AN INVESTIGATION OF THE DISTRIBUTION OF GASTRIN-LIKE ACTIVITY, AND ITS NATURE, IN THE GASTROINTESTINAL TRACT OF THE SHEEP

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SUMMARY

Using a modification of the extraction procedure of Gregory and Tracy (1964), and a perfused rat stomach bioassay, gastrin-like activity has been located in the ovine rumen, reticulum, omasum, abomasal body, antrum, duodenum and caecum. The antrum showed the highest concentration of gastrin-like activity, but substantial concentrations were found in the rumen, reticulum, omasum and caecum and proximal duodenum declining progressively with distance from the pylorus. A lesser concentration occurred in the abomasal body. Consideration of the relative total content of gastrin-like activity showed that the amount of extra-antral activity was substantial.

The active principle was unlikely to have been cholecystokinin as the extracts had isoelectric points of a more acidic nature than that of cholecystokinin. Similarly, bioassay using an isolated guinea-pig ileum showed histamine to be absent from the extracts.

Extraction of rumenal, reticular, omasal, abomasal body and antral mucosa and muscle separately showed that the gastrin-like activity resided almost completely in the mucosa.

An investigation of the gastrin-like activity of air dried and freeze dried extracts showed that the freeze dried

extracts were approximately 50% more active than the air dried extracts. Bioassay of a freeze dried antral extract over a twenty four week period showed that the activity was maintained for the initial six weeks, and that 80% of the activity was then lost over the following eighteen weeks.

Studies using columns of Sephadex have shown that extracts from all regions of the ovine gastro-intestinal tract investigated were similar and consisted of several components of differing molecular weight. By a comparison of their respective molecular weights, to those reported for components separated from extracts of non-ruminant origin, it seems likely that components corresponding to MG, HLG, BG, BBG and component I were present in varying amounts.

The response to infusions of gastrin extract in fed and fasted sheep were different. In fasted sheep infusions of extract, after a relatively long latent period, caused a small, but prolonged increase in acid secretion. In contrast pentagastrin caused an immediate and pronounced response. In fed sheep the responses were variable.

Experiments on a single dolphin (Delphinus delpis) have shown there to be gastrin-like activity present in the forestomach, mainstomach, pyloric stomach, duodenal ampulla and intestine of this mammal.

The distribution of gastrin-like activity reported in

the present work has been compared to previous reports concerning the distribution of gastrin activity in animals with a more simple stomach, and the possible function of gastrin in the ruminant has been discussed.

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ABBREVIATIONS

Ab.body	abomasal body
Abs.	absorbance
Ach	acetycholine
A.D.	air dried
Ala	alanine
am	before noon
An.	antrum
Asp.	aspartic acid
BBG	'big big gastrin'
BG	'big gastrin'
b.w.	body weight
^{o}C	degrees celsius
Ca.	caecum
CCK	cholecystokinin
CCK/PZ	cholecystokinin/pancreozymin
cm	centimetre
com.1	component 1
Du.	duodenum
et al.	and others
ext.	extract
F.D.	freeze dried
Fig.	figure
Fu.	fundus
g	gram
GI	gastrin I
GII	gastrin II

Glu.	glutamic acid
Gly.	glycine
H^+	hydrogen ion
H-LG	heptadecapeptide-like gastrin
hr	hour
i.e.	that is
Kg	kilogram
l	litre
Leu.	leucine
log	logarithm
M	molar
m-equiv	milli-equivalent
Met.	methionine
MG	'mini gastrin'
mg	milligram
min	minute
ml	millilitre
mm	millimetre
m mol	millimole
muc	mucosa
mus	muscle
M.W.	molecular weight
ng	nanogram
nm	nanometre
Om.	omasum
penta.	pentagastrin
%	per cent
pg	picogram

Phe	phenylalanine
Pro	proline
Re	reticulum
Ru	rumen
S.D.	standard deviation
S.D.S.	sodium dodecyl sulphate
sec	second
S.E.M.	standard error of the mean
Trp.	tryptophane
Tyr.	tyrosine
µ-equiv	microequivalent
µg	microgram
µl	microlitre
µ mol	micromole
Val.	valine
vol.	volume
wt.	weight
wt./vol.	weight for volume
Z.E.	Zollinger-Ellison

CHAPTER I:

GASTRIN

Introduction:

In 1824 an English chemist named Prout, discovered that the highly acid nature of gastric juice was due to the presence of hydrochloric acid. William Beaumont, from 1825 to 1833, contributed greatly to the knowledge of gastric secretion by his pioneering experiments on Alexis St. Martin who had a fistulous opening in his stomach as a result of a gun shot wound. Since that time physiologists have elucidated many of the mechanisms of the production and of the control of this acid secretion.

Gastric secretion is regulated by both neural and hormonal mechanisms. The gastric mucosa is innervated by both divisions of the autonomic nervous system. The parasympathetic innervation provides the pathways for secretory stimuli to the gastric mucosa, while the sympathetic pathways regulate gastric secretion indirectly, due to their control over vasomotor mechanisms and blood flow to the mucosa.

A number of hormonal mechanisms are also involved in the regulation of gastric secretion. For example, the participation of the hormone gastrin in the control of gastric acid secretion is definitely established. The

conclusion is that gastrin is produced and released by the antral glandular mucosa into gastric venous blood, in response to nervous and chemical stimuli (Grossman, 1967). The nervous stimuli include vagal, vago-vagal and local reflexes, while the local action of protein digestion products and alcohol provide a chemical stimulation of gastrin release.

History of Gastrin

As early as 1879 Heidenhain reported that the presence of food in the stomach caused a separate pouch to secrete. Heidenhain's conclusion was that the secretagogues were products of digestion and were absorbed from the stomach into the blood and carried to the pouch (Johnson, 1973).

J.S. Edkins (1905, 1906) formulated the gastrin hypothesis when he reported to the Royal Society that extracts of pyloric mucosa stimulated gastric secretion when injected intravenously into anaesthetized cats. He concluded that the active principle of the extracts (to which he gave the name gastrin) exercised a hormonal role in the stimulation of gastric secretion following a meal. However, those workers who attempted to confirm Edkin's reports tested their extracts on conscious dogs provided with gastric fistulas or pouches, and generally found that their extracts were inactive when given intravenously, or that extracts not only from the antral mucosa, but also from most other tissues, displayed similar activity

to Edkin's extracts, (Gregory, 1967). When this ubiquitous secretagogue was eventually identified as histamine (Barger & Dale, 1910; Dale and Laidlaw, 1911; Popielski, 1919) it was generally believed that the activity of Edkin's extracts was most probably attributable to this substance. The situation was further confused by the failure of apparently well-designed attempts to prove by physiological experiment that an antral hormone existed (Ivy & Whitlaw, 1922; Priestley & Mann, 1932). Probable reasons for such failures include the acidification of the antral contents, which suppress the release of gastrin (Woodward, Lyon, Landor and Dragstedt, 1954). Secondly, the antral pouches may have contained some mucosa from the body of the stomach and hence the contents were always acid. Finally, with antral and fundic vagal denervation it becomes more difficult to show a release of gastrin (Gregory, 1968).

Komarov (1938, 1942a and b) demonstrated that gastrin was of protein nature and distinct from histamine, and had probably been discarded at the onset of extractions by previous workers who had sought to clarify their crude extracts by deproteinization. From aqueous extracts of antral mucosa Komarov was able to precipitate a protein fraction, which contained no significant amount of histamine, but was a potent stimulant of acid secretion when injected intravenously into an anaethetized cat.

Komarov's work was confirmed by Uvnäs (1943a and b,

1945a), and Munch-Peterson, Ronnow and Uvnäs (1944) who used the same initial method of extraction, followed by various methods of concentrating the activity, based on precipitation from aqueous solutions. Harper (1946) reported a method of preparing gastrin by a methanol extraction. These workers tested the activity of their extracts by intravenous injection into anaesthetized cats and also noted a stimulant effect on pepsin secretion, and a small amount of secretin-like activity.

Jorpes, Jalling and Mutt (1952) introduced a method for the extraction of gastrin which involved the treatment of the antral mucosa with acid methanol which gastrin was said to be soluble in. The pH was adjusted and an active precipitate was obtained. However, initially the antra were boiled to stop enzyme action which, in the light of the subsequent work (Gregory and Tracy, 1961), would also have removed most of the gastrin present.

Gregory and Ivy (1941) offered the first substantial physiological evidence for the existence of a gastric hormone, but were unable to show that it originated from the antral region. Conclusive proof that a gastric antral hormone existed was eventually provided by Grossman, Robertson and Ivy (1948), and this was soon supported by further evidence provided by Dragstedt, Woodward, Oberhelman, Stover and Smith (1951) and Oberhelman, Woodward, Zubiran and Dragstedt (1952). Although these studies established the existence of an antral hormone

the methods of extraction and purification were still relatively crude.

The final purification was left to Gregory and colleagues (Gregory and Tracy, 1964; Tracy and Gregory, 1964) who described the isolation, purification and synthesis of gastrin from porcine antral mucosa. The active material consisted of two almost identical heptadecapeptide amides, each of which was a potent stimulant of gastric acid secretion.

