from a 1:1 ratio. This ratio, however, differed from the regression coefficients calculated between PRCl and PRTA in the feeding trials, of 2.43, 2.25 and 1.58 in Feeding Trials 1, 2 and 3, respectively (Figures 3, 5 and 7). As is discussed in Section 2.3.8, composition of gastric juice changes with secretory rate. At a higher secretory rate chloride concentration was found to increase slightly, but hydrogen

ion concentration was more directly related to secretory rate. Whereas in monogastric animals gastric juice hydrogen ion concentration was low (20 mmol/litre) at a basal secretion rate, hydrogen ion concentration increased to about 150 mmol/litre under more stimulated conditions. In sheep, maximal hydrogen ion concentrations in abomasal juice collected from an innervated abomasal fundic pouch of 150 mmol/litre were reported (McLeay & Titchen, 1975). This non-linear ratio between hydrogen and chloride ion concentration with variable abomasal secretion rates is also consistent with the results of Hill (1968). Based on these findings, the ratio between PRCl and PRTA is expected to be higher at lower abomasal secretion rates, as could also be concluded when the regression coefficients of Figure 7 were compared with those of Figures 3 and 5. In these feeding trials, a higher regression coefficient was calculated, when the average abomasal secretion rate was lower. Since this ratio is expected to decrease when abomasal secretion rates increase, the ratio between the additional amounts of chloride and hydrogen ions secreted, as induced by the various intra-abomasal infusions, could approach equimolarity.

In the present experiments, abomasal secretion of acid was substantially increased by several compounds infused into the abomasum. In none of the experiments, however, stimulation of acid secretion resulted in a decreased duodenal pH. These results were consistent with the effects on duodenal pH found in the feeding trials (Chapter 3). Apparently, the stimulative effects were not strong enough to induce such a decrease in the duodenal pH. Besides, however, these intra-abomasal infusions caused a higher passage rate of digesta through the abomasum, which might have resulted in a decreased average retention time of abomasal digesta, and consequently in a less decreased pH of the digesta leaving the abomasum.

5 Gastrin in blood plasma as related to abomasal secretion of acid

5.1 INTRODUCTION

The hormonal aspects of gastric and abomasal secretion of acid were discussed extensively in Section 2.3.5. In this context, the different gastrins were described as active stimulators of acid secretion. In sheep, the presence of gastrin was found to be not only confined to the antral mucosa. Anderson et al. (1962) demonstrated that gastrin is present in the ovine fundic mucosa as well.

In monogastric animals, the release of gastrin was found to be stimulated by vagal or local cholinergic stimulation. Also digesta components are involved in the stimulation of gastrin release. Proteins and their degradation products were found to be active gastrin releasers. The release of gastrin is regulated by a potent feedback mechanism related to the degree of acidity within the antrum. In dogs, gastrin release was totally inhibited at pH 2. In sheep, acidification (pH 2) of antral pouches was also demonstrated to inhibit fundic pouch acid secretion (McLeay & Titchen, 1975).

Addition of inorganic buffering compounds to the ration (Section 3.4) induced an increased abomasal secretory activity of acid, which was less than the increase in buffering capacity of digesta flowing into the abomasum, resulting in an increased abomasal and thus duodenal pH (Table 8). When $KHCO_3$ buffers of various concentrations were infused directly into the abomasum (Section 4.5) a comparable response in acid secretion and pH of duodenal digesta was noticed. It is not likely that inorganic buffering compounds stimulate acid secretion in a highly specific way. It is more obvious that inorganic buffers stimulate abomasal secretion of acid in a less specific way through their effect on the pH of abomasal contents. The pH of abomasal contents possibly acts on abomasal secretion of acid through neural pathways too. Acid-sensitive mucosal receptors in the ovine abomasum were demonstrated by Harding & Leek (1973). Konturek et al. (1976) demonstrated that perfusion of denervated fundic pouches in dogs with L-amino acids stimulated acid secretion, the secretory response decreasing with the pH of the test solution (Section 2.3.4). Since, however, the release of gastrin is gradually inhibited at a lower pH of abomasal digesta, stimulation of abomasal secretion of acid by inorganic buffering compounds can also be effectuated by a less inhibited release of gastrin.

When protein content of the ration was increased higher abomasal secretory rates of acid were achieved (Chapter 3). Highly positive significant relationships were found between the amount of crude protein passing through the abomasum and abomasal secretory activity of acid (Tables 7, 12 and 15). Obviously, proteins act on acid secretion also on behalf of their buffering capacity, but probably also in a more specific way through a selective stimulation of gastrin release by protein breakdown products. In this context, the specific stimulative effect of different proteins (Section 4.4) on acid secretion was

suggested to be caused by different potencies of the respective protein degradation products on gastrin release. Protein degradation products were found to be the most active gastrin releasers (Section 2.3.5). After infusion into the abomasum of a soya protein suspension, which was partly hydrolysed in advance (Section 4.7), abomasal secretion of acid was more actively stimulated when compared with the reference soya protein suspension, not hydrolysed in advance, possibly through a more active stimulation of gastrin release. Also particular amino acids have been mentioned as active gastrin releasers. Elwin (1974) showed in dogs that β-alanine and glycine were active gastrin releasers, whereas α-alanine was less effective. The potencies of these respective amino acids, however, could not be confirmed in our intra-abomasal infusion experiments (Section 4.6). In these experiments, β-alanine and glycine were not found to affect acid secretion, whereas α-alanine tended to stimulate it.

In order to collect evidence for an involvement of gastrin in the regulation of abomasal secretion of acid in sheep, plasma gastrin concentration was studied as affected by intra-abomasal infusion of a soya protein suspension, of a $KHCO_3$ buffer and of solutions of α-alanine, β-alanine and glycine in $KHCO_3$.

5.2 METHODS

Animals. Plasma gastrin concentration was studied in Texel wethers, weighing 45-60 kg. An abomasal infusion tube was fitted into the abomasal fundus (Section 4.2) several weeks before the experiments, which were started after the sheep had been well accustomed to the experimental procedure. Sheep were kept individually in metabolism cages.

Rations. In these experiments, plasma gastrin was studied as affected by various intra-abomasal infusions. Rations were kept constant and consisted of 600 g hay and 300 g concentrates per day (Section 4.2). Sheep received equal portions twice a day at 8:00 and 16:00 h.

Experimental procedure. Intra-abomasal infusions were carried out continuously, starting about 40 h before blood sampling. About 16 h before the first blood sampling period, a catheter (E-Z catheter, Deseret Pharmaceutical Co, Sandy) was inserted into a jugular vein. The catheter was kept open by a continuous infusion of physiological saline at a rate of about 20 g/h. Per intra-abomasal infusate per sheep, blood samples were taken every hour, from 8:00 to 17:00 h, on three consecutive days. After clotting, samples were centrifuged (Christ UJ3 centrifuge, 2000 g), and the supernatant serum was pooled per sampling day and kept at -20 °C.

In the first series of experiments, a 10% soya protein suspension in saline, partly hydrolysed in advance (promine DH, Section 4.7), and a $KHCO_3$ buffer 0.30 mol/litre were infused continuously into the abomasum of three sheep, in comparison with a control (no intra-abomasal infusion) according to a 3 x 3 Latin square design.

In the second series of experiments, solutions 0.10 mol/litre of α-alanine, β-alanine and glycine in $KHCO_3$ 0.15 mol/litre were infused continuously into the abomasum of four sheep versus control infusions of $KHCO_3$ 0.15 mol/litre according to a 4 x 4 Latin square design.

Analyses. Buffering capacity (IRBC) of the intra-abomasal infusates was determined as described in Section 4.2.

Serum gastrin concentrations were determined radioimmunologically by Lamers and coworkers, using the method described by Lamers & van Tongeren (1975) and Stadil & Rehfeld (1973). The gastrin antibody was raised by Rehfeld in a rabbit against synthetic human gastrin I containing residues 2 through 17, conjugated to bovine albumin (Rehfeld et al., 1972). In human serum, gastrin-34 (big gastrin) and heptadecapeptide gastrin could be demonstrated using this antibody (Rehfeld et al., 1974). The antibody reacted with sulphated and non-sulphated gastrins with an almost equimolar potency. Cross-reactivity of the antibody with porcine cholecystokinin was negligible and absent with porcine secretin (Lamers, 1976).

The antibody reacted with ovine gastrin. No data on potencies of binding of the ovine gastrins with the antibody are available. Walsh (1975) stated that in general ovine gastrin exhibits a lower affinity for human gastrin antibodies, resulting in an underestimation of ovine blood plasma gastrin concentration. In the present experiments, however, we were not interested in blood plasma gastrin levels as such, but in the effect of certain abomasal digesta components on gastrin release, and thus in an increased or decreased blood plasma gastrin level. Such differences in plasma gastrin level may be underestimated as well, using this human gastrin antibody, but decreased or increased gastrin levels in blood plasma may be well detected.

Synthetic human gastrin I (Imperial Chemical Industries, Rotterdam) was iodinated with Na ^{125}I (Behring Werke, Marburg) according to the method described by Stadil & Rehfeld (1972). Synthetic human gastrin I was used as standard gastrin preparation. In a total volume of 2.5 ml, 250 µl serum was incubated with 2 ml of gastrin antiserum, properly diluted, and 1-3 pg ^{125}I-labelled synthetic human gastrin. All solutions were made up in veronal buffer 0.02 mol/litre pH 8.4, containing 30 µmol human albumin and 0.6 mmol thiomersal per litre. Incubation was carried out at 4 ^{o}C for 4 days. Free and bound hormone were separated by adsorption of free gastrin to anionic exchange resin (Amberlite resin CG-B4, Rohm and Haas Company, Philadelphia), followed by decantation of the supernatant. Both the resin bound fraction and the supernatant were counted in a gammaspectrometer (Baird Atomic MS 588). Control mixtures containing no antiserum were set up for all samples to measure aspecific binding of free gastrin. For human gastrin the sensitivity of the assay was less than 10 pg/ml serum.

Statistics. Data were analysed statistically according to the analysis of variance for a Latin square design (Snedecor & Cochran, 1962). Statistical comparisons of the data after the intra-abomasal infusions versus the respective control values were made using the two-tailed Student t test (* , $P < 0.05$; ** , $P < 0.01$; *** , $P < 0.001$). Standard deviations are indicated by SD.

5.3 RESULTS

Infusion rate of the respective infusates (IR) and of their buffering capacity (IRBC), and serum gastrin level after infusion of a 10% soya protein suspension and a $KHCO_3$ buffer

Table 25. Infusion rate of the infusates (IR) and of their buffering capacity (IRBC), and serum gastrin concentration, with infusion of a 10% soya protein (promine DH) suspension in saline and $KHCO_3$ 0.30 mol/litre into the abomasum. For significances (two-tailed Student t_{20}) see Methods (Section 5.2).

	IR g/h	IRBC mmol/h	Serum gastrin pg/ml
Control	-	-	99.9
Promine DH	63.3	12.93	138.8***
$KHCO_3$	60.5	18.04	133.8***
SD	0.3	0.06	3.9

Table 26. Infusion rate of the infusates (IR) and of their buffering capacity (IRBC), and serum gastrin concentration, with intra-abomasal infusion of solutions 0.10 mol/litre of α-alanine, β-alanine and glycine in $KHCO_3$ 0.15 mol/litre, in comparison with control infusion of $KHCO_3$ 0.15 mol/litre. For significances (two-tailed Student t_{38}) see Methods (Section 5.2).

	IR g/h	IRBC mmol/h	Serum gastrin pg/ml
Control	77.0	11.86	102.9
α-Alanine	75.8	13.11	97.4
β-Alanine	76.4	17.04	96.8
Glycine	77.3	13.53	102.2
SD	0.5	0.09	4.0

0.30 mol/litre into the abomasum are given in Table 25. Both intra-abomasal infusion of soya protein and of $KHCO_3$ increased significantly serum gastrin concentration. Infusion of the soya protein suspension tended to increase serum gastrin level in excess of $KHCO_3$, despite the buffering capacity infused was lower.

IR and IRBC and serum gastrin level after infusion of solutions 0.10 mol/litre of α-alanine, β-alanine and glycine into the abomasum are given in Table 26. Though buffering capacity of the amino acid infusates differed significantly from that of the control infusate of $KHCO_3$ 0.15 mol/litre, effects of these amino acids on serum gastrin level were not significant.

5.4 DISCUSSION

In Chapter 7, the stimulative effect of a $KHCO_3$ buffer 0.30 mol/litre on abomasal secretion of acid is compared with the stimulative effect of a 10% soya protein suspension in saline, not hydrolysed in advance. Both intra-abomasal infusates exhibited in these experiments an almost equal potency in stimulating acid secretion. In Section 4.7 a 10% soya protein suspension (promine D) as such, was compared with a 10% soya protein suspension (promine DH), partly hydrolysed in advance, regarding the respective stimulating effects on acid secretion. Promine DH was a more active stimulator of acid secretion. Therefore from the two experiments, we may conclude that a 10% promine DH suspension in saline is a more active stimulator of abomasal secretion of acid than $KHCO_3$ 0.30 mol/litre, when

infused into the abomasum at equal rates.

As indicated in Table 25, intra-abomasal infusion of a 10% promine DH suspension tended to stimulate gastrin release, and thus to increase serum gastrin level, in excess of $KHCO_3$ 0.30 mol/litre. This agrees with promine DH being a more active stimulator of abomasal secretion of acid than $KHCO_3$, despite buffering capacity infused was higher when $KHCO_3$ was infused into the abomasum. This finding leads to the conclusion that obviously the release of gastrin is stimulated by $KHCO_3$ in a less specific way through its effect on the pH of abomasal contents. Promine DH, however, will not only stimulate the release of gastrin through its buffering capacity, but also through a selective stimulation caused by its proteolytic degradation products. Apparently, part of the stimulative effects of both intra-abomasal infusates on abomasal secretion of acid is achieved by their stimulative effects on gastrin release.

Elwin (1974) showed in dogs that β-alanine and glycine were active gastrin releasers, whereas α-alanine was less effective, as measured by the acid output of vagally denervated fundic pouches after instillation of these amino acids into an antral pouch. In our intra-abomasal amino acid infusion experiments (Section 4.6), these effects could not be confirmed. Of the amino acids used, no stimulative effects on gastrin release could be demonstrated either (Table 26), despite buffering capacities differed, although less substantially than in the experiments cited in Table 25. That gastrin release in sheep under the present experimental conditions was not stimulated by these amino acids was in line with the results of the intra-abomasal infusion experiments (Section 4.6), but not with the findings of Elwin in dogs. In these experiments, however, amino acids were acutely infused into the antral pouch, and at concentrations (0.29-0.80 mol/litre), which were higher than in the present experiments. Besides, differences may exist between the regulation of gastrin release in sheep and dogs.

6 Duodenal pepsin activity as related to abomasal secretion of pepsinogen

6.1 INTRODUCTION

In the preceding chapters, abomasal secretory activity of acid was studied as influenced by ration composition (Chapter 3) and by intra-abomasal infusion of different substances (Chapter 4). The ruminant abomasum, however, also secretes pepsinogen by the chief cells of the fundic tubular glands, whereas in juice collected from a sheep innervated antral pouch pepsin activity was demonstrated by McLeay & Titchen (1975). In an earlier paper (McLeay & Titchen, 1974), these workers concluded the cephalic phase of abomasal secretion to be involved in the stimulation of abomasal secretion of pepsinogen in sheep. In the same experiments, pepsin output from an innervated fundic pouch was increased when sheep were offered higher amounts of lucerne chaff. With equal amounts of lucerne chaff or of a mixture of lucerne and wheaten chaff, the highest pepsin outputs from innervated fundic pouches were achieved when lucerne chaff was supplied. As lucerne chaff had a higher crude protein content than wheaten chaff, in these experiments abomasal pepsin output was positively related to protein content of the ration and probably to the amount of protein entering the abomasum.