It is believed that these peptides represent some form of the antral hormone (Gregory, 1968) and are usually referred to as gastrins I and II.

The Actions of Gastrin

The crude gastrin extracts obtained by many of the early workers, in addition to being stimulants of gastric acid secretion, displayed a number of motor and secretory actions, which were assumed to be caused by impurities. Recent studies with purified gastrin, the terminal tetrapeptide and pentagastrin have shown a wide range of physiological and pharmacological actions. These have been summarised by Thompson (1969), and the more important actions are discussed below.

Stimulation of Gastric Acid Secretion:- Both gastrins I and II are potent stimulants of gastric acid secretion.

In dogs with Heidenhain pouches, gastrin is 570 times as potent as histamine on a molar basis (Davidson, Daves, Lemmi, Miller and Thompson, 1966), although the maximal acid output is slightly less than that with histamine (Cooke, 1967). In man, gastrin is 500 times as potent as histamine on a molar basis, and results in a greater maximal acid output than histamine (Markhlouf, McManus and Card, 1964).

In conscious dogs, secretion from a fundic pouch of the body of the stomach can be maintained in response to slow intravenous infusion or repeated subcutaneous injections of gastrin. If, during this time, there is superimposed a rapid intravenous injection of gastrin, a complete inhibition of the acid secretion will occur (Gregory and Tracy, 1964b). In man and also in the cat this effect cannot be observed, but it can occur in the anaesthetized rat (Barrett, Raventos and Sidall, 1966).

Pepsin Secretion:- The gastrin peptides are moderate stimulants of pepsin secretion when given in physiological doses, but they stimulate strongly when given in pharmacological doses (Gregory and Tracy, 1964). Their action, however, is much weaker than vagal stimulation, or the action of cholinomimetic drugs, but considerably greater than histamine (Cooke, 1967). It has been demonstrated that endogenous gastrin stimulates pepsin secretion (Dragstedt, Walton and Woodward, 1963), although recent evidence

indicates that the presence of acid stimulates pepsin secretion (Johnson, 1972).

Pancreatic Secretion:- It has been reported that gastrin weakly stimulates pancreatic volume flow and enzyme secretion (Gregory and Tracy, 1964; Preshaw, 1966), and that this response is not due to the entry of acid into the duodenum (Preshaw, Cooke and Grossman, 1965).

Biliary Tract:- In the anaesthetized dog a slight contraction of the gall bladder and stimulation of hepatic bile flow in response to intravenous injections of gastrin have been reported (Gregory and Tracy, 1964).

Gastrointestinal Tone and Motility:- Rapid intravenous injections of gastrin will stimulate tone and motility in a completely denervated fundic pouch, and contraction, followed by prolonged inhibition, in a Thiry loop of jejunum in conscious dogs (Gregory and Tracy, 1964). Pharmacological doses of gastrin will cause contractions of isolated guinea-pig ileum (Bennett, 1965; Mikos and Vane, 1967).

Metabolic Effects:- It has been reported that the chronic administration of pentagastrin produces parietal cell hyperplasia in the rat (Crean, Marshall and Rumsey, 1969) and that pentagastrin (Johnson, Aures and Yen, 1969) and synthetic human gastrin (Johnson, Aures and Hakanson, 1969)

stimulates the *in vitro* uptake of leucine into the protein of gastric and duodenal mucosa, but not into the liver, or skeletal muscle. The evidence presented by Martin, MacLeod and Sircus (1970) indicating that antrectomy leads to atrophy of the gastric mucosa and a decrease in the mass of both parietal and chief cells, and the close association between serum and antral gastrin levels (measured by radioimmunoassay), and the growth of gastrointestinal tract tissue (Johnson, 1976) would indicate that this trophic action of gastrin is physiological. As these metabolic actions of gastrin are not duplicated by histamine (Willems, Van Steenkiste and Limbosch, 1972) it seems likely that the observed trophic action of gastrin is independent of its secretory effect.

A more detailed account of the metabolic effects of the gastrointestinal hormones can be found in a recent review (Johnson, 1976).

Structure and Chemistry of Gastrin

Gastrin is an acidic peptide amide having seventeen linearly arranged amino acids and a molecular weight of approximately 2,100 (Anderson, Barton, Gregory, Hardy, Kenner, MacLeod, Preston, Sheppard and Morley, 1964). Gastrin exists in two nearly identical forms, differing only in the presence of (gastrin II), or absence of (gastrin I) a sulphate group on the tyrosyl residue at position 12. However, the two gastrins have a similar

Table 1 Species variation in some mammalian gastrins (from Thompson, 1973).

Species	Approximate molecular weight		Position														
	Gastrin I	Gastrin II	1				5				10					15	
Pig	2114	2194	Glu.	Gly.	Pro.	Trp.	Met.	Glu.	Glu.	Glu.	Glu.	Glu.	Ala.	Tyr.	Gly.	Trp.	Met.	Asp.	Phe. NH_2
Human	2096	2176					Leu.							SO_3H					
Cow and Sheep	2024	2104					Val.						Ala.						
Dog	2038	2118					Met.												
Cat	2040	2120					Leu.						Ala.						

potency in stimulating acid secretion (Gregory and Tracy, 1964). Gastrin has been isolated from a number of mammals and Table 1 summarises the known sequence variation between species in the gastrin molecule.

Radioimmunoassay techniques have shown that gastrin exists in several forms (Yalow and Berson, 1971a and b); one with a molecular weight of approximately 2,100 which corresponds to Gregory and Tracy's (1964) heptadecapeptide gastrin, and another more basic form which has a molecular weight of about 7,000. Further reports have indicated a gastrin peptide with a molecular weight which is less than heptadecapeptide gastrin, and greater than 30,000 (Walsh, 1975).

Structure function studies have shown that the entire range of physiological activities displayed by the natural gastrin peptides are possessed by the C-terminal tetrapeptide, Try. Met. Asp. Phe.-NH_2 (Tracy and Gregory, 1964). It is believed that the positions occupied by Trp., Met. and Phe. in the tetrapeptide are concerned with binding the hormone to its site of action, as substitutions of these amino acids do not result in the loss of activity (Morley, Tracy and Gregory, 1965; Morley, 1968a). It appears that the Asp. position plays an important part in the function of gastrin, as substitution at this position leads to complete inactivation of the molecule (Morley et al., 1965). On the basis that substitution of the

terminal amide group, and oxidation of the sulphur in the methionine group decreased activity, Morley (1968 (b)) has suggested that the ionized beta carboxyl group of Asp. brings about the action of gastrin by a proton transfer mechanism.

Although the N-terminal thirteen amino acids do not affect the range of activities of the gastrin molecule, they do affect the potency. The first five N-terminal amino acids have little effect, but the removal of further residues results in a marked reduction of potency, such that the C-terminal tetrapeptide is about one-twelfth as potent as the whole hormone (Gregory and Tracy, 1964). An acyl derivative of the tetrapeptide, N-tert-butyloxy carbonyl-B-Ala. Try. Met. Asp. Phe.-NH_2, known as pentagastrin, is commercially available ("Peptavlon", I.C.I., England). Pentagastrin produces the same maximal acid responses as the natural gastrins, and has largely taken over from histamine in the assessment of gastric secretion in man and animals.

Mutt and Jorpes (1967) have reported that the C-terminal amino acid sequence of cholecystokinin/ pancreozymin (CCK/PZ) is identical to that of gastrin. This offers an explanation for the CCK/PZ like activities of gastrin, and the stimulatory effects of CCK/PZ on acid secretion (Preshaw and Grossman, 1965). On the basis of these reports, and the similar actions of gastrin,

CCK/PZ and secretin, Grossman (1970) has hypothesised that these hormones act on one receptor, and this receptor has two interacting sites. One site has affinity for gastrin and CCK/PZ and the other for secretin. The study of the efficacies of gastrin, CCK/PZ and secretin on their targets would support such a view (Johnson, 1973).

Gastrin Release

The release of antral gastrin is mediated by vagal, vago-vagal and local reflexes, and by the local action of some chemical substances. The local reflexes, via neurons in the stomach wall, are stimulated by distension of the stomach and by chemical substances (Grossman, 1967) which cause the release of sufficient gastrin to produce maximal rates of acid secretion (Cooke, Nahrwold, Preshaw and Grossman, 1967). Short chain aliphatic alcohols with two or three carbon atoms, particularly ethanol, and the amino acids glycine and B-alanine (Elwin, 1969) are potent stimulants of gastrin release.

In contrast to local reflexes, vagal stimulation releases only very small amounts of gastrin (PeThein and Schofield, 1959). However, gastrin and direct vagal stimulation of the parietal cell act synergistically causing an acid secretory response greater than either agonist acting alone (Grossman, 1973).

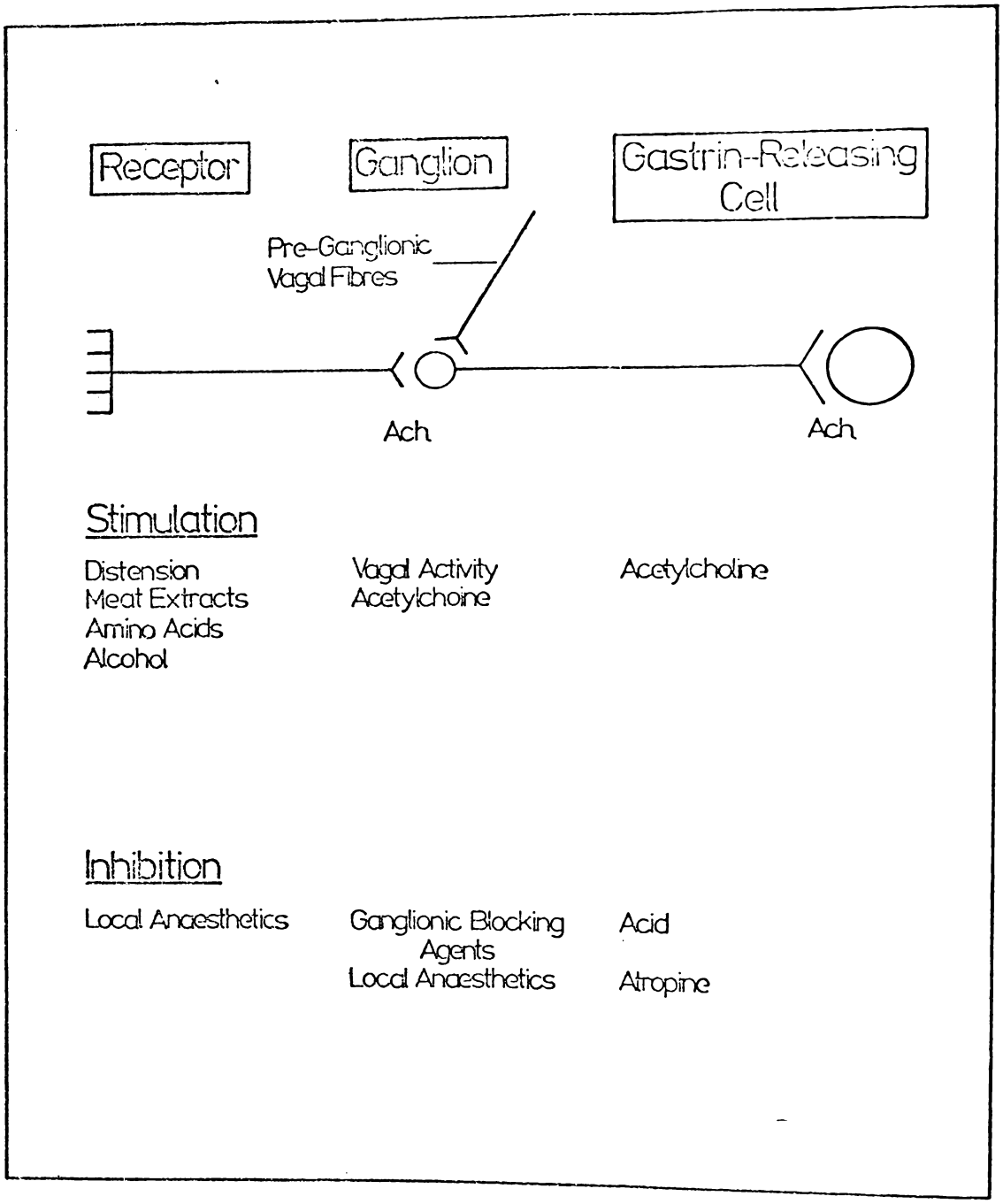

Fig. 1. Hypothetical scheme for the release of antral gastrin. (Grossman, 1967).

Antral release of gastrin is also influenced by a number of drugs. Local anaesthetics block the action of the nervous, mechanical and chemical stimuli, except that of acetylcholine. Atropine, applied locally, blocks the action of vagal, mechanical and chemical stimuli, and also that of acetylcholine. Ganglionic blocking agents given parenterally block the action of chemical and mechanical stimuli, but not the action of acetylcholine (Grossman, 1967). From the available evidence, Grossman (1967) has proposed the hypothetic release mechanism of antral gastrin shown in Fig. 1.

The release of antral gastrin is inhibited when the pH of the mucosa drops below 3.5. The mechanism of this inhibition is obscure, but it is known that a nervous reflex is not involved as local anaesthesia of the mucosa does not affect the response (Schofield, 1966). Most attention has been focussed on either the entrance of hydrogen ions into the mucosa, or on the effects of acid on mucosal receptors (Grossman, 1967). A recent report, however, has suggested that increasing acidity results in a reduced penetration into the mucosa of the stimulants of gastrin release (Berkowitz, Buetow, Walder and Praissman, 1971).

The Gastrin Cell

Gastrin activity of the human antrum is concentrated in the mucosa (Gregory and Tracy, 1966), and reports indicate

that the highest concentration of gastrin is located in the middle and basal parts of the antral mucosa of the cat, the dog and man (Broome, Fyro and Olbe, 1968). McGuigan (1968), using a technique of immunofluorescent staining has demonstrated the presence of "gastrin-cells" close to the basement membrane in porcine and human antral mucosa.

The Distribution of Gastrin

The most abundant source of gastrin is the antral or pyloric gland region of the stomach. Gastrin has been extracted, and chemically characterised, from the antral mucosa of the hog, human, cow, sheep, dog and cat (Kenner and Sheppard, 1973).

Studies on the distribution of gastrin-like activity in the gastrointestinal tract have given contradictory results. It has been reported that extracts from the cardiac mucosa of the hog (Edkins, 1905; Gregory and Tracy, 1961) and the cat (Lim, 1922; Emås and Fyrö, 1968) are stimulants of acid secretion. However, Uvnäs (1945) did not find any gastrin activity in extracts of hog cardia. Radioimmunoassay has shown there to be only very small amounts of gastrin in the cardia of the human, dog, cat and hog (Nilsson, Yalow and Berson, 1973). Gastrin activity has been isolated from the duodenum of the dog, hog, human and cat by some workers (Komarov, 1938, 1942; Uvnäs, 1945b; Harper, 1946; Lia, 1964; Emås and Fyrö, 1968; Nilsson, Yalow and Berson, 1973), but not in the hog and dog by others

(Uvnäs, 1943, 1945a; Gregory and Tracy, 1961; Elliot, Endahl, Knoernshild, Grant, Goswitz and Zollinger, 1963). Its presence in 1 of 4 extracts of cat duodenum was reported by Uvnäs (1943).

The contradictory reports concerning the distribution of extra-antral gastrin may represent differences in the extraction and assay procedures used. Radioimmunoassay has unequivocally shown the presence of gastrin in human, dog, cat and hog duodenum, jejunum and ileum, although there are considerable differences between these species in the amounts of extra-antral gastrin. Largest amounts of extra-antral gastrin, almost 40% of that in the antrum, were found in the human proximal duodenum (Nilsson et al., 1973).

The occurence of the intestinal phase of gastric acid secretion is well established (Grossman, 1967), but the role played by duodenal gastrin is unknown. Following gastrectomy in dogs, feeding produces a significant rise in serum gastrin level, indicating the release of extra-antral gastrin (Millar, Jackson and Thompson, 1970). Stern and Walsh (1973) have reported that serum gastrin levels measured by radioimmunoassay increase in patients with gastrectomies and gastro duodenal anastomosis, in response to feeding. Little change was noted in the post prandial serum gastrin levels of patients with gastrojejunal anastomosis. These results, together with the studies of

Yalow and Berson (1973) indicating the close similarity in the distribution of gastrin components in plasma to that in duodenal extracts, suggest that duodenal gastrin is of significance in the stimulation of gastric acid secretion.

Early reports indicated that the normal pancreas did not contain biological gastrin activity (Elliot et al., 1963; Hallenbeck, Code and McIlrath, 1963; Emås and Fyrö, 1968). However, radioimmunoassay has shown gastrin-activity to be present in the normal human pancreas, the average concentration being 4.0×10^{-10} g $.g^{-1}$ tissue, but no certain immunoreactivity has been detected in pancreatic tissue from the dog, cat or hog (Nilsson et al., 1973). The significance of gastrin, present in the pancreas, is unknown.

Reports from different laboratories have shown that the fasting serum gastrin concentrations in normal subjects range between $30 - 120 \times 10^{-12}$ g $.ml^{-1}$ (Yalow and Berson, 1970; Hansky and Cain, 1969; Gangali and Hunter, 1972). This variation is probably due to the different standards and antibodies used for radioimmunoassay (Berson, Walsh and Yalow, 1973).