In Section 2.3.9, gastric secretion of pepsinogen was discussed as affected by neural and hormonal mechanisms. Abomasal secretion of pepsinogen was shown to be increased by cholinergic stimulation. Under normal feeding conditions, however, little is known about abomasal secretion of pepsinogen, except of the results of McLeay & Titchen (1974).

Pepsin exhibits its highest activity under acid conditions. Since acidity of digesta in the proximal duodenum does not seem to differ from the acidity level of the abomasal contents (Weston, 1976), it is not likely that proximal duodenal pepsin activity differs from abomasal pepsin activity. Therefore pepsin activity was studied in duodenal digesta collected from the duodenal re-entrant cannulas in relation to ration composition and intra-abomasal infusion of proteins and inorganic buffers.

6.2 METHODS

Pepsin activity of duodenal digesta was determined in Feeding Trials 1 and 2 (Sections 3.3 and 3.4). The experimental lay out of these feeding trials is described in Section 3.2.

Duodenal digesta pepsin activity was also determined after intra-abomasal infusion of a 10% soya protein suspension in saline (Section 4.3), after intra-abomasal infusion of different proteins and of a phosphate buffer (Section 4.4), and after intra-abomasal infusion of $KHCO_3$ buffers of various concentrations (Section 4.5). The experimental lay out of these intra-abomasal infusion experiments is described in Section 4.2.

Inhibition of pepsin activity by proteins and by duodenal digesta was studied in ex-

periments in vitro. Remaining pepsin activity was determined in the supernatant solution (Christ UJ3 centrifuge, 2000 g). Duodenal pepsin activity, and remaining pepsin activity in the experiments in vitro were determined by the method of Baars (1962), as described in Section 3.2.

Statistical comparisons of the data were carried out as described in Sections 3.2 and 4.2, respectively.

6.3 RESULTS

When sheep were offered the three rations of Feeding Trial 1 (Table 1), duodenal passage rate of pepsin (PRPE) was highest with ration C, with the lowest content of crude protein and with the lowest duodenal passage rate of crude protein (PRCP). PRPE was lowest with ration B, with the highest content of crude protein causing the highest PRCP (Table 6).

With the sixteen rations of Feeding Trial 2 (Table 2), PRPE was significantly decreased after addition of soya protein to the basic ration (Table 8). After the rations containing maize starch, PRPE was significantly increased, whereas for the dual factor effects significantly positive and negative regression coefficients were calculated.

After continuous infusion into the abomasum of a 10% soya protein suspension in saline, PRPE showed a tendency to decrease at the lower infusion rate and was slightly decreased at the higher infusion rate (Table 17).

After continuous infusion into the abomasum of different proteins and of a phosphate buffer, PRPE was significantly decreased after intra-abomasal infusion of 5% suspensions in saline of α-protein, promine D, casein and gluten (Table 19). After intra-abomasal infusion of the phosphate buffer, no effect on PRPE was noticed, whereas intra-abomasal infusion of a 2.5% solution in saline of gelatin resulted in a significantly increased PRPE.

When continuously infused into the abomasum $KHCO_3$ buffers of 0.15, 0.30, 0.45 and 0.60 mol/litre, PRPE tended to be increased after infusion of the lower concentrated buffers. After $KHCO_3$ 0.60 mol/litre PRPE showed a slight increase (Table 20).

In conclusion, it seems that inorganic buffers slightly stimulate pepsinogen secretion and that pepsinogen secretion is depressed when protein content of abomasal digesta is increased. As will appear, however, from the experimental results of the effects in vitro of proteins on pepsin activity, no conclusions may be drawn from duodenal pepsin activity about abomasal secretion of pepsinogen.

6.4 INHIBITION IN VITRO OF PEPSIN ACTIVITY BY PROTEINS AND DUODENAL DIGESTA

After addition to a pepsin solution 0.06 mg/ml in HCl (pH 3), 2.5 mg/ml of the proteins infused into the abomasum as described in Section 4.4, and without preliminary incubation, remaining pepsin activity was decreased 20, 16, 16, 13 and 27%, respectively, for α-protein, promine D, casein, gelatin and gluten. Inhibition of pepsin activity, however, was not only confined to these proteins, but was also caused by duodenal digesta. When pepsin concentration of duodenal digesta (crude protein content 1.10%) was increased by addition of porcine pepsin 0.04 mg/ml (Merck, Darmstadt; 35000 U/g), an inhibition of pepsin activity of about 90% was calculated.

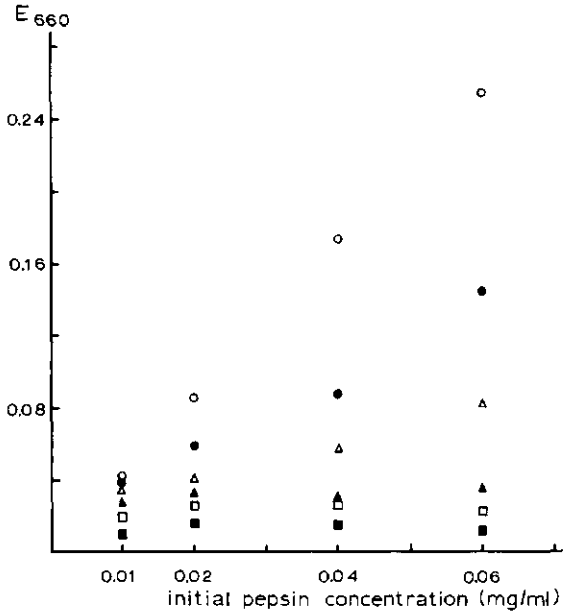

Fig. 19. Remaining pepsin activity (E_{660}) after preliminary incubation for 60 min (pH 3, 40 °C) with various promine D concentrations (○, 0%; ●, 0.05%; △, 0.15%; ▲, 0.25%; □, 0.50%; ■, 0.75%).

When inhibition of pepsin activity by the different proteins mentioned was studied at various protein concentrations and pepsin activities, and preliminary incubation for 60 min (pH 3, 40 °C), the remaining pepsin activity was lower at higher protein concentrations. Inhibition of pepsin activity was also more distinct at higher initial pepsin activities, as examplified in Figure 19 for promine D. When these inhibitions of pepsin activity found were compared with the inhibitions calculated previously, when no preliminary incubation at 40 °C was carried out (16% for promine D), percentages of inhibition were increased after preliminary incubation and thus coincident with a partial hydrolysis of the protein. At higher promine D concentrations, pepsin activity was almost completely inhibited at all initial pepsin concentrations. At lower promine D concentrations, inhibition tended to increase with the initial pepsin concentration, probably indicating that inhibiting factors were released at a higher concentration when the initial pepsin concentration was higher.

Such a pattern, as indicated in Figure 19, was also noticed when the other proteins (Section 4.4) were preliminarily incubated under comparable conditions, suggesting that the factor which is released is not a specific pepsin inhibitor. Inhibition of pepsin activity was therefore probably caused by normal products of protein hydrolysis.

6.5 DISCUSSION

It is obvious that no conclusions may be drawn regarding abomasal secretory activity of pepsinogen from pepsin activity of digesta leaving the abomasum. As was concluded in Section 6.4, pepsin activity was inhibited by addition of proteins. After preliminary incubation of these proteins with pepsin inhibition increased. The strongest inhibition was achieved when protein concentration and initial pepsin activity were highest. This suggests that a pepsin inhibiting factor was released during protein hydrolysis, and since this inhibiting activity was not confined to a single protein, pepsin activity was probably inhibited by the normal products of protein peptic hydrolysis.

When soya protein was hydrolysed in vitro with pepsin, Steinhart & Kirchgessner (1973) found no linear relation between initial pepsin activity and rate of proteolysis. They concluded that when the ratio of pepsin to substrate was increased, pepsin autolysis was increased, resulting in a decreased pepsin activity. On the assumption, however, that at a pepsin substrate ratio of 1:50 autolysis of pepsin may be neglected, they found at a pepsin substrate ratio of 1:10 a rate of hydrolysis of about 25% of the expected rate of hydrolysis, when no inhibition or autolysis of pepsin had occurred. When pepsin activity was increased further, they were hardly able to demonstrate an increased rate of protein hydrolysis. In these experiments, substrate availability was probably not limiting either, regarding the amount of protein, which was not dialysable after an 8-h incubation period (pH 2.2, 37 ^{o}C).

In the experiments of Steinhart & Kirchgessner, at an enzyme substrate ratio of 1:10 the initial concentrations of pepsin and soya protein were 0.5 mg/ml and 5 mg/ml, respectively. Determann et al. (1969) incubated a higher concentrated pepsin solution and without addition of a substrate at room temperature (14.3 mg/ml, pH 2). They found that even after an incubation period of 10 weeks less than 70% of pepsin was degraded by autolysis and that less than 50% of the initial pepsin activity was lost. Therefore the findings of Steinhart & Kirchgessner (1973), that rate of proteolysis did not increase linearly with initial pepsin activity, cannot be explained by an increased autolysis of pepsin when initial pepsin activity was higher. A more reliable explanation is that the inhibition of pepsin activity was increased at higher concentrations of protein degradation products. That pepsin activity is indeed inhibited by protein degradation products was also demonstrated by Determann et al. (1969). When pepsin was incubated at room temperature (14.3 mg/ml, pH 2) after 10 weeks about 40% of the initial pepsin activity was lost. When, however, pepsin (7 mg/ml, pH 2) was incubated at room temperature, and pepsin degradation products, released during incubation, were removed continuously by dialysis, after a shorter period of incubation (22 days), only 15% of the initial pepsin activity was left. Moreupon, products of pepsin autolysis were found to inhibit peptic degradation of hemoglobin. Inhibition of peptic hemoglobin degradation was also found after addition of the totally hydrolysed products of pepsin autolysis. These results indicate that pepsin activity was inhibited by protein degradation products and apparently also by particular amino acids. Of phenylalanine and tyrosine, however, Determann et al. (1969) were not able to demonstrate a pepsin activity inhibiting potency.

Studying peptic hydrolysis of N-acetyl-L-phenylalanyl-L-phenylalanyl-glycine, it was

found that N-acetyl-L-phenylalanine inhibited pepsin activity non-competitively but the corresponding D-phenylalanyl derivative competitively at pH 2.1 (Kitson & Knowles, 1971). At pH 4.3, however, the N-acetyl derivatives of L- and D-phenylalanine were found to exhibit both a competitive inhibitory activity (Kitson & Knowles, 1971a), the inhibitor constant (K_i) not differing substantially between the corresponding derivatives of both stereoisomers. The pH dependence of binding of the L-derivative with the enzyme was suggested to correspond to deprotonation of the inhibitor at a higher pH.

Fruton (1971) concluded that effective binding of an inhibitor or substrate to the active site of pepsin requires the presence of two adjacent hydrophobic groups. Of L-amino acid derivatives at pH 4 and 37 ^{o}C, inhibitor constants (K_i) for Phe-O-Methyl, Phe-O-Ethyl, Phe-Phe-O-Methyl, Gly-O-Methyl, and Gly-Gly-Phe-O-Methyl of 22, 10, 0.25, 100, and 25 mmol/litre were calculated, respectively.

Breukink (1973) found a decreasing pepsin activity in bovine abomasal digesta after addition of increasing amounts of concentrates to a basic ration of hay. He concluded that concentrates induced a higher abomasal passage rate of digesta resulting in a higher dilution rate of pepsin. Although no analyses of the rations are available, a more reliable explanation may be an increased inhibition of pepsin activity, induced by an increased abomasal digesta protein content after addition of increasing amounts of concentrates to the basic hay ration. Besides, a decreased abomasal pepsin activity in these experiments was noticed when changing the ration from a hay and concentrates ration to a protein rich ration of meadow grass.

In this context, the lowest duodenal pepsin activity found with the ration with the highest protein content in Feeding Trial 1 (Table 6) can also be explained. The same effect was found after addition of soya protein to the rations in Feeding Trial 2 (Table 8). After intra-abomasal infusion of different proteins, resulting in an increased protein content of abomasal digesta, in general significant decreases of duodenal pepsin activity were found. After infusion of $KHCO_3$ buffers at various concentrations into the abomasum, duodenal pepsin activity was found to increase, which was probably caused by a decreased protein content of abomasal digesta.

Regarding these experimental results in vivo and in vitro, we must conclude that abomasal secretion of pepsinogen cannot be studied by determining duodenal pepsin activity. In order to study abomasal secretion of pepsinogen, we have to exclude these pepsin inhibiting factors. The inhibiting protein degradation products should be no longer involved when collected pure abomasal juice from abomasal pouches, and possibly they can also be excluded by dialysis of duodenal digesta, in advance of pepsin determination.

7 Blood flow in the abomasal mucosa as related to abomasal secretion of acid

7.1 INTRODUCTION

As discussed previously, regulation of blood flow in the gastric mucosa has been proposed to be related to gastric secretory activity, although this supposed relationship is open to serious doubt (Section 2.4). To study this relationship in sheep, we carried out both a feeding trial and an intra-abomasal infusion experiment. In Feeding Trial 3 (Section 3.5), per sheep and per ration one of the five sampling periods was randomly chosen to study blood flow in the abomasal mucosa with the aminopyrine clearance technique, as described in Section 2.4. Abomasal aminopyrine clearance was also studied after intra-abomasal infusion of a 10% soya protein suspension in saline and of a $KHCO_3$ buffer 0.30 mol/litre.

Determination of blood flow in the gastric mucosa by the clearance of aminopryine is based upon the selective permeability of lipoidal membranes to undissociated compounds. Since there is a sizable pH gradient from plasma to abomasal or gastric juice, weakly basic drugs with a suitable pK_a, such as aminopyrine (pK_a = 5), are transported from plasma into gastric juice, where the ionized molecules are trapped. Therefore the aminopyrine method can be used to estimate blood flow in the gastric or abomasal mucosa, as pointed out in Section 2.4.

Archibald et al. (1975) compared in dogs blood flow in the gastric mucosa as determined by aminopyrine clearance and by γ-labelled microspheres. At periods of basal secretion the 'aminopyrine' mucosal blood flow was about 50% of the 'γ-labelled microspheres' mucosal blood flow, indicating that under these conditions blood flow in the gastric mucosa was probably underestimated by the aminopyrine clearance method. After stimulation of gastric secretion of acid by a continuous intravenous histamine infusion, however, a close agreement was found between both methods, suggesting that during periods with active secretion, blood flow in the gastric mucosa as determined by the aminopyrine clearance method was more reliably estimated. This disadvantage of the aminopyrine clearance method, in the sense that blood flow in the gastric mucosa may be underestimated during periods of less active secretion, is probably of less importance in studying the relationship between blood flow in the abomasal mucosa and acid secretion in ruminants, since abomasal secretion of acid is continuously induced by the fairly continuous passage of digesta from the forestomachs into the abomasum (Section 2.3.3).

Harper et al. (1968) and Curwain (1972) found that equilibration of aminopyrine between erythrocyte cytoplasm and physiological saline was reached within 1 min. This suggests that aminopyrine not only in plasma but also in the erythrocyte cytoplasm will be cleared almost quantitatively.