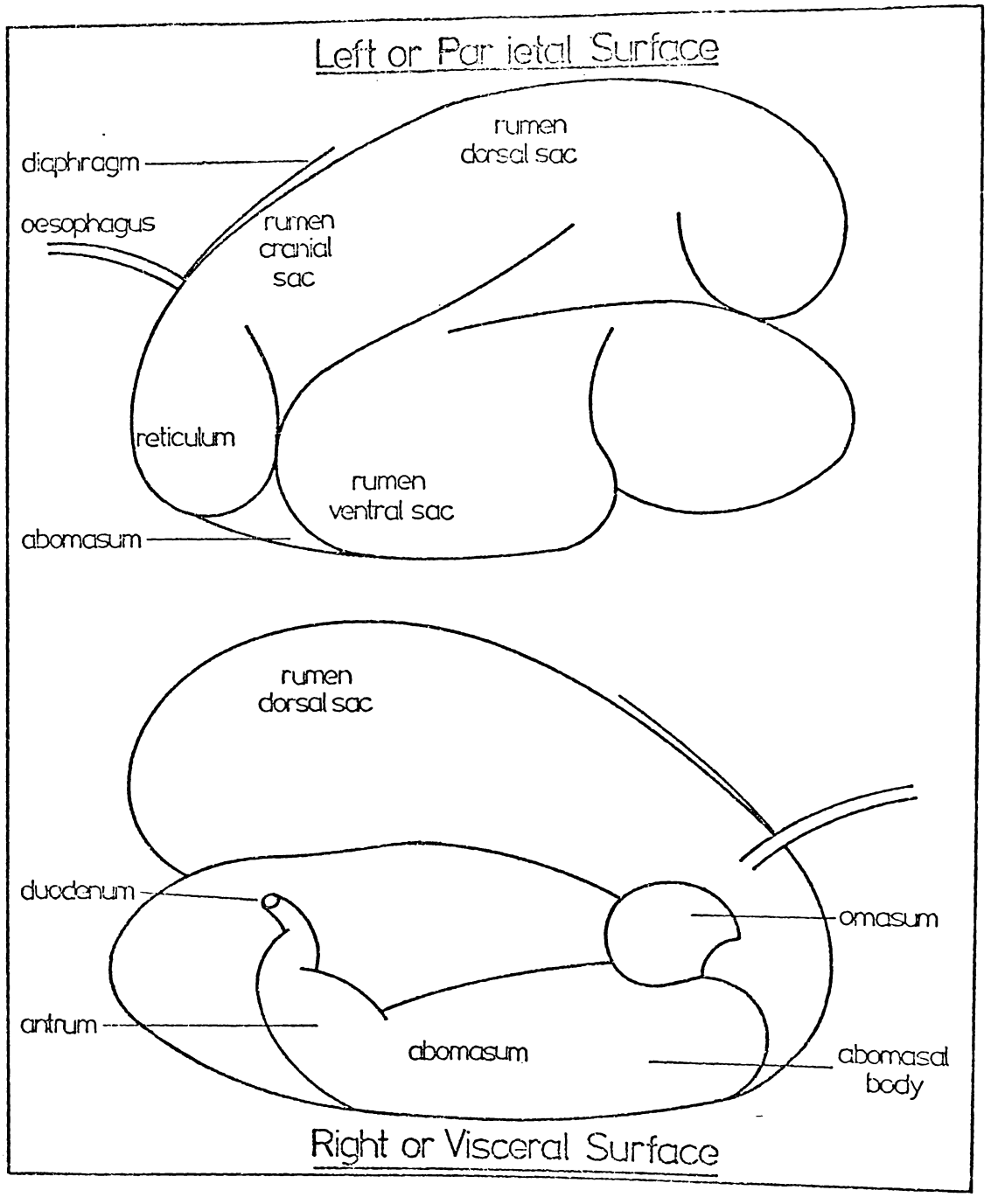

Fig. 2. The ruminant stomach (adapted from Habel, 1970)

THE RUMINANT

Introduction:

The ruminant stomach is divided into four chambers (Fig. 2); the rumen, the reticulum, the omasum and the abomasum. The rumen and reticulum form a fermentation vat where microbial digestion occurs on a large scale. The soluble products of fermentation are largely absorbed, and the material leaving the rumen represents a mixture of food residues, bacteria, protozoa and some soluble fermentation products.

From the omasum there is absorption of water, bicarbonate ions, and some fermentation products.

The abomasum constitutes the fourth division of the ruminant stomach, and corresponds morphologically and functionally to the gastric secretory stomach of other animals. The body region of the abomasum is comparatively wide, communicating cranially with the omasum through the omaso-abomasal orifice, and it is responsible for acid and pepsin secretion. The antral region communicates cranially with the fundic region and produces a mucous and slightly alkaline secretion. In the abomasum, material from the omasum is attacked and digested under acid conditions by the enzymes of the gastric juice.

The similarity of the stimuli involved in abomasal secretion (Hill, 1960, 1968; Ash, 1961a, 1961b; McLeay and Titchen, 1970, 1975, 1977) to the mechanisms involved in

the control of gastric secretion in simple stomached animals suggests the importance of gastrin in the control of abomasal secretory activity. The presence of gastrin activity in antral extracts of the abomasum (Anderson, Fletcher, McAlexander, Pitts, Cohen and Harkens, 1961; Anderson, Fletcher, Pitts and Harkens, 1962; Agarwal, Beacham, Bently, Gregory, Kenner, Sheppard and Tracy, 1968) supports this view.

Gastrin in the Ruminant

The number of papers dealing with the extraction and assay of gastrin from the ruminant gastrointestinal tract is small. Anderson et al. (1961) reported that a gastrin-like gastric secretory stimulant could be isolated from the pyloric and abomasal body mucosa of the bovine abomasum. Using a similar method of extraction and assay, Anderson, et al. (1962) reported that a gastrin-like stimulant could also be isolated from antral and abomasal body mucosa of the ovine abomasum. The bovine gastrin, although slightly less active, had a longer latency and a more prolonged effect than porcine gastrin prepared by the same method, when injected into conscious dogs (Anderson et al., 1961). This delayed secretory response was not displayed by their ovine gastrin extracts (Anderson et al., 1962).

Using the method of Gregory and Tracy (1964), Agarwal et al. (1968) have described the isolation of two gastrins

differing only in the presence of (gastrin II) or absence of (gastrin I) a sulphate group on the tyrosyl residue at position 12 (Table 1), from ovine and bovine antral mucosa. The activity of these two gastrins is identical with that described for the porcine gastrins.

Jury and McLeay (1974) isolated, by a modification of the method of Gregory and Tracy (1964), gastrin-like activity from the ovine antrum and abomasal body. Bioassay of the extracts using a perfused rat stomach preparation indicated that on a unit weight basis the antrum, contained five times more gastrin activity than the abomasal body. However, taking into account the relative weights of the respective regions it was apparent that a substantial amount of gastrin-like activity was present in the abomasal body.

Results published by McLeay and Titchen (1975) showed that a significant acid secretion occurred from pouches of the body of the abomasum after removing the antral region of the ovine abomasum. This further supported the presence of extra-antral gastrin, and studies on the distribution of gastrin in the sheep gastro-intestinal tract appeared to be warranted.

In the present study gastrin-like activity (hereafter referred to as gastrin activity) has been isolated from the antral and body regions of the ovine abomasum, and from ovine duodenum, caecum, rumen, reticulum and omasum. The

relative potencies of these extracts have been determined using a perfused rat stomach preparation (Smith, Lawrence, Colin-Jones and Schild, 1970), and compared to that of pentagastrin. Gastrin extracts from each of these regions have been assayed for histamine activity, and characterised by determination of their molecular weight and isoelectric point. The effect of gastrin extracts on gastric acid secretion in sheep provided with pouches of the body of the abomasum has also been examined.

CHAPTER II: BIOASSAY OF EXTRACTS WITH GASTRIN-LIKE ACTIVITY

INTRODUCTION

Biological and radioimmunological techniques have been used in the measurement of substances affecting gastric acid secretion.

Biological Assay:- Biological assay methods utilising conscious cats (Keeton, Koch and Lukhardt, 1920; Friedman, Hochschild and Carpenter, 1952; Emas, 1960), dogs (Gregory and Tracy, 1964), rats (Lane, Ivy and Ivy, 1957; Komarov, Bralow and Boyd, 1963 Gangali and Marshall, 1967), and anaesthetized cats (Edkins, 1906; Komarov, 1942; Uvnas, 1942; Jalling and Jorpes, 1947; Blair, Keenlyside, Newell, Reed and Richardson, 1968) and rats (Shay, Sun and Gruenstein, 1954; Gosh and Schild, 1958; Kim and Shore, 1963; Lai, 1964; Barrett, Raventos and Siddall, 1966; Pissidis and Clark, 1967; Hansky, 1968; Smith, Lawrence, Colin-Jones and Schild, 1970) have been reported. These techniques have been used to assess the physiological effects of gastrin extracts, to measure the relative quantities of gastrin in gastric mucosa and to test the action of synthetic analogues of gastrin.

Many of the biological assay methods cited above, (for example the techniques utilising conscious preparations)

are at best only semi-quantitative as a complete statistical comparison (allowing for assessment of variation in response), of the test substance and standard cannot be completed on the same day under strictly comparable conditions. Jalling and Jorpes (1947), first studied dose response relationships in the assay of gastric extracts and suggested using groups of animals to overcome individual variations in response. These concepts have been incorporated in assays using pyloric - oesophageal ligated rats (Shay et al., 1954; Kim et al., 1963; Hansky, 1968). The main disadvantage of this technique is that large numbers of experimental animals are required to eliminate between-animal variation, as only one test can be performed with each animal.

Gosh and Schild (1958) developed a method of assaying stimulants of gastric acid secretion, consisting of a continuous perfusion of the stomach of an anaesthetized rat. By continuous measurement of the pH of the perfusate, acid secretion could be monitored. As the anaesthetized rat responds to a gastric secretory stimulant, and then returns to basal secretion in a relatively short time (Gosh et al., 1958), more than one dose of test substance could be given to each animal, thus reducing the factor of individual variation. A disadvantage of these methods, based on the continuous measurement of pH change, is that the differential record obtained leads to a cumbersome calculation of the total acid secreted in response to

stimulant test substances.

Smith et al. (1970) increased the sensitivity of the anaesthetized rat perfused stomach assay technique by integrating the acid secreted using a reperfusion system. A buffer displaying a linear relationship between pH and acid added, was recirculated through the stomach, and the cumulative pH was measured using a glass electrode and chart recorder.

Methods of assay involving perfusion of the anaesthetized rat stomach have limitations. They measure total acid secreted, but give no indication of the volume and acid concentration of the fluid secreted, and the artificial conditions under which the animal is maintained, namely controlled body temperature and anaesthesia may affect acid secretion. However, perfusion methods have the advantages of high sensitivity (Smith et al., 1970, report that their preparation is sensitive to 10-20 ng synthetic human gastrin I), and of following the time course of secretion closely, so that successive doses of stimulants can be administered as soon as the effect of a previous dose subsides.

Radioimmunoassay:- One of the more recent and most sensitive methods of gastrin assay reported has been that of radioimmunoassay. A recent review (Rehfeld, 1973) describes the technique involved and the results obtained

by radioimmunoassay of gastrin.

In the present work, gastrin extracts obtained from certain regions of the ovine gastrointestinal tract have been assayed using a method based on the perfused rat stomach preparation described by Smith et al. (1970). The characteristics of this bioassay system were defined by the preparation of dose response curves and an investigation of the reproduceability of individual responses.

METHODS

The bioassay system used was developed from that described by Smith et al. (1970). The stomach of an anaesthetized rat was continuously reperfused with a buffer and the change in pH was monitored. This enabled the basal or stimulated acid output to be calculated.

Animal Preparation: Male rats of the Wistar strain weighing between 400 and 600 grams were used. During the 12 hours preceding the start of the assay, rats were allowed free access to water, but the normal pellet diet (5 1/2 pellets, Northern Roller Mills, Tauranga) was replaced by cane sugar cubes for 12 hours, and the animal then fasted for 12 hours. This feeding regime facilitated clearing of food debris from the stomach.

Anaesthesia: General anaesthesia was induced by giving urethane in 25% solution (wt. vol^{-1}), the usual dose being 0.6 ml. $100g^{-1}$ body weight of the rat. Rats varied in their sensitivity to urethane, and some difficulty was encountered in obtaining similar levels of anaesthesia for all animals. The following scheme was found most satisfactory; all but 0.5 ml of the calculated dose was given intraperitoneally, and if the limb withdrawal reflex was absent after 10 minutes, the dose was considered correct. However, if after 10 minutes reflexes still persisted, the remainder of the calculated dose plus a

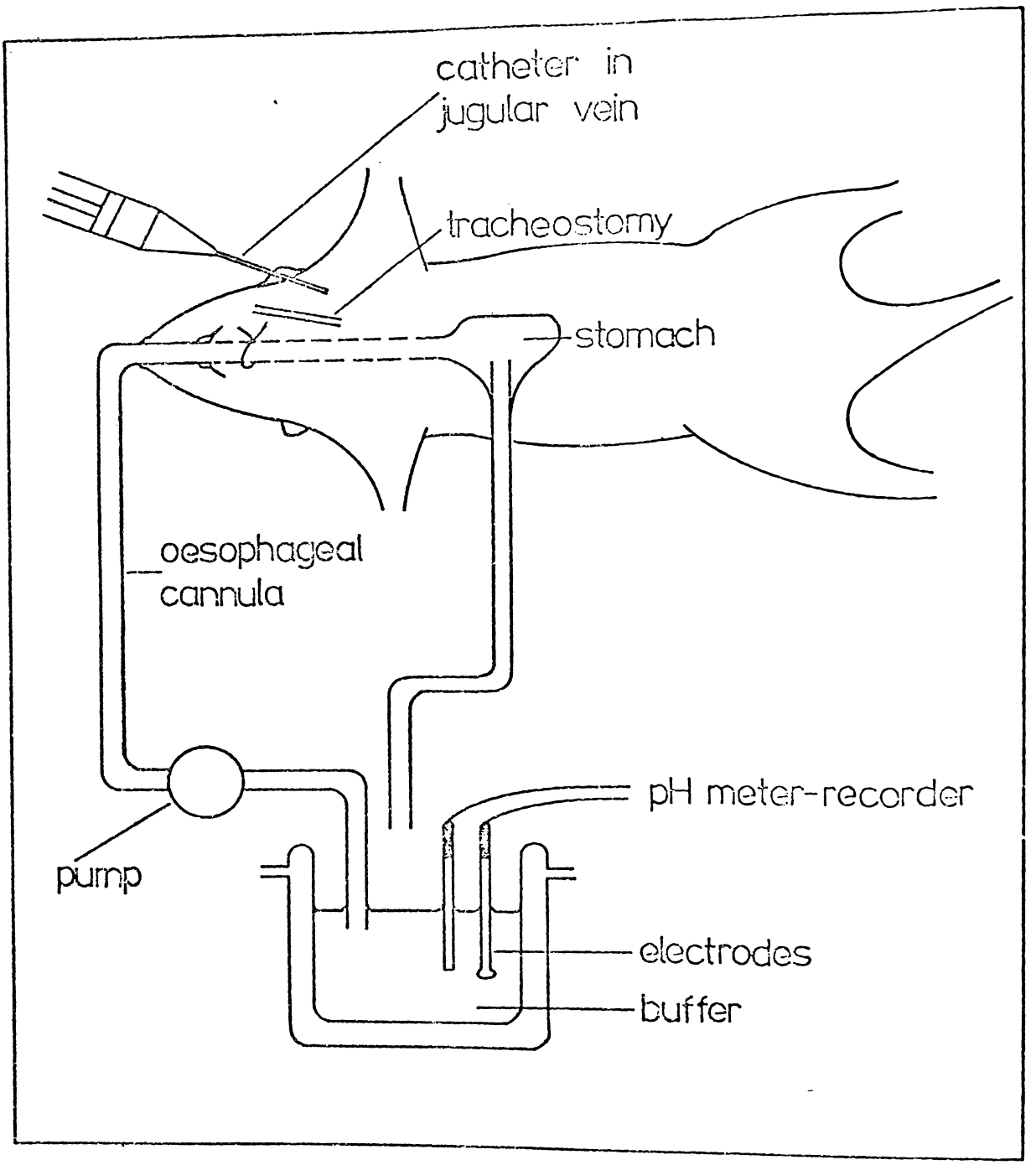

Fig. 3. Diagram of reperfusion apparatus (after Smith et al., 1970). By means of a peristaltic pump the stomach was continuously reperfused with 20ml of a buffer maintained at 30°C. Change in pH of the buffer was recorded, and the acid secretion calculated in m mol H^+. This figure, although published in Smith et al., 1970 and Jury, 1974, is included here for clarity.

further 0.5 ml was given intraperitoneally.

Body temperature was maintained at 34°C (rectal temperature) by means of a 100 watt light bulb suspended approximately 20 cm above the animal.

Operative Procedure: The trachea was exposed and cannulated with a 13 gauge hypodermic needle, and any secretions collecting in the respiratory passages were removed by means of a syringe and fine polythene tube. A polythene tube (1 mm external diameter) was used to cannulate the external jugular vein. The abdomen was opened, and a glass cannula (6 mm in external diameter with a waist 2 cm from the tip 4.5 mm in diameter which snugly fitted the pylorus; the intragastric portion contained numerous perforations) was passed through an incision in the duodenum, approximately 1 cm from the pylorus, and gently slipped into the stomach. The cannula was tied in place with a ligature around the duodenum. A polythene tube (2.5 mm external diameter) was then passed down the oesophagus and tied in place in the cervical region, excluding the vagal nerves. Care was taken to avoid handling the glandular portion of the stomach, and the gastric contents were washed out gently using a syringe and warmed saline. Surgical wounds were sutured.

Perfusion System: The arrangement used is shown diagrammatically in Fig. 3. The stomach was continuously

perfused with 20 ml of propionic-succinic buffer maintained at 30°C in a jacketed reservoir. The buffer was recirculated at a rate of 3 $ml.min^{-1}$ by means of a peristaltic pump (Watson, Marlow; "Flow inducer", MHRE 7).

The stock solution of propionic-succinic buffer (Appendix I), after pH adjustment to 5.5 with 1M NaOH or 1M HCL was diluted 5 ml to 1 litre of distilled water for each experiment.

Methods of Assay: A pH meter (Electronic Instruments Ltd; 7020) and glass electrode coupled to a chart recorder (Honeywell-Brown "Electronik") was used to continuously monitor the change in pH during reperfusion. The recorder was calibrated so that full scale deflection equalled 1.0 pH unit and, knowing the volume and concentration of buffer, the secretion was calculated in μ mol H^+ from the change in pH.