7.2 METHODS

Animals. As in the previous experiments Texel wethers, weighing 45-60 kg, were used. Re-entrant cannulas were fitted into the proximal duodenum and an abomasal infusion tube into the abomasal fundus (Sections 3.2 and 4.2). Surgery was performed several weeks before the start of the experiments. Sheep were kept individually in metabolism cages and care was taken to start the experiments, after the sheep had been well trained.

Rations. Composition of the rations of Feeding Trial 3 is given in Table 4. Rations were offered to the sheep in 4 equal portions at 7:00, 9:00, 17:00 and 19:00 h.

When abomasal secretion of acid was stimulated by intra-abomasal infusion of a soya protein suspension and of a $KHCO_3$ buffer, sheep were fed as described in Section 4.2.

Experimental procedure. In the feeding trial, duodenal digesta were collected and sampled for 12-h periods (7:00-19:00 h). Ten percent samples were removed and replaced by donor digesta collected from the same sheep one week in advance and kept at -20 ^{o}C. Samples were pooled per 3-h period and stored at -20 ^{o}C (Section 3.2).

In the intra-abomasal infusion experiment, a 10% suspension of soya protein (promine D) in saline and a $KHCO_3$ buffer 0.30 mol/litre were continuously infused into the abomasum, in comparison with a control (no intra-abomasal infusion), according to a 3 x 3 Latin square design. Per intra-abomasal infusate and when no intra-abomasal infusion was carried out, duodenal digesta were collected for three 8-h periods (8:00-16:00 h) on three consecutive days. Ten percent samples were removed and not replaced by donor digesta. Samples were pooled per sampling period and stored at -20 ^{o}C. Intra-abomasal infusions were started about 40 h in advance of the first sampling period (Section 4.2).

About 11 h before the sampling period in the feeding trial, and about 16 h in advance of the first sampling period in the intra-abomasal infusion experiment, catheters (E-Z catheter, Deseret Pharmaceutical Co, Sandy) were inserted into both jugular veins. Through one of these catheters a 0.5% solution of aminopyrine in physiological saline was infused continuously at a rate of about 20 g/h. The other catheter, which was used to draw blood samples, was kept open by a continuous infusion of physiological saline at the same infusion rate. Simultaneously with the duodenal sampling periods, jugular blood samples were drawn from this catheter every hour, starting at 7:00 h in the feeding trial and at 8:00 h in the intra-abomasal infusion experiment. Blood samples were heparinized and the supernatant plasma (Christ UJ3 centrifuge, 2000 g) was pooled per 3-h period in the feeding trial and per sampling period of 8 h in the intra-abomasal infusion experiment. Pooled plasma samples were kept at -20 ^{o}C.

Analyses of rations. Samples of the rations of Feeding Trial 3 were analysed as described in Section 3.2. Composition of these rations is given in Table 4.

Analyses of abomasal infusates. Samples of the 10% soya protein suspension in saline and of the $KHCO_3$ buffer 0.30 mol/litre were analysed as described in Section 4.2. For the soya protein suspension and for the $KHCO_3$ buffer 0.30 mol/litre, average infusion rates

(IR) of 64.9 and 66.9 g/h (SD 0.5) were calculated, respectively. Infusion rates of buffering capacity (IRBC) were 9.88 and 20.52 mmol/h (SD 0.12), infusion rates of dry matter (IRDM) 6.39 and 2.01 g/h (SD 0.04), and osmotic pressures (OPI) 361.7 and 510.3 mosm/kg (SD 0.6), respectively. For the soya protein suspension infusion rates of crude protein (IRCP) of 5.48 g/h (SD 0.03) and of chloride (IRCl) of 7.76 mmol/h (SD 0.07) were calculated.

Analyses of digesta. Duodenal passage rate of digesta (PRD) was measured during the experiments. The average per hour was calculated for each sampling period of 8 or 12 h. Duodenal passage rates of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), and of chloride (PRCl) were determined as described in Section 3.2.
Corrections of PRTA and PRCl were carried out as described in Section 4.2. The duodenal pH was measured before total acid concentration was determined. The estimation of duodenal osmotic pressure (OPD) was described in Section 4.2.

Analyses of aminopyrine. Aminopyrine concentration in plasma (Christ UJ3 centrifuge, 2000 g) and in duodenal supernatant samples (MSE 65 ultracentrifuge, 70000 g) were estimated according to the method of Brodie & Axelrod (1950). After alkalinization of the samples, aminopyrine was extracted in 1,2-dichloro-ethylene and the organic solvent solution washed with sodiumborate 0.2 mol/litre. Finally, aminopyrine was transferred into HCl 0.1 mol/litre and estimated at 260 nm (Beckman DU spectrophotometer), against the extinctions of standard solutions run through the same procedure. Concentration values of aminopyrine were not corrected for the aminopyrine metabolite 4-amino-antipyrine, since the average ratios between duodenal and plasma concentrations of aminopyrine and 4-amino-antipyrine, respectively, did not differ substantially, as was also reported by Jacobson et al. (1966), who found ratios between aminopyrine and its metabolite in dog gastric juice, which did not differ either from those in blood plasma. In 7 determinations, recoveries of aminopyrine in duodenal digesta and plasma were 94.4 and 93.8%, respectively.

Blood flow in the abomasal mucosa (BF) was calculated by dividing the amount of aminopyrine passing through the proximal duodenum (PRD x concentration of aminopyrine x (100 - percentage of dry matter)/100) by plasma aminopyrine concentration. The ratio (R) was calculated by dividing duodenal aminopyrine concentration by plasma aminopyrine concentration.

Statistics. Data were analysed statistically according to the analysis of variance for a Latin square design (Snedecor & Cochran, 1962). Statistical comparisons of the data, as affected by ration composition, and after both intra-abomasal infusates versus the control duodenal data were made using the two-tailed Student t test (* , $P < 0.05$; ** , $P < 0.01$; *** , $P < 0.001$). Standard deviations are indicated by SD.

7.3 RESULTS

Amount and composition of duodenal digesta as affected by the rations of Feeding Trial 3, and calculated from the sampling periods in which aminopyrine was infused intravenously are given in Table 27. The data on blood flow in the abomasal mucosa and on the

Table 27. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), and of chloride (PRCl), the pH of duodenal contents, blood flow in the abomasal mucosa (BF), and the ratio (R) between duodenal and plasma aminopyrine concentration with the rations of Feeding Trial 3 (Table 4). Significant differences (two-tailed Student t_2, for critical levels see Section 7.2) are indicated by ✱ (A-B), ∇ (B-C), and † (A-C).

		Ration			SD
		A grass	B hay and grass	C hay and semisynthetics	
PRD	g/h	645.6	632.2$^{\nabla}$	698.3†	6.5
PRDM	g/h	20.93	24.25	26.82†	0.57
PRTA	mmol/h	38.15	32.91	33.07	0.88
pH		2.83	2.81$_{\nabla\nabla}$	2.91$_{\dagger\dagger}$	0.02
PRCP	g/h	7.46**	6.02$^{\nabla}$	6.56†	0.03
PRCl	mmol/h	70.57*	65.87$^{\nabla}$	70.65	0.56
BF	litre/h	9.83	11.11	9.33	1.34
R		15.86	18.90	13.39	1.47

ratio between duodenal and plasma aminopyrine concentration are presented in the same table. The 'duodenal digesta' data were commented in Section 3.5. As a consequence of the limited number of sampling periods, few degrees of freedom were left, and significant differences regarding abomasal secretory activity of acid as indicated by duodenal passage rate of total acid (PRTA) and of chloride (PRCl) were hardly noticeable. No significant differences in blood flow in the abomasal mucosa (BF) were detectable either, possibly due to the rather high coefficients of variation of these values. Tendencies did not parallel the slight differences in abomasal secretory activity of acid.

Amount and composition of duodenal digesta as affected by intra-abomasal infusion of the soya protein suspension and of the $KHCO_3$ buffer are given in Table 28. After both intra-abomasal infusates, the increases in duodenal passage rate of digesta (PRD) were higher than the respective infusion rates (IR) as given in Section 7.2. The increases in PRD were discussed in Section 4.9. The increases in duodenal passage rate of dry matter (PRDM) and of crude protein (PRCP) did not differ significantly from the amounts of dry matter and crude protein infused, respectively (Section 7.2). Like with the experiments of Chapter 4, abomasal digesta entry rates were obviously not affected by these continuous intra-abomasal infusions. As in these intra-abomasal infusion experiments, stimulation of abomasal secretion of acid by the soya protein infusion, as indicated by the respective increases in duodenal passage rate of total acid (PRTA) and of chloride (PRCl), almost equalled buffering capacity infused (IRBC, Section 7.2). Nevertheless, the pH of duodenal digesta was significantly increased, suggesting that the buffering capacity of the soya protein suspension was increased by a partial peptic hydrolysis. After intra-abomasal infusion of $KHCO_3$, the increases in PRTA and PRCl were less than IRBC (Section 7.2) resulting in a significantly increased duodenal pH. As was to be expected after intra-abomasal infusion of $KHCO_3$ 0.30 mol/litre, duodenal osmotic pressure (OPD) showed a significant decrease. From this decrease in OPD, PRTA was corrected as described in Section 4.2. Using the same formula, a theoretical osmotic pressure (OPT) after soya protein infusion of 257.1 mosm/kg could be calculated, which was lower than the value measured. This same difference was found and

Table 28. Duodenal passage rate of digesta (PRD), of dry matter (PRDM), of total acid (PRTA), of crude protein (PRCP), and of chloride (PRCl), the pH and osmotic pressure (OPD) of duodenal contents, blood flow in the abomasal mucosa (BF) and the ratio (R) between duodenal and plasma aminopyrine concentration, as affected by continuous intra-abomasal infusion of a 10% soya protein (promine D) suspension in saline and of $KHCO_3$ 0.30 mol/litre. For significances (two-tailed Student t_{20}) see Methods (Section 7.2).

		Control	Soya protein	$KHCO_3$	SD
PRD	g/h	559.1	656.7**	697.3***	14.8
PRDM	g/h	19.77	26.50***	21.14	0.53
PRTA	mmol/h	31.54	39.69***	41.48***	0.94
pH		2.82	2.99***	3.13***	0.02
PRCP	g/h	5.30	10.84***	5.16	0.14
PRCl	mmol/h	63.80	73.39**	73.57**	1.51
OPD	mosm/kg	245.6	267.9***	239.4**	1.0
BF	litre/h	11.17	9.87	13.37	1.03
R		20.85	16.27	20.45	1.63

discussed in Section 4.7.

The respective responses of abomasal secretory activity of acid after intra-abomasal infusion of the soya protein suspension and of the $KHCO_3$ buffer did not differ substantially from the effects described in Chapter 4. Also in this intra-abomasal infusion experiment, the respective increases in PRTA and PRCl did not differ significantly from equimolarity.

From the average of the increases in PRTA and PRCl in comparison with PRTA of the control experiments, both of the intra-abomasal infusates stimulated acid secretion by about 30%. These secretory responses were not followed by simultaneous significant increases in mucosal blood flow (BF).

7.4 DISCUSSION

With the rations of Feeding Trial 3, no substantial differences in abomasal secretory activity of acid and in mucosal blood flow were induced (Table 27). Therefore from this experiment we were not able to conclude if abomasal secretion of acid and blood flow in the abomasal mucosa (BF) were related directly. After both of the intra-abomasal infusions, however, abomasal acid secretory activity was increased by about 30%, and also in this experiment no significant effect on BF was detectable. After intra-abomasal infusion of soya protein BF tended even to decrease. These findings indicated that under the present experimental conditions an increase in acid secretion was not followed or preceded intimately by an increased mucosal blood flow. This implies that acid secretion was not limited by abomasal mucosal circulation, and that abomasal secretory activity of acid and blood flow in the abomasal mucosa were probably not related directly, but regulated through separate pathways.

These findings disagree with the positive relationships between gastric secretion of acid and blood flow in the gastric mucosa, cited in Section 2.4. They agree, however, with the results of Jacobson & Chang (1969), who found in dogs that after stimulation of gastric secretion of acid at comparable secretory rates of acid after intravenous infusion of histamine or porcine gastrin, a significantly higher mucosal blood flow was observed

after histamine, indicating that mucosal blood flow and acid secretion were not necessarily related directly. They also agree with the results of Domanig et al. (1966), who showed acid secretion in dogs to be stimulated in excess of mucosal blood flow after a prolonged intravenous histamine infusion, resulting in a decreased gastric venous oxygen saturation. In the present experiments, however, differences in abomasal secretory activity of acid were almost lacking in the feeding trial, while in the intra-abomasal infusion experiment stimulation of abomasal secretion of acid was possibly not pronounced enough to induce simultaneous increases in mucosal blood flow. Therefore our suggestion that a direct relationship between blood flow in the abomasal mucosa and abomasal secretory activity of acid is obviously absent, needs confirmation when secretory activity of acid is more actively stimulated.

In experiments with dogs, Jacobson et al. (1966) found ratios (R) of blood flow in the gastric mucosa to gastric secretory rate, or gastric juice to plasma aminopyrine concentration, of up to 40 during active or maximal secretion. In the present experiments, we were not able to determine this ratio, since no abomasal juice but digesta leaving the abomasum were collected. Abomasal acid secreting glands are continuously active (Section 2.3.3), and so if a ratio between abomasal juice and plasma aminopyrine concentration could have been determined, we might have expected a comparable one, as published by Jacobson et al. In our experiments, we found ratios of aminopyrine proximal duodenal (abomasal) digesta to plasma concentration of up to 20. These ratios were expected to be lower as a result of the dilution of abomasal juice by digesta present in the abomasum. Assuming, however, that R is about 40 during continuous abomasal secretion of acid, digesta leaving the abomasum should consist for about 50% of abomasal juice, which fits well with the data on secretory rate of abomasal juice calculated in Chapter 3.

8 Abomasal motility

8.1 INTRODUCTION

As was discussed in Section 2.5.4, propulsion of abomasal digesta into the duodenum is proposed to be effectuated by the contractile activity of the abomasum. Since generally no vigorous contractions are noticed in the abomasal fundus (Section 2.5.3), abomasal antral and pyloric contractile activity are expected to be primarily responsible for propulsion of abomasal digesta into the duodenum.

Abomasal inflow rate of digesta and composition of abomasal digesta are related to abomasal secretory activity, and thus to amount and composition of digesta leaving the abomasum, as has been demonstrated in Chapters 3 and 4. Consequently, changes in amount and composition of digesta entering the abomasum may modify not only abomasal secretory activity, but abomasal contractile activity as well.

In order to study the proposed relationship between abomasal motility and abomasal outflow rate of digesta, experiments were carried out, in which contraction activity of the antral part and of the pylorus was recorded under varying conditions.

8.2 METHODS

The experiments were carried out with Texel wethers, weighing 45-60 kg. As described previously (Section 4.2), a silicone abomasal infusion tube was inserted into the abomasal fundus. Strain gauges (Hellige, Freiburg, Type F102/F102S, resistance 120 Ohm) were fixated on a small bended metal sheet and incorporated in silicone paste (Possehl Eisen und Stahl, Lübeck) (Fig. 1). They were sutured on the serosal side of the abomasum in such a way that deformation of the strain gauges was induced by contraction of the circular muscle layer. Strain gauge resistance variability was measured against three reference resistances with a Wheatstone bridge. After amplification (SE Labs Ltd, Feltham, Transducer-Converter SE 905/2/1, Amplifier SE 423/1E) the signals were recorded on paper (SE Labs Ltd, UV Recorder SE 3006/DL). Per time period, frequency (counting) and intensity (amplitude) of contraction activity were counted. The number of contractions per time period is indicated as N and the average amplitude, calculated as the sum of the amplitudes divided by the number of contractions, is indicated as A.