The basal acid output was recorded until a constant baseline was obtained and the gastric stimulant administered. Basal secretion was assumed to have continued unchanged during the stimulation period, and the stimulated acid secretion during the following 30 minutes could be estimated by extrapolating the baseline and measuring the ordinate below the extrapolated line. The pH was monitored for 30 minutes after administering the secretory stimulant, then the buffered reperfusion solution

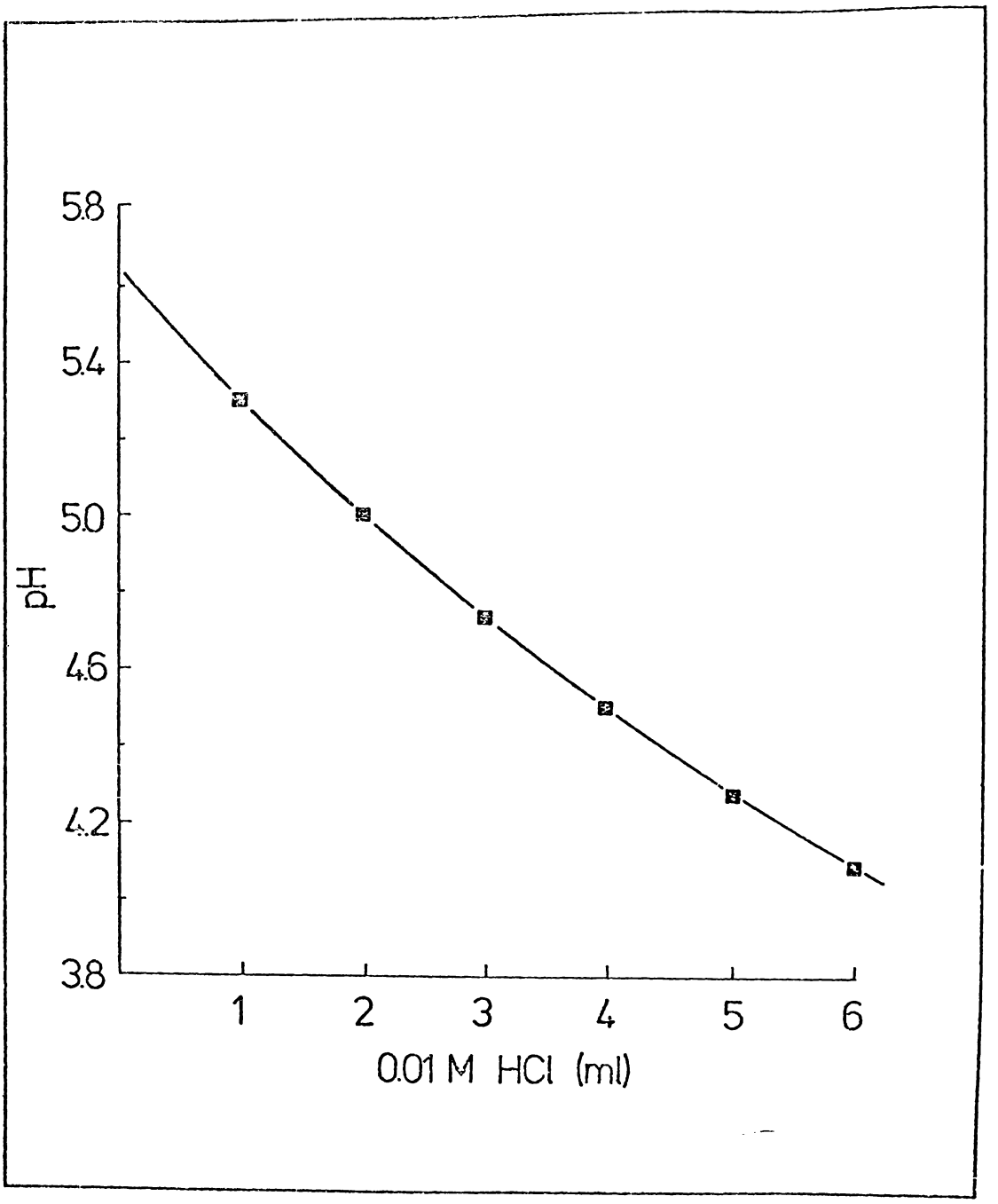

Fig. 4. Titration curve for 20ml propionic-succinic buffer and 0.01M HCl. This buffer has three pK valves over the pH range 5.6 - 4.4, so that as hydrogen ions accumulate the pH change is almost linear.

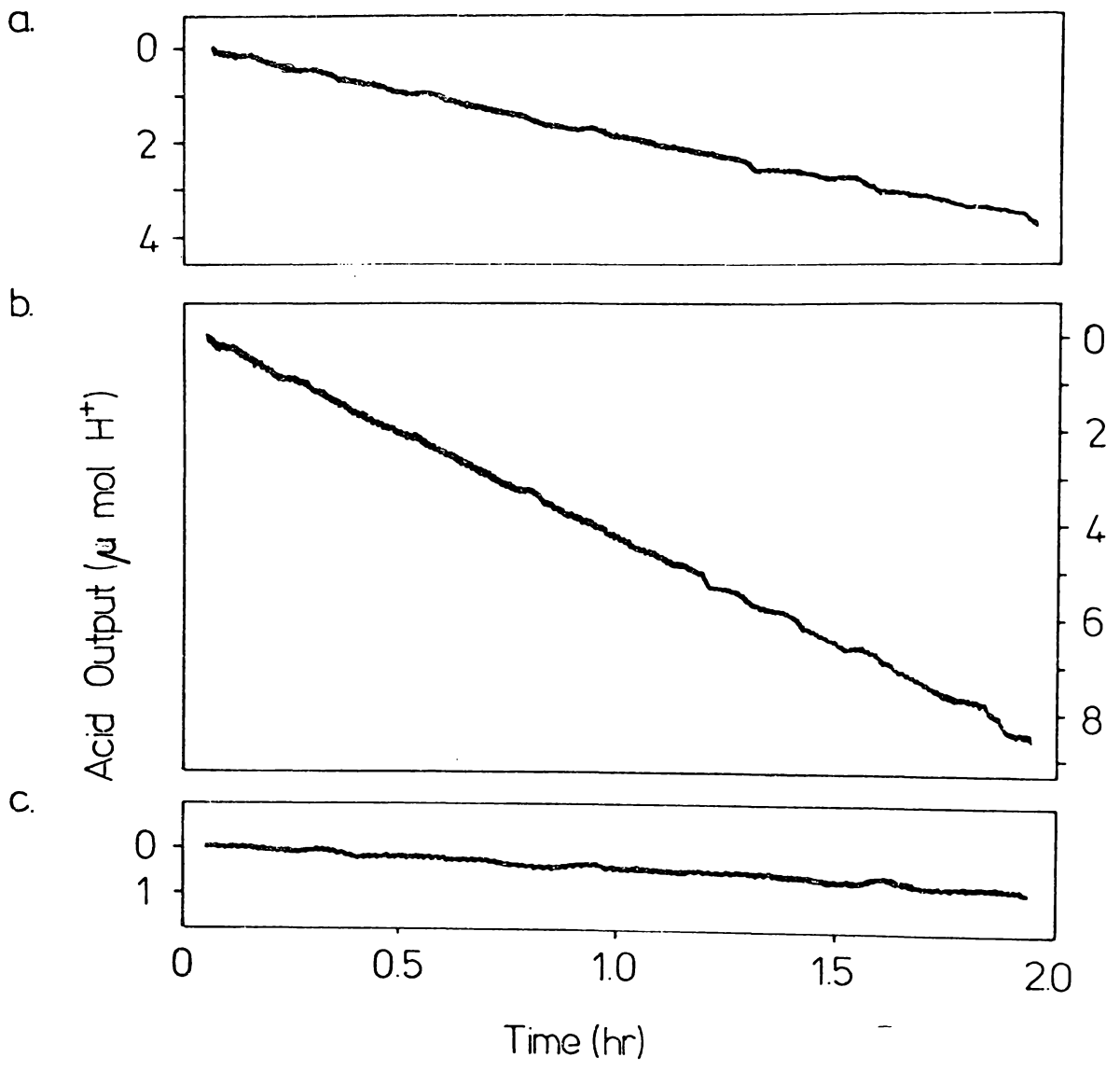

Fig. 5. The unstimulated basal rate of gastric acid secretion, for each of three rats, recorded over two hours.

a) 0.33 μ mol H^+.10 min^{-1}

b) 0.70 μ mol H^+.10 min^{-1}

c) 0.03 μ mol H^+.10 min^{-1}

Table 2. Response of the reperfused rat stomach (measured as μ mol H^+ secreted) to seven successive 2.0×10^{-5} g doses of pentagastrin in each of three rats. The mean, and S.E.M. for doses 2-7 in each preparation are shown.

	μ mol H^+ secreted		
	Rat 1	Rat 2	Rat 3
Dose 1	19.98	27.61	26.72
Dose 2	11.66	15.0	12.50
Dose 3	11.32	14.98	13.86
Dose 4	9.99	15.56	11.71
Dose 5	10.66	14.10	12.50
Dose 6	10.99	15.90	11.90
Dose 7	11.32	16.00	12.83
Mean; doses 2-7	10.99	15.2	12.55
SEM; doses 2-7	0.24	0.29	0.31

was changed and the routine was repeated.

Stability of Basal Secretion: The basal rate of acid secretion was observed in each of 3 rats over 2 hours to assess any spontaneous change in secretion.

Constancy of Response to Pentagastrin: In each of 3 rats six successive 2.0×10^{-6}g doses of pentagastrin (a synthetic analogue of the active C-pentapeptide of gastrin, Peptavlon, I.C.I. England) were administered and the secretory response was calculated to assess the constancy of the response to gastrin.

Dose Response Curve: In two groups of rats, doses of pentagastrin in the range 5.0×10^{-8}g, 5.0×10^{-7}g, 1.0×10^{-6}g, 5.0×10^{-5}g (n=3), and 5.0×10^{-6}g, 1.0×10^{-5}g, 2.0×10^{-5}g and 4.0×10^{-5}g (n=6) were administered, to enable a dose response curve to be constructed.

Sensitivity to Histamine: Sensitivity of the preparation to 1.0×10^{-5}g, 2.0×10^{-5}g and 4.0×10^{-5}g histamine diphosphate (Ethicals Ltd.) was tested.

RESULTS

The Anaesthetized Animals: The rats were usually fully anaesthetized (determined by the lack of the limb withdrawal reflex) within 15 minutes, during which respiration slowed to approximately 92. min^{-1}. Body temperature was maintained at 34°C with very little variation during the course of the experiment.

Buffer Solution: The propionic-succinic buffer used has three pk values distributed over the pH range used (Smith et al., 1970) so that the pH change was nearly linear as hydrogen ions accumulated (Fig. 4).

Stability of Basal Secretion: The basal rates of acid secretion recorded over 2 hours were 0.03, 0.33 and 0.70 μ mol H^+. 10 min^{-1} for three rats. Although there was some variation between animals, there was no spontaneous change in acid output, the rate of secretion being constant over the recording period (Fig. 5).

Sensitivity and Constancy of the Response to Pentagastrin: Table 2 shows the response of the reperfused rat stomach (measured as μ mol H^+ secreted) to seven successive 2.0×10^{-5} g doses of pentagastrin in each of three rats. The first response to stimulation was variable and was disregarded. The six responses that followed showed little variation, although the magnitude of the responses between

Table 3. The responses (as μ mol H^+.30 min^{-1}, mean (S.E.M.)) of two groups of rats to varying doses of pentagastrin.

Group 1: (n = 3) Pentagastrin doses of 5.0×10^{-8}g, 5.0×10^{-7}g, 1.0×10^{-6}g and 5.0×10^{-5}g.

Dose Pentagastrin (g)	μ mol H^+.30 min^{-1}
5.0×10^{-8}	1.14 (0.09)
5.0×10^{-7}	1.84 (0.17)
1.0×10^{-6}	3.35 (0.16)
5.0×10^{-5}	36.63 (1.07)

Group 2: (n = 6) Pentagastrin doses of 5.0×10^{-6}g, 1.0×10^{-5}g, 2.0×10^{-5}g and 4.0×10^{-5}g.

Dose Pentagastrin (g)	μ mol H^+.30 min^{-1}
5.0×10^{-6}	4.54 (0.39)
1.0×10^{-5}	9.05 (1.38)
2.0×10^{-5}	17.40 (2.22)
4.0×10^{-5}	30.30 (3.11)

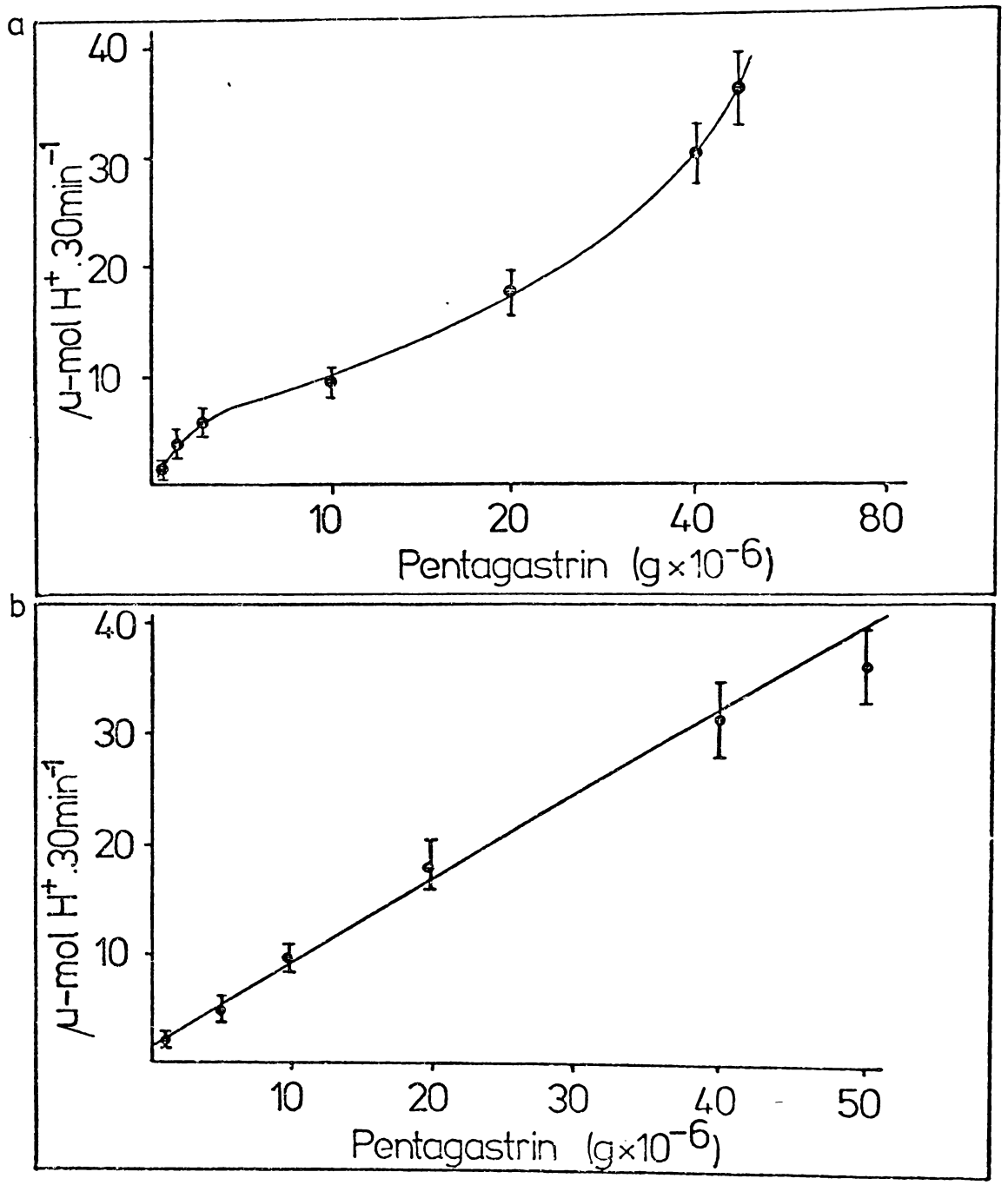

Fig. 6. Construction of dose response curves from data obtained from two groups of rats (Table 3).

(a) Plot Response (μ mol H^+) verus log dose pentagastrin (g x 10^{-6}).

(b) Plot Response (μ mol H^+) verus dose pentagastrin (g x 10^{-6}).

Table 4. Response of a single rat to 1.0×10^{-5} g 2.0×10^{-5} g and 4.0×10^{-5} g of pentagastrin and histamine diphosphate, measured as μ mol H^+ secreted in 30 min.

Dose ($\times 10^{-5}$ g)	μ mol $H^+ \cdot 30\ min^{-1}$	
	Pentagastrin	Histamine
1.0	10.7	0
2.0	14.3	1.6
4.0	29.1	9.2

animals differed.

Dose Response Curve: To enable a dose response curve to be constructed, the responses of two groups of rats to 5.0×10^{-8}g, 5.0×10^{-7}g, 1.0×10^{-6}g, 5.0×10^{-5}g and 5.0×10^{-6}g, 1.0×10^{-5}g, 2.0×10^{-5}g and 4.0×10^{-5}g were determined (Table 3). Dose response curves constructed from this data are shown in Fig. 6a and b.

Sensitivity to Histamine: The reperfused rat stomach preparation is relatively insensitive to histamine. Table 4 shows the acid secretory response to 1.0×10^{-5}g, 2.0×10^{-5}g and 4.0×10^{-5}g histamine diphosphate.

DISCUSSION

The reperfused rat stomach preparation described was sensitive to pentagastrin over a wide range of doses, and provided a sensitive method for the assay of gastrin extracts obtained from the ovine gastro-intestinal tract.

A buffer was continuously recirculated through an anaesthetized rat's stomach so that the pH change of the buffer, as hydrogen ions accumulated, provided an integrated record of the secretory response. To allow the simple measurement from such a record, of the amount of acid secreted, it was necessary that the buffer used showed a linear change in pH as hydrogen ions accumulate. The propionic-succinicbuffer (Smith et al., 1970) used, exhibited such a relationship.

An advantage of this assay technique was that the time course of the secretory response can be followed continuously, thus enabling a further stimulant to be administered as soon as the previous response subsides. This was usually within 60 minutes, and hence a 2 + 2 dose assay of standard and test substance could be completed with the one animal.