Surgery was performed about three weeks before the start of the experiments. Experiments were started after sheep had well recovered and had been well accustomed to the experimental procedure. During the recordings any disturbance of the animals was prevented as good as possible. Sheep were kept individually in metabolism cages.

In the first series of experiments (Section 8.3), abomasal antral (3 cm proximally to the pylorus) and pyloric contractile activity were recorded for 14 recording periods

of 12 h (18:30-6:30 h). Motility recordings were carried out during night hours with one sheep. In advance, the experimental sheep, in companionship with another sheep, had been accustomed to a 24-h light regime, in order to limit possible differences between day and night. The ration consisted of 600 g hay and 300 g concentrates per day, during the first 8 recording periods, and of 720 g hay and 360 g concentrates per day, during the last 6 recording periods (Section 4.2). The sheep was offered equal portions twice a day at 8:00 and 17:00 h. No intra-abomasal infusions were carried out.

In the second series of experiments (Section 8.4), abomasal antral and pyloric contractile activity were recorded from the same sheep under the same experimental conditions. These recordings were carried out alternately with the recordings of the first series. The ration consisted of 600 g hay and 300 g concentrates per day (Section 4.2). Starting about 48 h in advance of the first of 4 consecutive recording periods of 12 h (18:30-6:30 h), a 10% soya protein (promine D) suspension in saline (77.6 g/h, SD 3.0) was infused continuously into the abomasum. Abomasal antral and pyloric motility were compared with 6 representative control recording periods (Section 8.3).

In the third series of experiments (Section 8.5), abomasal motility was recorded with two sheep. The ration consisted of 600 g hay and 300 g concentrates per day (Section 4.2). Both experimental sheep were offered equal portions twice a day at 7:30 and 15:30 h. About 2 weeks after surgery, 2 of the strain gauges failed, in one of the sheep the antral (3 cm proximally to the pylorus), and in the other the pyloric strain gauge. Therefore in these experiments, antral motility was recorded from one sheep and pyloric motility from the other. Antral and pyloric contractile activity were recorded in dependence of discontinuous intra-abomasal infusions (Table 29), which were started at the same time as the recording periods. On 4 consecutive days, abomasal antral and pyloric motility were recorded for two 5.5-h recording periods (9:00-14:30 and 17:00-22:30 h). Intra-abomasal infusions, in comparison with the respective controls, were randomized over the sheep per day according to a 2 x 2 Latin square design repeated once in the inverse sequence. Data of these experiments were analysed statistically according to the analysis of variance for a Latin square design (Snedecor & Cochran, 1962). Statistical comparisons were made using the two-tailed

Table 29. Infusion rate (IR_1) of the substances, a 10% soya protein (promine D) suspension in saline, $KHCO_3$ 0.30 mol/litre, solutions 0.10 mol/litre of α-alanine, β-alanine, glycine and methionine, solutions 0.083 mol/litre of acetic and butyric acids, and a suspension 0.083 mol/litre of stearic acid in $KHCO_3$ 0.15 mol/litre, infused into the abomasum in the experiments of Section 8.5, in comparison with the amount (IR_2) of $KHCO_3$ 0.15 mol/litre applied in the control experiments.

	IR_1 g/h	IR_2 g/h	SD
Promine D	102.0	-	1.9
$KHCO_3$	94.9	-	0.3
α-Alanine	99.5	99.5	0.4
β-Alanine	100.2	100.5	0.6
Glycine	102.3	101.4	0.4
Methionine	101.6	100.0	0.4
Acetic acid	100.2	96.3	1.8
Butyric acid	96.8	99.9	1.6
Stearic acid	98.9	100.1	0.6

Student t_9 test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Standard deviations are indicated by SD, unless otherwise stated.

8.3 ANTRAL AND PYLORIC MOTILITY

Smooth muscular slow wave electrical activity was proposed (Section 2.5.2) not to trigger smooth muscular contractions directly, but to be involved in rhythmicity control, regulating probability of spike discharge and consequently contractile activity. They spread distally and therefore a contraction, if present, will be recorded first on the antral strain gauge, as illustrated in Figure 20. Time interval between the antral and pyloric contraction differed, but may be as high as 0.05 min, which indicated a propagation velocity of about 1 cm/sec. Rhythmicity of the contractions was found to be rather regular in time, but not contraction intensity. Variability in contraction intensity was highest on the antral strain gauge.

In general, an antral contraction was followed by contraction of the pylorus, although in some cases pyloric contractions were recorded when no antral contractions could be detected. From time to time an antral contraction was noticed, which was not propagated to the pylorus.

As shown in Figure 20, periods of contractile activity were alternated with longer or shorter periods of almost inactivity. Especially during these periods of inactivity, frequency of pyloric contractile activity was higher than of the antrum.

During feeding, both the abomasal antrum and pylorus contracted actively as demon-

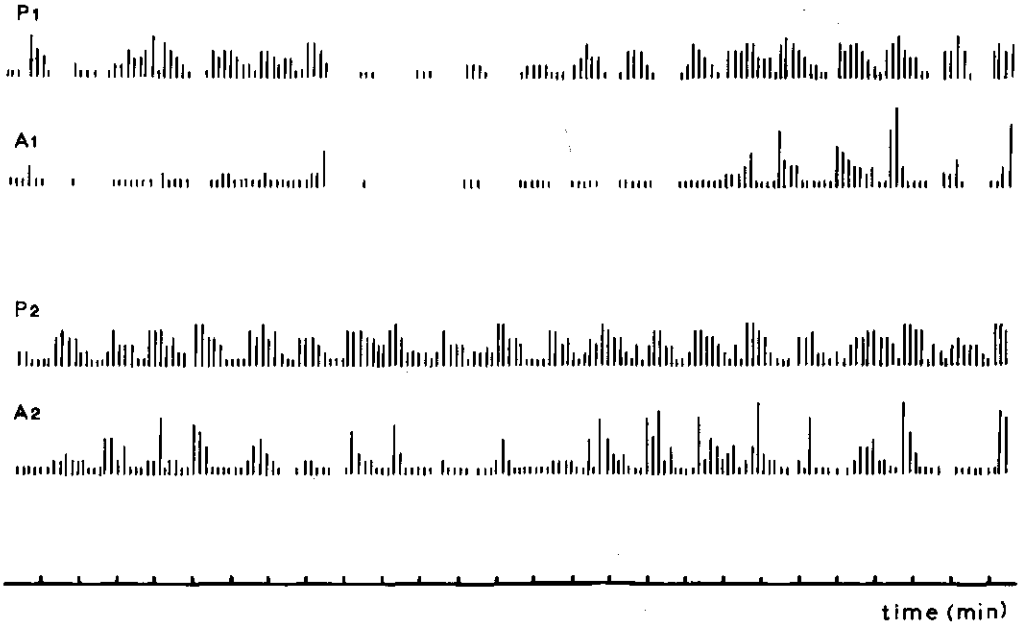

Fig. 20. Antral (A) and pyloric (P) contractile activity. A_1 and P_1, and A_2 and P_2 were recorded simultaneously. Amplitudes are expressed in arbitrary units.

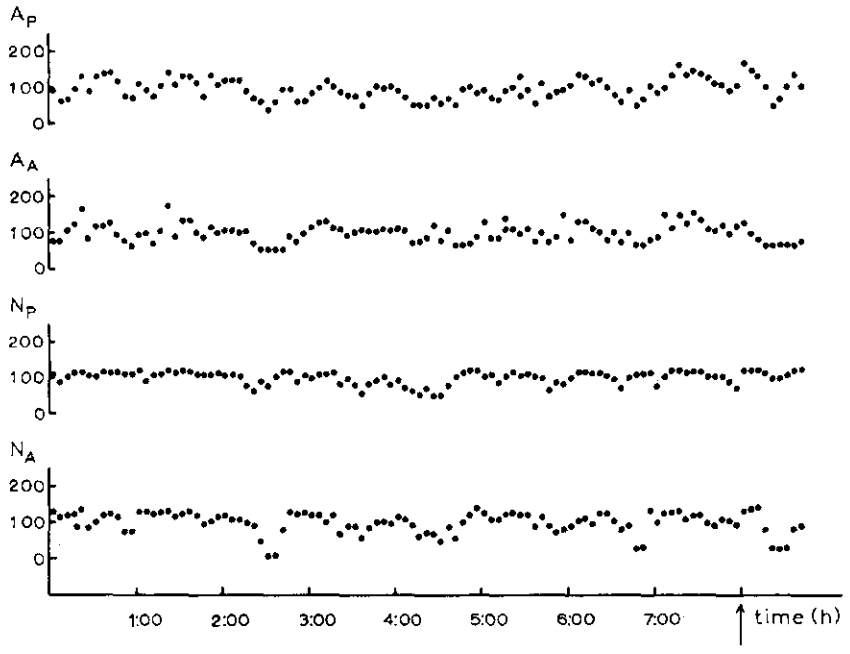

Fig. 21. Frequency (N) and intensity (A) of antral (A) and pyloric (P) contractile activity, counted per 5-min periods. Data are expressed as a percentage of the average over the whole recording period. Feeding time is indicated by the arrow.

strated in Figure 21 for a single recording period, which was counted until 45 min after the ration was supplied. We were not able to standardize strain gauge signals, and therefore frequency and intensity of the different strain gauges may not be compared as such. In Figure 21, this evil was accomodated by plotting relative values (100% corresponds with the average of the recording period) of the number of contractions (N) per 5 min and the average amplitude (A) of the same 5-min period against time. Both frequency and intensity of antral and pyloric contractile activity increased after feeding for a short period. The most striking response was noticed in the intensity of pyloric contractile activity (A_P). Fifteen to twenty minutes after the start of feeding, however, when intake of the supplied ration was completed, a period of less activity was recorded again on both strain gauges. Variability in intensity and frequency of contractile activity was found to be highest on the antral strain gauge, as could also be concluded from Figure 20.

Strain gauge sensitivity decreased with time after surgery. Therefore it was not possible to conclude whether frequency and intensity of contraction activity were decreasing with time, from the first to the fourteenth recording period, as indicated in Table 30. The first recording was made about 3 weeks and the fourteenth about 2 months after surgery. Probably, the lower frequency of both antral and pyloric contractile activity of the last 6 recording periods were due to an underestimation, since weak contractions were no longer detected. This decrease in the number of contractions was most substantial on the pyloric strain gauge, but it did not agree with the respective decreases of the average amplitude (A), which decreased most substantially on the antral strain gauge. Therefore we have to

Table 30. Frequency (N) and intensity (A) of abomasal antral (A) and pyloric (P) contractile activity of fourteen 12-h recording periods. Data were calculated as averages per h. N is expressed as the number of contractions per h and A is expressed in arbitrary units.

	N_A	N_P	A_A	A_P
1	266	240	2.08	2.09
2	307	320	2.56	2.71
3	334	349	2.54	2.80
4	316	346	2.29	3.03
5	319	359	1.68	2.42
6	306	338	1.56	2.33
7	305	350	1.46	2.10
8	305	310	1.42	2.12
9	295	270	1.25	1.63
10	218	184	1.17	1.37
11	263	220	1.20	1.51
12	267	194	1.12	1.31
13	232	177	1.13	1.31
14	266	188	1.09	1.36

conclude that the decreases in frequency and intensity of abomasal antral and pyloric motility were not only caused by decreases in sensitivity of both strain gauges. Regarding the different quantities of the rations supplied (Section 8.2), we possibly may conclude that with feeding at a lower level frequency of pyloric motility predominates, and that with feeding at a higher level frequency of antral motility predominates.

For this decrease in sensitivity of the strain gauges, corrections were made by expressing the individual hourly data as a percentage of the hourly average per recording period. These proportional data of 14 recording periods of 12 h were averaged per hour and plotted in Figure 22 against time. The experimental sheep was fed twice a day and therefore a pattern in the duodenal passage rate of digesta was to be expected, as described in Section 3.5. Consistent differences between the hourly motility values, however, were not present, but a tendency was noticed for both frequency (N) and intensity (A) to decrease with time after feeding. This decrease was most substantial on the pylorus, since in general frequency and intensity of pyloric motility were relatively higher during the first hours after feeding, but in the last hours the opposite was noticed. This deviation between antral and pyloric motility in relation to time after feeding might possibly explain a particular duodenal flow pattern in relation to time after feeding. In Feeding Trial 3, and to a lesser extent in Feeding Trial 1, duodenal passage rate of digesta increased with time after feeding (Figures 2 and 6). Comparing this duodenal flow pattern with antral and pyloric motility as affected by time after feeding, the highest abomasal emptying rates should occur during periods of predominance of abomasal antral motility.

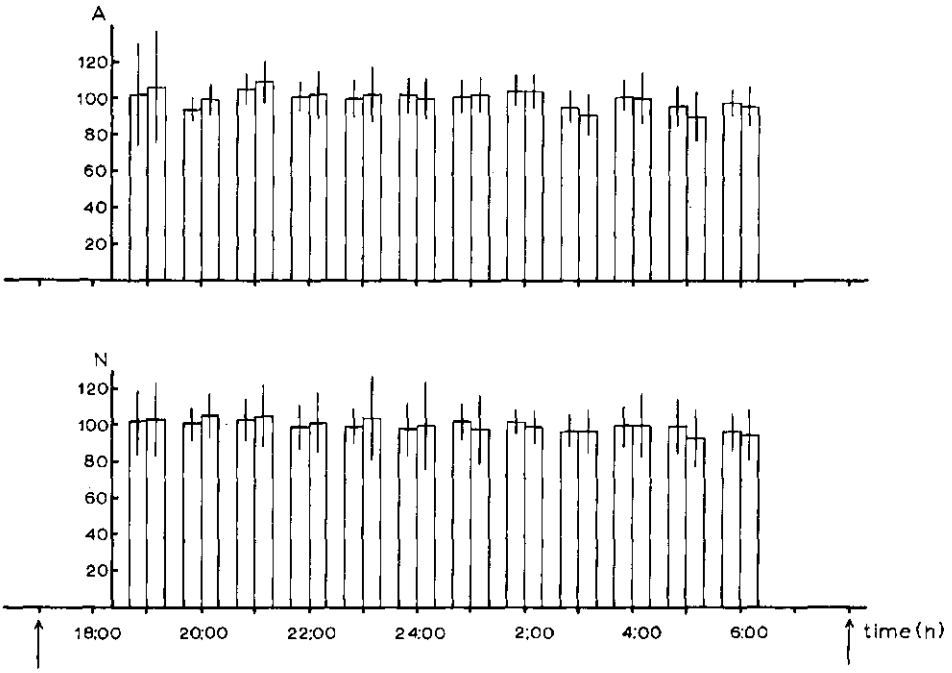

Fig. 22. Frequency (N) and intensity (A) of antral and pyloric motility, as affected by feeding time. Per recording period, hourly data were standardized on the hourly average per recording period. Of each set of columns the left represents antral and the right one pyloric motility. Times of feeding are indicated by the arrows. Standard deviations are indicated by the vertical bars (± 1 SD).

8.4 ANTRAL AND PYLORIC MOTILITY AS AFFECTED BY CONTINUOUS INTRA-ABOMASAL INFUSION OF SOYA PROTEIN

Intra-abomasal infusion of soya protein was shown (Sections 4.3, 4.4 and 4.7) to induce an increased abomasal secretory activity of acid, and thus an increased duodenal passage rate of digesta. Since abomasal digesta are supposed to be propelled into the duodenum by abomasal antral contractions, we were interested in the effect of continuous intra-abomasal infusion of a 10% soya protein (promine D) suspension in saline on abomasal antral and pyloric motility. In Table 31, frequency (N) and intensity (A) are given as affected by infusion of soya protein. All data were calculated from the hourly averages per 12-h recording period.

As in the preceding experiments (Section 8.3), a high variability between recording periods was noticed. On the data of Table 31, coefficients of variation of up to 30% could be calculated, which reduced strongly the detection of possible effects of intra-abomasal infusion of soya protein. Nor frequency (N), neither intensity (A) of abomasal antral and pyloric motility were significantly affected by intra-abomasal infusion of soya protein, however, not only as a result of the variability of the data, but also as a result of the minor differences between the average values.

As in the preceding experiments (Section 8.3), hourly data were expressed as a per-

Table 31. Frequency (N) and intensity (A) of abomasal antral (A) and pyloric (P) motility with continuous intra-abomasal infusion of a 10% soya protein (promine D) suspension in saline. Data were calculated as averages per h. N is expressed as the number of contractions per h and A is expressed in arbitrary units. Standard deviations (SD) are indicated between the parentheses.

	Control	Promine D
N_A	312 (12)	318 (34)
N_P	335 (17)	339 (30)
A_A	1.98 (0.56)	1.88 (0.16)
A_P	2.52 (0.38)	2.56 (0.34)

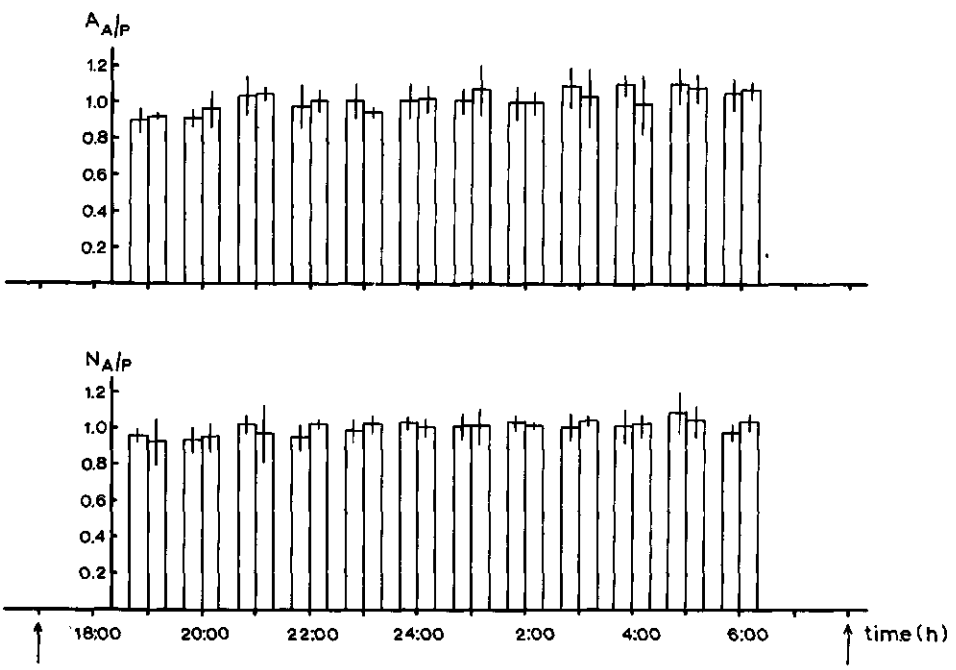

Fig. 23. Ratio between frequency (N) and intensity (A) of abomasal antral (A) to pyloric (P) motility, as affected by feeding time and intra-abomasal infusion of a 10% soya protein (promine D) suspension in saline. Of each set of columns the left represents the control experiments and the right one the soya protein infusion experiments. Feeding times are indicated by the arrows. Standard deviations are indicated by the vertical bars (± 1 SD).

centage of the respective hourly averages per recording period. When no intra-abomasal infusions were carried out, a tendency was noticed for pyloric motility to predominate during the first hours after feeding and antral motility during the last hours before feeding, and thus the ratio of frequency and intensity of antral to pyloric motility was found to increase with time after feeding. In Figure 23, this ratio of frequency (N) and intensity (A) of antral to pyloric motility were plotted against time, after continuous intra-abomasal infusion of soya protein and when no intra-abomasal infusion was carried out. As in the

preceding experiments (Section 8.3), the ratio of frequency and intensity of antral to pyloric motility tended to increase with time after feeding, a pattern which was not substantially influenced by intra-abomasal infusion of soya protein.

8.5 ANTRAL AND PYLORIC MOTILITY AS AFFECTED BY DISCONTINUOUS INTRA-ABOMASAL INFUSIONS

In the preceding experiments (Section 8.4), abomasal antral and pyloric motility were recorded during continuous intra-abomasal infusion of a soya protein suspension, which was started in advance of the recording periods. Under comparable experimental conditions intra-abomasal infusions of soya protein (Sections 4.3, 4.4 and 4.7) were found to stimulate abomasal secretion of acid, not to affect abomasal digesta entry rate, and thus to increase abomasal outflow rate. This increased abomasal outflow rate after continuous intra-abomasal infusion of soya protein was not achieved by an increased abomasal motor activity. Continuous intra-abomasal infusions probably stimulate acid secretion partly through a stimulated release of gastrin, they increase abomasal outflow rate, but they will also stimulate neural or hormonal factors, which originate from the small intestine and which inhibit abomasal motor activity. Apparently, both stimulating and inhibiting factors were induced by these continuous intra-abomasal infusions, resulting in a new steady-state situation, which did not differ from the pre-infusion steady state.

In the present experiments, abomasal antral and pyloric contractile activity were recorded after discontinuous intra-abomasal infusions. Under these conditions, before or while the new steady-state situation is being reached, more substantial effects of the respective intra-abomasal infusions on abomasal antral and pyloric motility could well be expected. Different substances, which were also used in studying abomasal secretion of acid (Chapter 4) were infused into the abomasum, and simultaneously with the start of the infusions abomasal motility was recorded for 5.5-h periods. As was pointed out under Methods (Section 8.2), antral motility was recorded in one sheep and pyloric motility in another one.

Frequency (N) and intensity (A) of abomasal antral and pyloric motility were counted over 15-min periods. In Figure 24, the data after the intra-abomasal infusions, expressed as a percentage of the respective control values, were plotted against time after the start of infusion. Besides, the effects of the intra-abomasal infusions on the average frequency and intensity of abomasal antral and pyloric motility per recording period were determined, calculated as 15-min averages per 5.5-h period. These 15-min average values, expressed as a percentage of the control values, are given in Table 32.

In general abomasal antral motility was found to be more sensitive to the intra-abomasal infusions than pyloric motility. Depending on the intra-abomasal infusate and on the time interval after the start of infusion, stimulation or inhibition was noticed. Frequency and intensity of antral or pyloric motility were not always affected in the same way. After soya protein infusion intensity of pyloric motility (A_P) was slightly decreased, whereas intensity of antral motility (A_A) tended to be increased (Table 32). These opposite effects are also noticed in Figure 24. When stearic acid was infused into the abomasum in a particular 15-min period, frequency of antral motility increased significantly, but intensity decreased significantly.

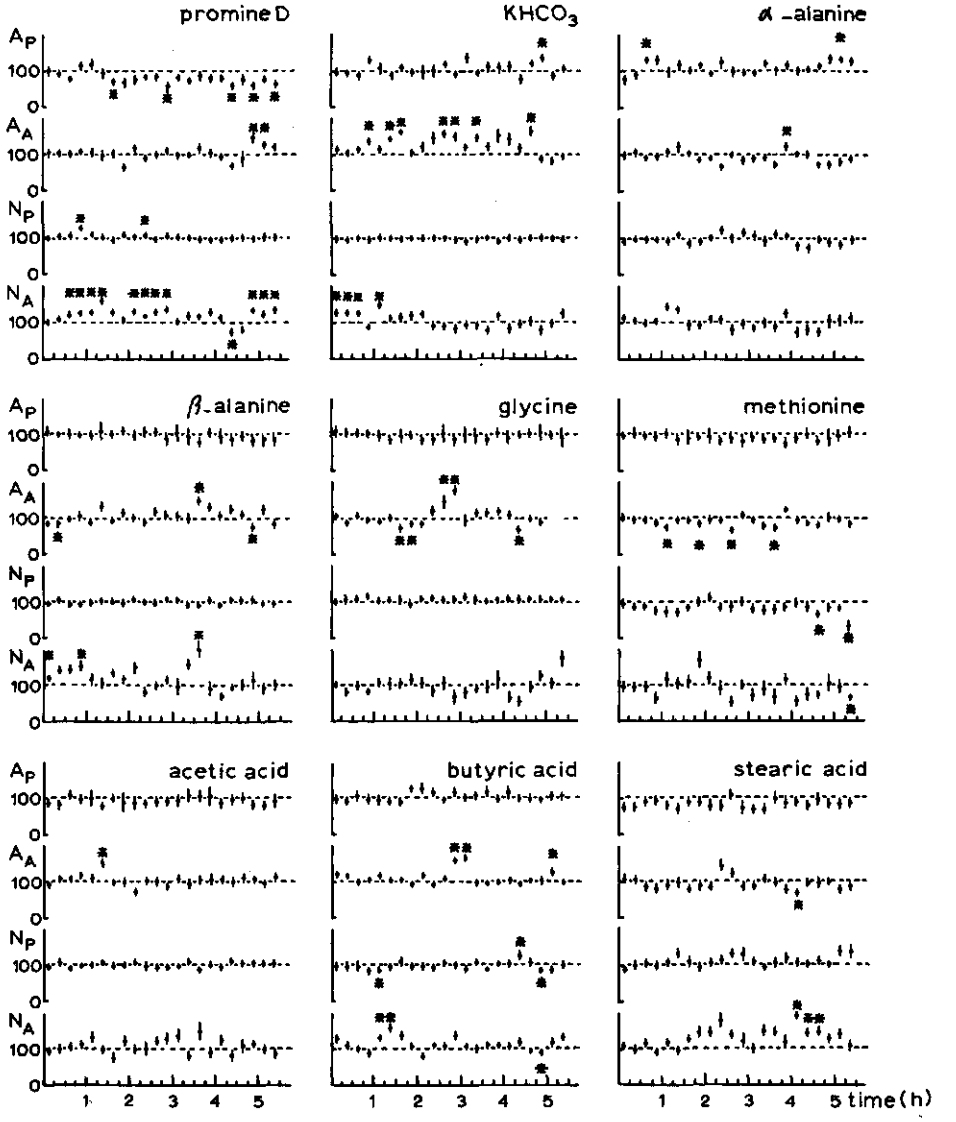

Fig. 24. Frequency (N) and intensity (A) of abomasal antral (A) and pyloric (P) motility, as affected by discontinuous intra-abomasal infusions (Table 29). Data are expressed as percentages of the control values. Standard deviations (+ 1 SD) are indicated by the vertical bars. Significances (two-tailed Student t_9) are indicated by the stars ($P < 0.05$).

The effects of the various intra-abomasal infusions on abomasal antral and pyloric motility, as expressed for the individual 15-min periods in Figure 24, did not always coincide with the overall infusion effects, as shown in Table 32. For example, intra-abomasal infusion of a solution of glycine was not found to affect the average intensity of antral motility (A_A, Table 32), but in Figure 24 both significant increases and decreases are present. When a solution of butyric acid was infused into the abomasum, frequency of antral contractile activity (N_A) was slightly increased, whereas in Figure 24 not only

Table 32. Frequency (N) and intensity (A) of abomasal antral (A) and pyloric (P) motility, as affected by discontinuous intra-abomasal infusions (Table 29). Data are expressed as percentages of the control values. For significances (two-tailed Student t_9) see Methods (Section 8.2). Standard deviations (SD) are indicated between the parentheses.

	N_A		N_P		A_A		A_P	
Promine D	119.7**	(3.3)	105.0	(2.6)	110.7	(4.6)	81.5*	(4.8)
$KHCO_3$	105.1	(4.6)	102.1	(2.3)	124.4**	(3.5)	108.2	(3.6)
α-Alanine	100.2	(3.1)	101.1	(2.5)	98.9	(3.6)	113.4	(4.6)
β-Alanine	117.6	(5.8)	99.7	(2.6)	99.0	(2.9)	96.9	(5.2)
Glycine	96.5	(4.1)	105.5	(2.0)	101.7	(1.9)	98.4	(3.5)
Methionine	90.9	(4.1)	84.7**	(2.8)	92.1	(2.9)	92.8	(5.7)
Acetic acid	104.3	(3.0)	99.0	(1.5)	105.3	(1.8)	90.7	(2.6)
Butyric acid	109.2*	(2.2)	95.7	(1.8)	106.1	(3.9)	102.1	(7.4)
Stearic acid	124.1*	(5.5)	107.1	(5.1)	91.2	(8.0)	81.8	(11.6)

significant increases are present, but also a 15-min period, which shows a significant decrease.

8.6 DISCUSSION

In Section 2.5, the abomasal antrum and pylorus were described to contract in a rather regular pattern. In the present experiments, a comparable contractile pattern was found, although periods of less activity, which lasted several minutes, were detected, as were also reported by Bueno & Toutain (1974) in sheep and goats. Intensity of contractile activity was found to be more regular at the pylorus than at the abomasal antrum, where weak contractions were relieved by relatively strong contractions (Figures 20 and 21). These strong antral contractions may possibly act as the propelling mechanism, forcing abomasal contents into the proximal duodenum. In general, periods of high antral activity were paralleled by periods of high pyloric contractile activity. Recordings of antral and pyloric motility in the same sheep (Sections 8.3 and 8.4), however, showed deviations of this close relationship to occur rather frequently. In some periods, pyloric motility was found to predominate, while also periods were present with a more frequent or a more intensive antral contractile activity. Davenport (1971) suggested that whether digesta are propelled into the proximal duodenum by antral peristaltic contractions or not, depends on pyloric contractile activity. Therefore a high abomasal outflow rate of digesta is to be expected during periods of predominance of antral motility.

When sheep were fed less frequently (Section 3.5), duodenal passage rate of digesta was found to increase with time after feeding. Variability of this duodenal flow pattern from day to day was found to be high, however, and therefore it was not always possible to calculate significant differences between hourly amounts of digesta passing through the duodenum. Substantial variations in abomasal antral and pyloric contractile activity one day to the other were also shown (Table 30), even under most standardized conditions, which might possibly explain the variability in duodenal passage rate of digesta between days. When expressing the motility characteristics as relative values per recording period,

some pattern of antral and pyloric motility according to time after feeding was noticed. Both frequency and intensity of abomasal antral and pyloric motility tended to decrease with time after feeding. Pyloric motility, however, tended to predominate during the first hours after feeding but antral motility during the last hours before feeding (Fig. 22). Such a motility pattern is possibly consistent with a pattern of duodenal passage rate of digesta in relation to time after feeding as shown in Figure 6, suggesting abomasal outflow rate of digesta to be highest during periods of predominance of abomasal antral contractile activity.

After continuous infusion of a soya protein suspension into the abomasum, higher abomasal secretory rates of acid and consequently increased duodenal passage rates of digesta were demonstrated previously (Sections 4.3, 4.4 and 4.7). Under comparable experimental conditions (Section 8.4), intra-abomasal infusion of soya protein was not found to induce an increased contractile activity of the abomasal antrum nor of the pylorus, and therefore abomasal outflow rate of digesta is probably not determined only by abomasal antral and pyloric contractile activity.

Recordings of abomasal antral and pyloric motility, when intra-abomasal infusions (Section 8.5) were carried out discontinuously, showed minor effects on antral or pyloric contractile activity. On the assumption that duodenal passage rate of digesta be affected by discontinuous intra-abomasal infusions in the same way as when these infusions were carried out continuously, an increased duodenal passage rate of digesta after intra-abomasal infusion of soya protein should be achieved by an increased frequency of antral motility and by a decreased intensity of pyloric motility (Table 32). After intra-abomasal infusion of $KHCO_3$ 0.30 mol/litre, however, the same effect on abomasal emptying rate should be achieved by an increased intensity of antral motility. In previous experiments, α-alanine (Section 4.6), acetic and butyric acids (Section 4.8) were found to increase abomasal outflow rate of digesta. No consistent effects on abomasal antral and pyloric motility were present however, except after intra-abomasal infusion of butyric acid when frequency of antral motility was slightly increased. After intra-abomasal infusion of methionine (Section 4.6), a decreased duodenal passage rate of digesta was found and in the present experiments frequency of pyloric motility was significantly decreased, whereas frequency and intensity of antral motility and intensity of pyloric motility tended to decrease (Table 32). After intra-abomasal infusion of β-alanine and glycine (Section 4.6), no significant increases of abomasal outflow rate of digesta were induced and in the present experiments no significant effects on frequency and intensity of abomasal antral and pyloric motility were noticed. Obviously, increased duodenal passage rates of digesta do not always follow increased antral or pyloric motility, whereas after intra-abomasal soya protein and $KHCO_3$ infusion these increases in abomasal outflow rate of digesta seemed to be induced in different ways.

The absence of a significant effect on the average motility characteristics (Table 32) did not always indicate the absence of an effect of the intra-abomasal infusate at all. As shown in Figure 24, 15-min values deviated significantly from the reference value if no effect on the average value per recording period was noticed. Sometimes, as with intensity of antral motility after intra-abomasal infusion of glycine, significant decreases alternated with significant increases, suggesting that a new steady state was being sought after

the start of infusion. Such an oscillating pattern could also be noticed in the other plots of Figure 24.

Introduction of long chain fatty acids into the stomach of man was found to inhibit gastric emptying rate (Hunt & Knox, 1968). Ehrlein & Hill (1970) demonstrated that infusion of long chain fatty acids into the duodenum of goats inhibited both abomasal antral and pyloric motility. In our experiments, intra-abomasal infusion of a suspension of stearic acid in $KHCO_3$ 0.15 mol/litre did not inhibit abomasal motor activity, although intensity of abomasal antral and pyloric motility tended to decrease. On the contrary, frequency of abomasal antral contractile activity was found to be increased significantly. As was discussed previously (Section 2.5.3), different mechanisms are involved in the regulation of abomasal motor activity. Both neural and hormonal mechanisms have been shown to affect gastric motility. Davenport (1967) observed vigorous contractions of dog denervated fundic pouches after introduction of a solution 0.10 mol/litre of acetic acid, or when diffusion of hydrogen ions into the gastric mucosa was facilitated by topical application of acetylsalicylic acid. Fatty acids are not ionized under the acid abomasal conditions, and thus highly permeable to the abomasal mucosa. After diffusion of fatty acids into the abomasal mucosa, ionization occurs and hydrogen ions thus formed are trapped, possibly acting on motility through a neurally mediated pathway, or by a stimulated formation of histamine, as suggested by Davenport (1972). Such mechanisms may also account for the stimulative effect on abomasal antral motility after intra-abomasal infusion of butyric and stearic acids. Long chain fatty acids were mentioned as active releasers of cholecystokinin (Andersson, 1973). Cholecystokinin, however, is expected to inhibit abomasal motor activity (Section 2.5.3), and therefore it is difficult to explain the effect of stearic acid on abomasal motor activity through a stimulated release of cholecystokinin. This suggestion is in line with the results of Fisher et al. (1973), who found an increased pylorus pressure in the opossum after cholecystokinin, and such an effect was not noticed after intra-abomasal infusion of stearic acid.

After discontinuous intra-abomasal infusions (Section 8.5), frequency or intensity of antral and pyloric motility were occasionally affected in the same direction, but also opposite effects were noticed. After soya protein infusion for instance, frequency of antral motility was significantly increased, whereas intensity of pyloric motility was significantly decreased. After $KHCO_3$ infusion, intensity of antral motility was significantly increased, while an effect on intensity of pyloric motility could not be noticed. These different effects on antral and pyloric motility possibly indicate antral and pyloric motor activity to be regulated by different mechanisms, or to be affected in different ways by the same effector. Evidence for such different ways of regulation of antral and pyloric motility was supplied by Anuras et al. (1974), who found that in opossums, cats, dogs and humans upon electrical stimulation of isolated pyloric circular muscle strips relaxation occurred, whereas in antral strips occasionally contractions were induced.

Intra-abomasal infusion of soya protein and of $KHCO_3$ were found to stimulate abomasal secretion of acid partly through a stimulated release of gastrin (Chapter 5), which might explain a part of these opposite effects, since gastrin was reported to stimulate antral motility in pigs (Stadaas et al., 1974) but to relax the pylorus in the opossum (Fisher et al., 1973). Other factors, however, have to be involved, since abomasal antral and

pyloric motility were affected in different ways after intra-abomasal infusion of soya protein and $KHCO_3$.

When abomasal secretion of acid is stimulated, the amount of acid, entering the duodenum is also increased. This may stimulate the release of for instance secretin, which enterohormone is known to inhibit gastric motility. Besides, other inhibiting mechanisms originating from the duodenum, neurally or hormonally, may be activated, such as for instance the release of bulbogastrone (Andersson & Uvnäs, 1961). Possibly also cholecystokinin was involved in inhibiting abomasal motility after intra-abomasal infusion of methionine, which amino acid has been mentioned as an active releaser of cholecystokinin (Section 2.3.5). From our experimental results it is not possible to decide, which of these regulative mechanisms were involved after the different intra-abomasal infusions. Summarizing, however, we may conclude that abomasal outflow rate of digesta is probably not only determined by abomasal antral and pyloric motility. Other factors, such as tonic contractions of the fundus (Section 2.5.4), but also propagation velocity of the antral peristaltic contraction or the pressure gradient between abomasum and duodenum may be involved as well.

Weisbrodt et al. (1969) suggested that also coordination between antral and duodenal contractile activity plays an essential role in gastric emptying. Ehrlein (1976) concluded that in rabbits antral and pyloric motility were important for evacuation of meals with a high viscosity, but not after intragastric instillation of saline. Consistency of abomasal digesta is low and was under the present experimental conditions even decreased after intra-abomasal infusion of the suspensions or solutions in $KHCO_3$. In line with the results of Ehrlein, abomasal antral and pyloric contractile activity could not only be responsible for abomasal emptying.

Strain gauges detect fast contractile activity of antrum and pylorus. With this recording procedure we were not able to detect tonic contractions. Especially as far as the pylorus is concerned, contractile activity need not necessarily parallel the mode of opening of the pyloric canal. That a more quiet pylorus may facilitate evacuation of abomasal contents, was noticed for instance after intra-abomasal infusion of soya protein, but not after methionine.

More experimental results are needed in order to decide in what way propulsion of abomasal contents into the duodenum is related to abomasal motor activity. In those experiments, we would have to record abomasal motor activity simultaneously with the pattern of duodenal passage rate of digesta.

9 General discussion

In the present experiments, a clear relationship was demonstrated between composition of abomasal digesta and abomasal secretion of acid as estimated from the amounts of total acid and of chloride passing through the proximal duodenum. This method probably underestimates acid secretion in the abomasum as pointed out in Section 3.1, but decreases and increases in acid secretion can well be detected.

Continuous passage of digesta through the abomasum stimulates abomasal secretion of acid continuously. Distension of the abomasum showed (Section 2.3.3) to be involved as well, acting on abomasal secretion of acid through centrally or intramurally mediated reflexes and through stimulation of the release of gastrin. In non-ruminant mammals, especially in dogs, information is available on the contributions of the different mechanisms in stimulating secretion of acid. In experiments with dogs, distension of the antrum was shown to act on fundic acid secretion through a gastrin-dependent mechanism and through an antro-fundic cholinergic reflex mechanism (Debas et al., 1974). Release of gastrin was found to be stimulated by distension of the antrum, but also by distension of the fundus (Debas et al., 1975). Components of gastric digesta stimulate the release of gastrin (Section 2.3.5), but particular amino acids also stimulate acid secretion through a gastrin-independent mechanism (Konturek et al., 1976).

In sheep (Hill, 1960), less acid was secreted from vagally denervated fundic pouches of the abomasum than from vagally innervated pouches. Hill (1965) suggested that the vagus not only directly stimulates acid secretion from the parietal cells, but also helps to maintain parietal cell sensitivity to gastrin. McLeay & Titchen (1975) suggested that vagal innervation is of interest for the release of gastrin in sheep. Cholinergic stimulation of an antral pouch stimulated acid secretion from a fundic pouch. These experiments, however, were carried out in sheep with vagally innervated pouches and concentrations of gastrin in blood plasma were not measured. Hence more data from sheep fitted with vagally denervated antral pouches or on gastrin in blood plasma are needed for any definite conclusion that the release of gastrin in sheep is increased cholinergicly. The role of gastrin in the regulation of abomasal secretion of acid was demonstrated by McLeay & Titchen (1975), who showed that after antrectomy, volume and acidity of juice secreted by vagally innervated fundic pouches were decreased. Involvement of gastrin in abomasal secretion of acid was underlined by the present experimental results.

In Section 2.3.5, gastric secretion of acid was discussed as influenced by enterohormones, but probably reflexes originating in the small intestine are involved as well. Although not measured in these experiments, enterohormones are probably released as well upon ingestion of a meal so that the secretion of acid is governed by the interplay of stimulating and inhibiting neural and hormonal mechanisms. Ingestion of a meat meal in man was found to increase both gastrin and secretin in blood plasma (Tai et al., 1974). A

complete understanding of the regulation of abomasal secretion of acid requires identification of these neural and hormonal mechanisms. In order to determine their relative contributions, their combined effects on abomasal secretion of acid have to be measured as well.

Hormones released by the mucosa of the gastro-intestinal tract and reflexes arising from receptors in the gastro-intestinal wall are involved not only in abomasal secretion of acid but also in abomasal motor activity and in consequence in abomasal outflow of digesta. In Section 2.5.4, evacuation of gastric digesta was discussed as influenced by composition of gastric and duodenal digesta, acting on gastric emptying through neurally or hormonally mediated pathways. In man, for instance, cholecystokinin was mentioned as a governor of gastric emptying at doses submaximal for pancreatic secretion. Cholecystokinin is also known as an inhibitor of gastrin-stimulated secretion of acid. Perhaps factors that stimulate or inhibit abomasal motility, and thus abomasal outflow of digesta, influence abomasal secretion of acid in the same direction. So retardation of abomasal outflow of digesta may result in a lower steady-state acidity level of abomasal digesta, but simultaneous inhibition of abomasal secretion of acid would prevent the pH of abomasal digesta from falling to a level that could damage the abomasal mucosa.

In the present experiments, the interrelationship between average retention time of digesta in the abomasum and resulting steady-state acidity of abomasal digesta has not been studied. When abomasal secretion of acid was stimulated by increasing protein or inorganic buffering content of the rations, or by continuous infusion of different substances into the abomasum, abomasal outflow rates of digesta were higher, possibly concurrently with decreased retention times of digesta in the abomasum, resulting in a steady-state pH of abomasal digesta that did not change significantly or increased. Such studies on the relation between retention time of digesta in the abomasum and abomasal outflow of digesta have to be carried out simultanuously with recordings of abomasal contractile activity, since the present data do not indicate how propulsion of abomasal digesta into the duodenum is induced by abomasal motility.

In the introductory remarks, the proposed relationship was discussed between abomasal secretory activity of acid and the occurrence of abomasal ulcers with fatal haemorrhage in adult cattle. In none of the present experiments, however, was a significantly decreased pH of abomasal digesta observed upon stimulation of abomasal secretion of acid. On the contrary in Feeding Trial 2, the lowest pH of abomasal digesta was found when none of the variable ration components was added to the fixed basic ration, whereas in Feeding Trial 3 different abomasal secretory rates of acid were induced by different rations without significant deviations in the pH of the digesta flowing through the duodenum. These findings suggest that induction of abomasal ulcers by ration composition is not caused through the pH of abomasal contents. But the pH of abomasal digesta could still be involved in abomasal ulceration. The pH of abomasal digesta is more likely to decrease when acid-stimulating factors are present in abomasal digesta, which do not exhibit a high buffering capacity. Histamine in the ration or synthesized by rumino-reticular micro-organisms, has been suggested to be involved (O'Sullivan, 1968; Aukema, 1971). Ulcers in the abomasal mucosa could perhaps alternatively depend on amount and composition of mucus secreted by the mucosal mucus glands.

In Section 2.3.10, secretion of gastric mucus was discussed as influenced by neural

and hormonal factors. Mucus is broken down with pepsin. Unfortunately in the present experiments we could not characterize abomasal secretion rates of pepsinogen by determination of pepsin activities of duodenal digesta. The pepsin activities measured in duodenal digesta may, however, be representative for pepsin activity of abomasal digesta, since the proposed inhibition of pepsin by peptic protein degradation products is likely to occur in vivo as well. The pepsin activities found were in general lower when abomasal secretion of acid was stimulated by proteins. Abomasal ulcers cannot therefore be attributed to increased peptic degradation of abomasal mucus. The present experimental results do suggest that higher pepsin activities of abomasal digesta are to be expected when sheep receive rations with lower contents of protein. But Aukema & Breukink (1974) found that abomasal ulceration in adult cattle occurred frequently during the summer when the cows were on pasture and were supplied with a ration rich in protein. Inhibition of pepsin activity in abomasal or duodenal digesta by protein breakdown products suggests that in these digesta pepsin activity could be governed by an autoregulative mechanism.

The present experiments allow no firm conclusion about how abomasal secretion of pepsinogens is affected by amount and composition of digesta passing through the abomasum. In Section 6.5, it was suggested that abomasal secretion of pepsinogens could be estimated more reliably when abomasal juice is collected that is not contaminated by digesta entering the abomasum, for instance in sheep with abomasal pouches. Perhaps these factors inhibiting pepsin activity could be removed from abomasal or duodenal digesta by dialysis.

Summary

Information on the regulation of abomasal functioning in steady state is scanty. The increasing number of abomasal ulcers and haemorrhages in dairy cows, and the suggested relation of this disorder to composition of the ration, prompted an investigation of functioning of the abomasum. In sheep, the effects were studied of different components of the food and of rumen fermentation products on acid secretion, mucosal blood flow, motility and plasma gastrin level.

Chapter 2. The literature on abomasal functioning is reviewed: secretion of hydrochloric acid, of pepsinogen and of mucus; blood flow through the mucosa; motility in relation to evacuation rate of digesta, and of neural and hormonal regulation of these processes. As the literature on abomasal functioning is restricted and as the morphological and the functional similarity of the abomasum to the stomach of monogastric animals is evident, the review was extended to studies of simple stomachs.

In Chapters 3-8, the experiments and methods are described. The results of these experiments are summarized below.

Chapter 3. In three feeding trials, abomasal secretion of acid was studied in sheep, fitted with re-entrant cannulas in the proximal duodenum. Abomasal secretion of acid was estimated from the amounts of total acid and of chloride leaving the abomasum. Duodenal digesta were sampled for 12-h periods.

In the first feeding trial (Section 3.3), three sheep were fed on three different rations. Abomasal secretory rates were highest when fed on hay or grass. Content of crude protein in the third ration, composed of ryegrass straw and semisynthetic components (Table 1) was lowest, resulting in a less stimulated abomasal secretion of acid.

In the second feeding trial (Section 3.4), sixteen rations were fed to four sheep, fitted with a rumen cannula as well. These rations included a fixed basic ration, consisting of ryegrass straw and semisynthetic components, and four variable ration components, cellulose, soya protein, $KHCO_3$ and maize starch, added in all possible combinations (Table 2). The highest abomasal secretory rates of acid were found with addition of soya protein or $KHCO_3$. Cellulose and maize starch had less effect on abomasal secretion of acid, both directly and through formation of volatile fatty acids in the forestomachs. No relationship could be demonstrated between ruminal concentrations of volatile fatty acids and abomasal secretion of acid.

In the third feeding trial (Section 3.5), three rations (Table 4) were fed to three sheep. In that feeding trial, duodenal passage rate of digesta depended on feeding regime. The highest passage rates were measured the hours preceding the times of feeding (Fig. 6). The highest abomasal secretory rates of acid were induced by a ration of grass. When half the grass ration was replaced by hay, secretory rates of acid were lower. Replacement of grass in this second ration by semisynthetic components with a comparable chemical com-

position, resulted in an increased duodenal passage rate of digesta, but had no effect on abomasal secretion of acid.

In general, when protein content of the rations was increased in those feeding trials, abomasal secretory rates of acid were higher. There were highly significant positive correlation coefficients between duodenal passage rates of total acid and of crude protein and between passage rates of chloride and of crude protein (Tables 7, 12 and 15).

In the abomasum, the parietal cells of the fundic tubular glands secrete acid as hydrochloric acid, resulting in a decreased pH of abomasal digesta coincident with an increased abomasal digesta chloride concentration. There was a highly significant positive relationship of duodenal passage rates of chloride with those of total acid, but regression coefficients exceeded 1 significantly, probably because of chloride in the ration and of chloride secreted by the abomasal non-parietal cells (Figures 3, 5 and 7).

If concentration of chloride in abomasal juice be 155 mmol/litre, if concentration of chloride be doubled when digesta flow through the omasum as assumed in the second feeding trial, or if amounts of chloride secreted in the abomasum be 80% of the amounts of chloride leaving the abomasum as supposed in the other two feeding trials, more than half of the digesta leaving the abomasum would be abomasal juice.

Chapter 4. In sheep fitted with an abomasal infusion tube, with re-entrant cannulas in the proximal duodenum, and occasionally with a rumen cannula, abomasal secretion of acid was studied as affected by continuous intra-abomasal infusions. These infusions were started about 40 h in advance of the sampling period. Duodenal digesta were sampled for 8-h periods. Studying abomasal secretion by this infusion technique requires that abomasal entry rate of digesta is not influenced by the continuous intra-abomasal infusions. In the first experiment, neither ruminal concentration of polyethylene glycol (PEG), infused at constant rate into the rumen, nor duodenal recovery of PEG was found to change with intra-abomasal infusion of soya protein (Section 4.3). In that experiment but also in the others, the respective increases in duodenal passage rate of dry matter and of crude protein were in general not found to deviate from the respective amounts of dry matter and of crude protein infused into the abomasum. From these results it was concluded that continuous intra-abomasal infusions did not affect inflow rate of digesta into the abomasum, and that the effect of continuous intra-abomasal infusions on abomasal secretion of acid could be calculated from duodenal passage rates of total acid and of chloride in comparison with the respective amounts of total acid and of chloride passing through the proximal duodenum in the control experiments.

Intra-abomasal infusion of a soya protein suspension in saline in three sheep at two rates induced minor increases in abomasal secretion of acid (Section 4.3). The most substantial response was found when the soya protein suspension was infused into the abomasum at the highest rate (Table 17).

Proteins are supposed to act on abomasal secretion of acid in a dual way, as far as gastrin is concerned. In the literature, lower molecular protein fragments have been mentioned as active releasers of gastrin, a gastro-intestinal hormone that stimulates acid secretion. In general, proteins exhibit a high buffering capacity. Since at a higher pH the release of gastrin is known to be less inhibited, proteins are thought to stimulate the release of gastrin also in a less specific way by affecting the pH of abomasal con-

tents. After infusion of different proteins and of a phosphate buffer at similar rates into the abomasum of three sheep (Section 4.4), abomasal secretory rates of acid increased, which could not be explained completely by the respective buffering capacities of the different infusates (Fig. 8). Therefore proteins stimulate abomasal secretion of acid not only through their buffering capacity, but probably also through more specific factors such as amino acid composition.

That inorganic buffers increased abomasal secretory rate of acid indeed was confirmed (Section 4.5). After continuous infusion of $KHCO_3$ buffers of different concentrations into the abomasum of two sheep, duodenal passage rates of total acid and of chloride were higher (Table 20). Proportionally to the buffering capacities infused, however, acid secretion was less stimulated with a more concentrated buffer (Fig. 10). Obviously, acid secretion is less stimulated when the abomasum secretes at a higher rate. Probably, intra-abomasal infusions and subsequent increased abomasal secretory rates of abomasal juice influence digesta outflow from and average retention time of digesta in the abomasum. Besides, abomasal secretory activity will approach maximal secretory activity at higher secretion rates.

Amino acid composition of proteins was suggested to be involved in the stimulation of abomasal secretion of acid. Infusion of α-alanine, β-alanine, aspartic acid, arginine, glycine and methionine into the abomasum of three sheep (Section 4.6), however, did not affect abomasal secretion of acid substantially (Table 21). These results were surprising for β-alanine and glycine, since these amino acids have been mentioned as active releasers of gastrin in the dog. After infusion of methionine abomasal secretion of acid significantly decreased, probably indirectly by inhibition of the passage rate of digesta through the abomasum.

According to the literature the smaller molecular fragments of protein hydrolysates are highly potent stimulators of gastrin release in dogs. When soya protein, partially hydrolysed in advance, was infused into the abomasum of three sheep (Section 4.7) abomasal secretory rates of acid were higher than with soya protein, not hydrolysed in advance (Table 22).

Infusion of acetic, propionic, butyric and L-lactic acids into the abomasum of three sheep, in amounts which exceeded the amounts normally entering the abomasum (Section 4.8), stimulated abomasal secretion of acid poorly (Table 23). Under normal physiological conditions, volatile fatty acids and L-lactic acid thus seem unimportant in the regulation of abomasal secretion of acid, except for their involvement in the buffering capacity of digesta entering the abomasum.

If they stimulated secretion of acid, the intra-abomasal infusions induced increases in the amounts of total acid and of chloride leaving the abomasum, which did not differ significantly from one another (Figures 9, 11, 13, 16 and 18). This indicates that stimulation of abomasal secretion under the present conditions was probably confined to stimulation of acid abomasal juice secretion by the parietal cells of the fundic tubular glands, which are supposed to secrete almost isotonic hydrochloric acid.

Stimulation of abomasal secretion by continuous intra-abomasal infusions or, for instance, by increasing protein content of the rations, did not decrease the pH of abomasal digesta. The buffering capacities of abomasal digesta components stimulating abomasal secretion of acid were thus higher than the stimulatory effects on abomasal acid

secretion.

Chapter 5. Infusion of a soya protein suspension, partly hydrolysed in advance, and of a $KHCO_3$ buffer into the abomasum of three sheep, fitted with an abomasal infusion tube and with a catheter in one of the jugular veins increased blood plasma gastrin level. Proportionally to the buffering capacities infused, the protein suspension exhibited the highest potency in stimulating the release of gastrin (Table 25). Thus stimulation of abomasal secretion of acid by proteins and inorganic buffers is mediated, at least partly, by gastrin. After infusion of α-alanine, β-alanine and glycine into the abomasum of four sheep, gastrin concentration in blood plasma was unchanged in line with the effects of these amino acids on abomasal secretion of acid (Table 26).

Chapter 6. In some published experiments, pepsin activity in duodenal digesta was determined as well. When protein content of the rations was high, or when proteins were infused into the abomasum, duodenal pepsin activities were in general lower. After intra-abomasal infusion of $KHCO_3$ buffers duodenal pepsin activity was slightly increased. From these duodenal pepsin activities, however, no conclusions may be drawn about abomasal secretion of pepsinogen. In experiments in vitro, proteins inhibited pepsin activity, probably through the protein degradation products (Fig. 19).

Chapter 7. A soya protein suspension and a $KHCO_3$ buffer were infused into the abomasum of three sheep with an abomasal infusion tube, with re-entrant cannulas in the proximal duodenum and with a catheter in both jugular veins. After these infusions and in one of the published feeding trials, the relation was studied between abomasal secretion of acid and blood flow in the abomasal mucosa. Abomasal mucosal blood flow was estimated by the aminopyrine clearance method. Even when abomasal secretion of acid was increased by 30%, no increase in mucosal flow of blood was noticed (Tables 27 and 28). Hence abomasal secretion of acid and blood flow in the abomasal mucosa are not necessarily related directly under normal physiological conditions. Thus higher demands for oxygen and substrates, when abomasal secretory activity is increased, may be met by higher extraction rates of these substances from blood.

Chapter 8. Abomasal digesta are supposed to be propagated into the duodenum by abomasal antral and pyloric contractile activity. Abomasal antral and pyloric contractions were recorded with strain gauges in sheep, fitted with an abomasal infusion tube. Abomasal motility was found to be more regular than gastric motility in non-ruminant mammals. This regularity was highest for the pylorus. In the antrum, contractile activity was less regular in that strong contractions were interspersed by rather weak contractions. Contractile activity of both antrum and pylorus showed alternating periods of more and less activity (Figures 20 and 21).

Recordings of abomasal antral and pyloric contractile activity in relation to feeding time in a sheep, fed twice a day, showed no consistent pattern (Fig. 22). In the first interval after feeding, however, pyloric contractile activity tended to predominate, whereas later a tendency was noticed for antral motility to be highest. Perhaps higher abomasal outflow rates of digesta are induced during periods of predominance of antral motility, as suggested by the relation of motility pattern with feeding regime.

Continuous intra-abomasal infusion of soya protein resulted in an increased abomasal outflow rate of total acid, of chloride, but also of digesta. These continuous infusions

of soya protein into the abomasum of one sheep, however, did not alter abomasal antral and pyloric contractile activity (Table 31). Other factors are thus involved in outflow rate of digesta from the abomasum, such as for instance tonic fundic contractions.

Discontinuous infusions into the abomasum of two sheep, however, had little effect on abomasal antral and pyloric contractile activity (Fig. 24). After infusion of soya protein, frequency of antral motility increased and the pylorus showed a less intense contractile activity. When the abomasum was infused with a $KHCO_3$ buffer, however, intensity of antral motility increased. Since both of these intra-abomasal infusates stimulated the release of gastrin, it was concluded that these different effects cannot be explained by gastrin only.

Minor effects on abomasal motility were induced by infusion of α-alanine, β-alanine and glycine. Methionine, however, caused a significantly decreased frequency of abomasal pyloric contractile activity, possibly decreasing abomasal outflow rate of digesta as was found after continuous intra-abomasal infusion of methionine. These findings are consistent with methionine being an active releaser of the enterohormone cholecystokinin, which inhibits gastric motility in non-ruminant mammals. After infusion of butyric or stearic acids into the abomasum, frequency of antral motility was slightly but significantly increased. These results for butyric acid were ascribed to accumulation of butyric acid in the abomasal mucosa. In the literature, long-chain fatty acids have been mentioned as active releasers of cholecystokinin. The present data obtained after stearic acid infusion were not in line with such an effect on the release of cholecystokinin.

The infusion experiments allow no definite conclusion on the relationship between abomasal motility and propulsion of abomasal digesta into the duodenum. More data are needed, preferably from experiments in which abomasal motility and duodenal passage rate of digesta are recorded simultaneously.

Samenvatting

Betrekkelijk weinig gegevens zijn beschikbaar over het functioneren van de lebmaag onder fysiologische omstandigheden. Het toenemende aantal lebmaagulcera en -bloedingen bij melkkoeien en de verondersteld relatie van deze afwijkingen tot de samenstelling van het rantsoen, vormden de aanleiding een onderzoek te doen naar het functioneren van de lebmaag. Bij het schaap werden de effecten bestudeerd van verschillende rantsoencomponenten en van pensfermentatieprodukten op de zuursecretie in de lebmaag, de mucosadoorbloeding, de motoriek en de gastrine-concentratie in bloedplasma.

Hoofdstuk 2 geeft een overzicht van de literatuur over de secretie van zoutzuur, van pepsinogeen en van slijm, de doorbloeding van de mucosa en de motoriek in relatie tot de voortstuwing van de digesta. Tevens werd aandacht geschonken aan de neurale en hormonale regulatie van deze processen. Daar de literatuur over de lebmaag beperkt is en doordat de maag van éénmagige dieren en de lebmaag zowel morfologisch als functioneel in hoge mate vergelijkbaar zijn, werd eveneens gebruik gemaakt van gegevens over de enkelvoudige maag.

In de hoofdstukken 3 t/m 8 zijn de verschillende experimenten en de toegepaste methoden beschreven. De resultaten van deze experimenten zijn hieronder samengevat.

Hoofdstuk 3. In drie voederproeven werd de zuursecretie in de lebmaag bestudeerd van schapen die voorzien waren van re-entrant canules in het proximale duodenum. De zuursecretie in de lebmaag werd bepaald aan de hand van de hoeveelheden zuur en chloride die het proximale duodenum passeerden.

In de eerste voederproef (paragraaf 3.3) werden drie verschillende rantsoenen aan drie schapen verstrekt. De grootste zuursecretie in de lebmaag werd gemeten, wanneer hooi of gras werd aangeboden. Het gehalte aan ruw eiwit in het derde rantsoen, bestaande uit raaigrasstro en semisynthetische bestanddelen (tabel 1), was lager, resulterende in een minder sterk gestimuleerde zuursecretie in de lebmaag.

In de tweede voederproef (paragraaf 3.4) werden vier schapen die naast de hiervoor genoemde canules voorzien waren van een penscanule, gevoerd met zestien verschillende rantsoenen. Deze rantsoenen waren samengesteld uit een vast basisrantsoen van raaigrasstro en semisynthetische stoffen waaraan waren toegevoegd vier variabele rantsoencomponenten, cellulose, soja-eiwit, $KHCO_3$ en maïszetmeel, in alle mogelijke combinaties (tabel 2). De grootste zuursecretie in de lebmaag werd gemeten na toevoeging van soja-eiwit of $KHCO_3$ aan het basisrantsoen. Toevoeging van cellulose en maïszetmeel had een geringer effect op de zuursecretie, zowel direct als door middel van de produktie van vluchtige vetzuren in de voormagen. Geen relatie was aantoonbaar tussen de concentraties van de vluchtige vetzuren in de pens en de zuursecretie in de lebmaag.

In de derde voederproef (paragraaf 3.5) werd eveneens het effect van de samenstelling van het rantsoen op de zuursecretie in de lebmaag van drie schapen nagegaan. In deze proef bleek de digestadoorstroming in het duodenum afhankelijk te zijn van de tijdstippen waarop

de rantsoenen werden verstrekt. De grootste digestapassage in het duodenum werd waargenomen tijdens de uren voordat de dieren werden gevoerd (fig. 6). Verstrekken van gras resulteerde in de sterkst gestimuleerde zuursecretie in de lebmaag. Na vervanging van de helft van het grasrantsoen door hooi bleek de zuursecretie kleiner te zijn. Een rantsoen bestaande voor de helft uit hooi, aangevuld met semisynthetische componenten van een chemische samenstelling overeenkomende met die van het gras, bewerkstelligde een grotere digestadoorstroming in het duodenum in vergelijking met het hooi en grasrantsoen, doch een niet afwijkende zuursecretie.

In het algemeen resulteerde in deze voederproeven een hoger eiwitgehalte in het rantsoen in een sterker gestimuleerde zuursecretie in de lebmaag. Hoog significante positieve correlatiecoëfficiënten werden berekend tussen de duodenumdoorstroming aan zuur en ruw eiwit en tussen die aan chloride en ruw eiwit (tabellen 7, 12 en 15).

In de lebmaag wordt het zuur door de wandcellen van de tubuleuze fundusklieren gesecerneerd als zoutzuur, resulterende in een verlaging van de pH simultaan met een verhoging van de chlorideconcentratie van de lebmaagdigesta. De duodenumdoorstroming aan chloride was in deze voederproeven sterk positief gecorreleerd met die aan zuur, maar de regressiecoëfficiënten waren significant hoger dan 1, waarschijnlijk als gevolg van het chloride uit het voer en van het door de niet-wandcellen gesecerneerde chloride (figuren 3, 5 en 7).

Als men aanneemt dat de chlorideconcentratie in lebmaagsap gelijk is aan 155 mmol/liter, dat in de tweede voederproef de chlorideconcentratie van de pensnetmaag-digesta werd verdubbeld in de boekmaag, en dat in de eerste en in de derde voederproef de hoeveelheid in de lebmaag gesecerneerd chloride gelijk was aan 80% van de duodenumdoorstroming aan chloride, dan bestaat meer dan de helft van de lebmaagdigesta uit lebmaagsap.

Hoofdstuk 4. Bij schapen die voorzien waren van een lebmaag-infusieslang, van reentrant canules in het proximale duodenum, en in enkele gevallen van een penscanule, werd het effect gemeten van continue infusies in de lebmaag op de zuursecretie. Deze infusies werden gestart ongeveer 40 uur voordat de duodenuminhoud werd bemonsterd gedurende een periode van 8 uur. Het bestuderen van de lebmaagsecretie volgens deze infusietechniek vereist dat de hoeveelheid digesta die de lebmaag binnenstroomt, niet wordt beïnvloed door de continue infusies in de lebmaag. In het eerste experiment bleken na continue infusie van polyethyleenglycol (PEG) in de pens noch de PEG-concentratie in de pens, noch de in het duodenum teruggevonden hoeveelheid PEG te worden beïnvloed door infusie in de lebmaag van soja-eiwit (paragraaf 4.3). In dit experiment, evenals in de volgende, was in het algemeen de stijging van de hoeveelheid droge stof en ruw eiwit die het duodenum passeerden, vergelijkbaar met de in de lebmaag geïnfundeerde hoeveelheid droge stof en ruw eiwit. Uit deze resultaten werd geconcludeerd, dat de continue infusies in de lebmaag de digestapassage vanuit de boekmaag naar de lebmaag niet beïnvloedden, en dat het effect van continue infusies in de lebmaag op de zuursecretie berekend kon worden uit de duodenumpassage aan zuur en chloride in vergelijking met die in de controle-experimenten.

Infusie in de lebmaag van deze schapen van een soja-eiwit-suspensie in twee doseringen bewerkstelligde een geringe stimulatie van de lebmaag-zuursecretie (paragraaf 4.3). Het grootste effect werd gevonden na infusie van de grootste dosis soja-eiwit-suspensie (tabel 17).

Men neemt aan dat eiwitten de zuursecretie in de lebmaag via gastrine op tweeërlei

wijze beïnvloeden. In de literatuur zijn lager-moleculaire eiwitbrokstukken beschreven als actieve stimulatoren van de afgifte van gastrine, een maagdarmwandhormoon dat de zuursecretie stimuleert. In het algemeen hebben eiwitten een hoge buffercapaciteit. Doordat bij een hogere pH de afgifte van gastrine minder geremd is, wordt van eiwitten eveneens aangenomen, dat zij de afgifte van gastrine indirect stimuleren door hun invloed op de pH van de lebmaaginhoud. Na infusie in de lebmaag in vergelijkbare doseringen van verschillende eiwitten en van een fosfaatbuffer waren de effecten op de zuursecretie niet volledig te verklaren uit de buffercapaciteit van de verschillende lebmaaginfusen (fig. 8). Derhalve werd vastgesteld dat eiwitten van invloed zijn op de zuursecretie in de lebmaag niet alleen door hun buffercapaciteit, maar waarschijnlijk ook door meer specifieke factoren zoals hun aminozuursamenstelling.

Dat anorganische buffers inderdaad de zuursecretie in de lebmaag stimuleren, werd bevestigd (paragraaf 4.5). Na continu infunderen van $KHCO_3$-buffers van verschillende concentratie in de lebmaag van twee schapen waren de hoeveelheden zuur en chloride die het duodenum passeerden, vergroot (tabel 20). In verhouding tot de buffercapaciteit van het infuus echter werd de zuursecretie minder sterk gestimuleerd door de meer geconcentreerde buffers (fig. 10). Klaarblijkelijk wordt de zuursecretie minder sterk gestimuleerd bij een hogere secretie-activiteit van de lebmaag. Waarschijnlijk beïnvloeden infusies in de lebmaag en de dientengevolge verhoogde secretie-activiteit van de lebmaag de uitstroomsnelheid van de digesta vanuit en de retentietijd van de digesta in de lebmaag. Bovendien zal naarmate de secretie meer wordt gestimuleerd de lebmaagsecretie-activiteit haar maximale niveau benaderen.

Van de aminozuursamenstelling van eiwitten werd aangenomen, dat deze van belang is voor de stimulatie van de zuursecretie in de lebmaag. Infusie van α-alanine, β-alanine, asparaginezuur, arginine, glycine en methionine in de lebmaag van drie schapen bleek echter niet van invloed te zijn op de lebmaag-zuursecretie (paragraaf 4.6, tabel 21). Deze resultaten wekten verwondering voor wat betreft β-alanine en glycine, aminozuren die beschreven zijn als actieve stimulatoren van de gastrine-afgifte bij de hond. Na infusie van methionine was de lebmaag-zuursecretie significant verkleind, waarschijnlijk indirect door een remmende werking van methionine op de digestapassage door de lebmaag.

Volgens de literatuur zijn de lager-moleculaire fragmenten in eiwit-hydrolysaten actieve stimulatoren van de afgifte van gastrine bij de hond. Na infusie in de lebmaag van drie schapen van soja-eiwit dat gedeeltelijk was gehydrolyseerd, was de lebmaag-zuursecretie groter dan na infusie van soja-eiwit als zodanig (paragraaf 4.7, tabel 22).

Infusie in de lebmaag van drie schapen van azijnzuur, propionzuur, boterzuur en L-melkzuur in hoeveelheden die groter waren dan die normaliter vanuit de boekmaag de lebmaag bereiken, resulteerde niet in een sterk vergrote lebmaag-zuursecretie (paragraaf 4.8, tabel 23). Er werd derhalve verondersteld dat onder fysiologische omstandigheden aan de vluchtige vetzuren en aan L-melkzuur geen bijzondere betekenis voor de lebmaag-zuursecretie behoeft te worden toegekend, behalve dan dat ze medebepalend zijn voor de buffercapaciteit van de digesta die de lebmaag binnenstromen.

In de gevallen dat er sprake was van een stimulatie van de zuursecretie, resulteerden de infusies in de lebmaag in vergrotingen van de hoeveelheden zuur en chloride die het proximale duodenum passeerden, die onderling niet significant verschilden (figuren 9, 11,

13, 16 en 18). Dit duidt erop dat onder de gegeven experimentele omstandigheden de stimulatie van de lebmaagsecretie resulteerde in een stimulatie van de wandcellen van de tubuleuze fundusklieren, waarvan wordt aangenomen dat deze nagenoeg isotonisch zoutzuur afscheiden.

Stimulatie van de lebmaagsecretie door continue lebmaaginfusies of anderszins door het eiwitgehalte van het rantsoen te verhogen ging niet gepaard met een daling van de pH van de lebmaagdigesta. Blijkbaar was de buffercapaciteit van de lebmaagdigesta-componenten die de lebmaagsecretie stimuleerden, groter dan hun stimulatief effect op de lebmaag-zuursecretie.

Hoofdstuk 5. Infusie in de lebmaag van drie schapen die voorzien waren van een lebmaag-infusieslang en van een katheter in een van de uitwendige halsvenen, van een suspensie van gedeeltelijk gehydrolyseerd soja-eiwit en van een $KHCO_3$-buffer bewerkstelligde een verhoogde gastrine-concentratie in het bloedplasma. In verhouding tot de buffercapaciteit van de infusen stimuleerde de soja-eiwit-suspensie de afgifte van gastrine het sterkst (tabel 25). Geconcludeerd werd dat stimulatie van de lebmaag-zuursecretie door eiwitten en anorganische buffers deels tot stand komt via gastrine. Infusie van α-alanine, ß-alanine en glycine in de lebmaag van vier schapen bleek niet van invloed te zijn op de bloedplasma-gastrine-concentratie (tabel 26). Deze bevinding stemde overeen met de waarneming dat infusie van deze aminozuren in de lebmaag de zuursecretie niet deed toenemen.

Hoofdstuk 6. In sommige van de voorgaande experimenten werd eveneens de pepsine-activiteit bepaald in de duodenumdigesta. Bij een hoog eiwitgehalte in het rantsoen of na infusie van eiwitten in de lebmaag werd in het algemeen een lagere pepsine-activiteit in de duodenumdigesta gemeten. Infusie in de lebmaag van $KHCO_3$-buffers had een licht verhoogde pepsine-activiteit van de duodenumdigesta tot gevolg. Het bleek echter niet geoorloofd om uit de pepsine-activiteit van duodenumdigesta conclusies te trekken ten aanzien van de lebmaag-pepsinogeensecretie. In experimenten in vitro bleken eiwitten de pepsine-activiteit te remmen, waarschijnlijk door de eiwit-afbraakprodukten (fig. 19).

Hoofdstuk 7. In de lebmaag van drie schapen die voorzien waren van een lebmaag-infusieslang, van re-entrant canules in het proximale duodenum en van een katheter in de beide uitwendige halsvenen, werd een soja-eiwit-suspensie en een $KHCO_3$-buffer geïnfundeerd. Na deze infusies en in één van de beschreven voederproeven werd de relatie bestudeerd tussen de lebmaag-zuursecretie en de lebmaagmucosa-doorbloeding. De doorbloeding van de mucosa werd gemeten volgens de aminopyrine-clearance-methode. Zelfs na stimulatie van de lebmaag-zuursecretie met 30% was een verhoogde mucosa-doorbloeding niet waarneembaar (tabellen 27 en 28). De lebmaag-zuursecretie is onder fysiologische omstandigheden derhalve niet noodzakelijkerwijze direct gerelateerd aan de lebmaagmucosa-doorbloeding. Na stimulatie van de lebmaagsecretie-activiteit kan wellicht aan de grotere behoefte aan zuurstof en substraten worden voldaan door een grotere extractie van deze stoffen uit het bloed.

Hoofdstuk 8. Van lebmaagdigesta wordt aangenomen dat zij voortgestuwd worden naar het duodenum door de contractie-activiteit van het antrum en de pylorus van de lebmaag. De contracties van het antrum en de pylorus werden geregistreerd met behulp van rekstrookjes die waren aangebracht op de lebmaagwand van schapen voorzien van een lebmaag-infusieslang. De motoriek van de lebmaag bleek in vergelijking met die van de maag van niet-herkauwende zoogdieren regelmatiger te zijn. De regelmaat van de contractie-activiteit was het grootst op de pylorus in vergelijking met die in het antrum, waar sterke contracties werden afge-

wisseld met tamelijk zwakke contracties. De contractie-activiteit van zowel antrum als pylorus toonde afwisselende perioden van meer en minder sterke intensiteit (figuren 20 en 21).

Registraties van de antrum- en pylorusmotoriek in relatie tot het tijdstip van voeren in een schaap dat twee keer per dag werd gevoerd, gaven geen duidelijk patroon te zien (fig. 22). In de periode na het voeren echter tendeerde de pylorusmotoriek te domineren, terwijl dat later het geval was voor de antrummotoriek. Dit motoriekpatroon in relatie tot het tijdstip van voeren leidde tot de veronderstelling dat mogelijk de digestapassage in het proximale duodenum het grootst is gedurende perioden waarin de antrummotoriek domineert. Continu infunderen van soja-eiwit in de lebmaag resulteerde niet alleen in een vergrote duodenumpassage van zuur en chloride, maar ook in die van digesta. Deze continue infusies van soja-eiwit in de lebmaag van één schaap waren echter niet van invloed op de contractie-activiteit van antrum en pylorus (tabel 31). Derhalve zijn andere factoren mede bepalend voor de voortstuwing van de digesta vanuit de lebmaag, zoals bijvoorbeeld tonische contracties van de lebmaagfundus.

Discontinue infusies in de lebmaag van twee schapen gaven enig effect te zien op de contractie-activiteit van antrum en pylorus (fig. 24). Na infusie van soja-eiwit was de frequentie van de antrummotoriek verhoogd en de intensiteit van de pylorusmotoriek verlaagd. Infusie van een $KHCO_3$-buffer resulteerde in een verhoogde intensiteit van de antrummotoriek. Daar, zoals uit eerdere experimenten bleek, beide lebmaaginfusen de afgifte van gastrine stimuleren, werd geconcludeerd dat deze verschillende effecten niet alleen door gastrine te verklaren zijn.

Infusie van α-alanine, β-alanine en glycine hadden slechts geringe invloed op de motoriek. Methionine veroorzaakte echter een significante daling in de frequentie van de pylorusmotoriek, mogelijk resulterende in een verkleinde digestapassage door het duodenum, zoals werd gevonden na infusie van methionine in de lebmaag. Deze bevindingen waren in overeenstemming met de stimulerende werking van methionine op de afgifte van het maagdarmwandhormoon cholecystokinine dat de maagmotoriek in niet-herkauwende zoogdieren remt. Na infusie in de lebmaag van boterzuur en stearinezuur bleek de frequentie van de antrummotoriek licht te zijn gestegen. Voor boterzuur werd dit resultaat toegeschreven aan accumulatie van boterzuur in de lebmaagmucosa. In de literatuur zijn hogere vetzuren aangegeven als stimulatoren van de cholecystokinine-afgifte. De huidige resultaten waren echter niet in overeenstemming met een dergelijk effect van stearinezuur op de afgifte van cholecystokinine.

De uitgevoerde infusie-experimenten staan geen eenduidige conclusie toe met betrekking tot het verband tussen de lebmaagmotoriek en de voortstuwing van de digesta vanuit de lebmaag naar het duodenum. Meer gegevens zijn gewenst, bij voorkeur van experimenten, waarin de lebmaagmotoriek en de digestapassage door het duodenum tegelijkertijd worden geregistreerd.

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