As the response of the rat to a secretagogue was measured by acid secreted in excess of the basal rate, it had to be assumed that basal secretion was constant.

Maintenance of a steady base-line was facilitated by ensuring that the stomach was clear of food debris and that there was no obstruction to the flow of buffer from the stomach. Observation that the basal rate of secretion showed no spontaneous change over 2 hour recording periods indicated that the above assumption was justified.

The dose response curves for pentagastrin show that the perfused rat stomach was sensitive to small amounts of gastric secretagogue. All animals were sensitive to 5.0×10^{-8}g pentagastrin, and in some cases sensitivity to smaller doses was shown. Although the dose response curve shows a secretory response over the range of doses of pentagastrin 5.0×10^{-8}g to 5.0×10^{-5}g, the response was linear in the range of doses of 1.0×10^{-6}g to 4.0×10^{-5}g. The amount of acid produced by the ovine extracts fell within the range of that produced by 1.0×10^{-6}g to 4.0×10^{-5}g pentagastrin; i.e. on the linear part of the response curve.

The reperfused rat stomach used in the present work had approximately the same sensitivity as the cat preparation of Blair and Wood (1968), and greater sensitivity than the rat assay described by Barrett (1966). However the present system was slightly less sensitive than the reperfused rat stomach described by Smith et al. (1970), who were able to detect 1.0×10^{-8}g of synthetic human gastrin I. This difference in the minimal amount of

gastrin activity detectable was probably attributable to the greater dilution of the stock buffer used by Smith et al. (1970). In the anaesthetized rat, when compared to man and the dog, histamine is a much weaker stimulant of gastric acid secretion than gastrin or pentagastrin (Smith, 1973). The smallest amount of histamine detected by the perfused rat stomach preparation used in the present work was between 10 and 20 ug.

As observed by Lia (1964) and Smith et al. (1970) in the anaesthetized rat perfused stomach assay, the first secretory response was variable and hence was not included in the assay. Unlike the assay procedure of Lia (1964) where an increase in sensitivity with the fifth and subsequent responses was observed, the present preparation showed little variability with up to six doses, with no apparent trend in responsiveness. Smith et al. (1970) reported similar findings. This enabled a 2 + 2 assay of two test substances and pentagastrin to be completed in one day.

Gosh (1956) has reported that there is a lower mortality when the anaesthetized rat is maintained at a body temperature of 30°C. The maintenance of this low body temperature, and of the animal under anaesthesia could be criticised as these conditions result in a reduced gastric acid secretion by the rat (Maitrya, 1967; Barrett, Raventos and Siddal, 1966). However, in an assay system

where the test and the standard receive the same treatment in the one animal these considerations are less important.

CHAPTER III: THE EXTRACTION AND BIOASSAY OF GASTRIN-LIKE ACTIVITY FROM THE OVINE GASTROINTESTINAL TRACT

INTRODUCTION

Simple methods for the preparation of gastrin based on an initial aqueous extraction (Edkins, 1905, 1906; Blair, Harper, Lake, Reed and Scratchard, 1961) an acid extraction (Komarov, 1938; Munch - Peterson et al., 1944; Uvnäs, 1942, 1943, 1945) and an acid method extraction (Harper, 1946; Jorpes, et al., 1952) have been reported. These workers attempted to purify their extracts by precipitation with trichloroacetic acid (Komarov, 1938), and fractional precipitation from aqueous solutions (Komarov, 1938; Uvnas, 1942, 1943 a & b, 1945 a). Although there was some increase in activity gained, the yields were still low.

Gregory and Tracy (1959) introduced a more detailed and efficient method for the preparation of hog gastrin. Initially they treated strips of fresh antral mucosa with aqueous acetone containing picric acid or trichloroacetic acid. An aqueous fraction was recovered, and then refined by fractional precipitation at an alkaline pH. The final product was free of histamine and was more potent than histamine in stimulating gastric acid secretion. Following this Gregory and Tracy (1964) reported that the gastrin activity present in the crude aqueous extracts could be

recovered, free of histamine, by treatment with diethylaminoethyl cellulose floc. After removal from the cellulose floc with sodium hydroxide the crude protein material was subjected to an isopropanol extraction, gel filtration on Sephadex and gradient elution on diethylaminoethyl cellulose. This procedure led to the characterisation of the two gastrin peptides.

Anderson et al. (1961, 1962) reported the preparation of ovine and bovine gastrin by digestion of fresh abomasal material in aqueous alkali, and subsequent fractional precipitation. Agarwal et al. (1968) described the isolation of two gastrin peptides from ovine and bovine antral mucosa (by the method of Gregory and Tracy, 1964), although difficulty was experienced with the presence of a large amount of unidentified brown pigment. A subsequent modification involving separation of the pigment on an ion exchange resin has been reported (Jury and McLeay, 1974).

In the present study, gastrin activity has been isolated from the ovine rumen, reticulum, omasum, antral and body regions of the abomasum, duodenum and caecum using the method reported by Gregory and Tracy (1964), as modified by Jury and McLeay (1974).

The relative potencies of the extracts obtained from regions of the ovine gastro-intestinal tract were determined (using the bioassay described in the previous chapter), and

compared to the activity of pentagastrin.

The activity of air dried extracts (as obtained by Jury and McLeay, 1974) was compared to that of freeze dried extracts. All extracts were tested for the presence of histamine using the bioassay method described by Adam, Hardwick and Spencer (1954).

METHODS

Collection of Material: Tissue from sheep of both sexes, aged between 1 and 4 years was obtained from the Ruakura Agricultural Research Centre abattoir, A.F.F.C.O. Ltd., Horotiu, and Aotearoa Meats Ltd., Cambridge. Complete rumen, reticulum, omasum and caecum were collected. The antral and abomasal body regions of the abomasum were separated, the indistinct area of about 4 cm between the two regions being discarded to prevent overlapping. Duodena were collected, and four successive 20 cm segments of each duodenum were cut in the laboratory. In one extraction of rumen, reticulum, omasa, antra and abomasal body, the muscle and mucosal layers were dissected and extracted separately.

Gut contents were removed and the material was washed with cold running tap water. Adhering fat was removed and the tissue was either stored deep frozen at $-20^{o}C$ or the extraction of gastrin was begun within 2 hours of slaughter. No distinction was made between gut processed by either method.

Extraction of Ruminant Gastrin: The technique used for the extraction of gastrin was that of Gregory and Tracy (1964) as modified by Jury (1974). Whole gastrointestinal tissue or separated muscle and mucosa were boiled for 30 minutes in 300 - 800 ml of tap water. After cooling overnight, the

liquor was strained through nylon cloth and its volume made up to 600 - 1600 ml with cold tap water and it was then stirred for three hours with 1.5 g diethylaminoethyl cellulose floc (Whatman DE32). The floc was collected by filtration through fine nylon cloth and washed well with distilled water. The gastrin, together with inert protein was eluted by treating the floc with 25 ml 0.1 M NaOH. The eluant was brought to pH 7.0 with glacial acetic acid and then cooled to 10°C. The pH was then brought to 4.0 with glacial acetic acid, and after refrigeration overnight the precipitate was collected by centrifuging. This precipitate was redissolved in 20 ml tap water at pH 10.0 by the addition of 18 M ammonia solution, and its volume then made up to 100 ml with tap water. One gram di-potassium hydrogen phosphate (Analar) was dissolved in it and while stirring, 150 ml of isopropanol (Analar) was slowly added; stirring continued for 30 minutes. An aqueous phase was recovered by adding 2 volumes of diethyl ether (May and Baker), shaking, and collecting the lower phase. This aqueous phase was again extracted with two volumes of diethyl ether, and then residual ether was removed by vacuum aeration at room temperature.

After cooling to 10°C, the aqueous phase was brought to pH 4.0 by the addition of glacial acetic acid. Following refrigeration overnight the precipitate was collected by centrifugation and dissolved in a volume of water equivalent to about half that of the original aqueous residue (usually

about 25 ml), by adding several drops of 18 M ammonia solution. Precipitation at 10°C and pH 4.0 was then repeated. After refrigeration overnight the precipitate was collected by centrifuging, and dissolved in 3 ml distilled water by adding two drops of 18 M ammonia solution. The pH was then brought to 7.5 by the addition of glacial acetic acid.

This solution was passed through a column (1cm x 10cm) of diethylaminoethyl cellulose (Whatman DE32) packed in deionized water (pH 5.5). After placing the gastrin extract (pH 7.5) on the column, it was initially washed with distilled water, and then with 0.1 M ammonium bicarbonate.

Ammonium bicarbonate fractions showing absorption (at 210 nm) were pooled, and precipitated at 10°C and pH 4.0. After refrigeration overnight, the precipitate was collected by centrifuging, washed in diethyl ether and allowed to dry in air overnight.

Preparation of Freeze Dried Extracts of Gastrin: The technique described above was followed to the stage of fractionation on diethylaminoethyl cellulose. The ammonium bicarbonate fractions showing absorption (at 210 nm) were pooled, and then reduced to a volume of about 5 ml, under vacuum at 37°C. This concentrated protein solution was then frozen in liquid nitrogen and dried overnight under vacuum.

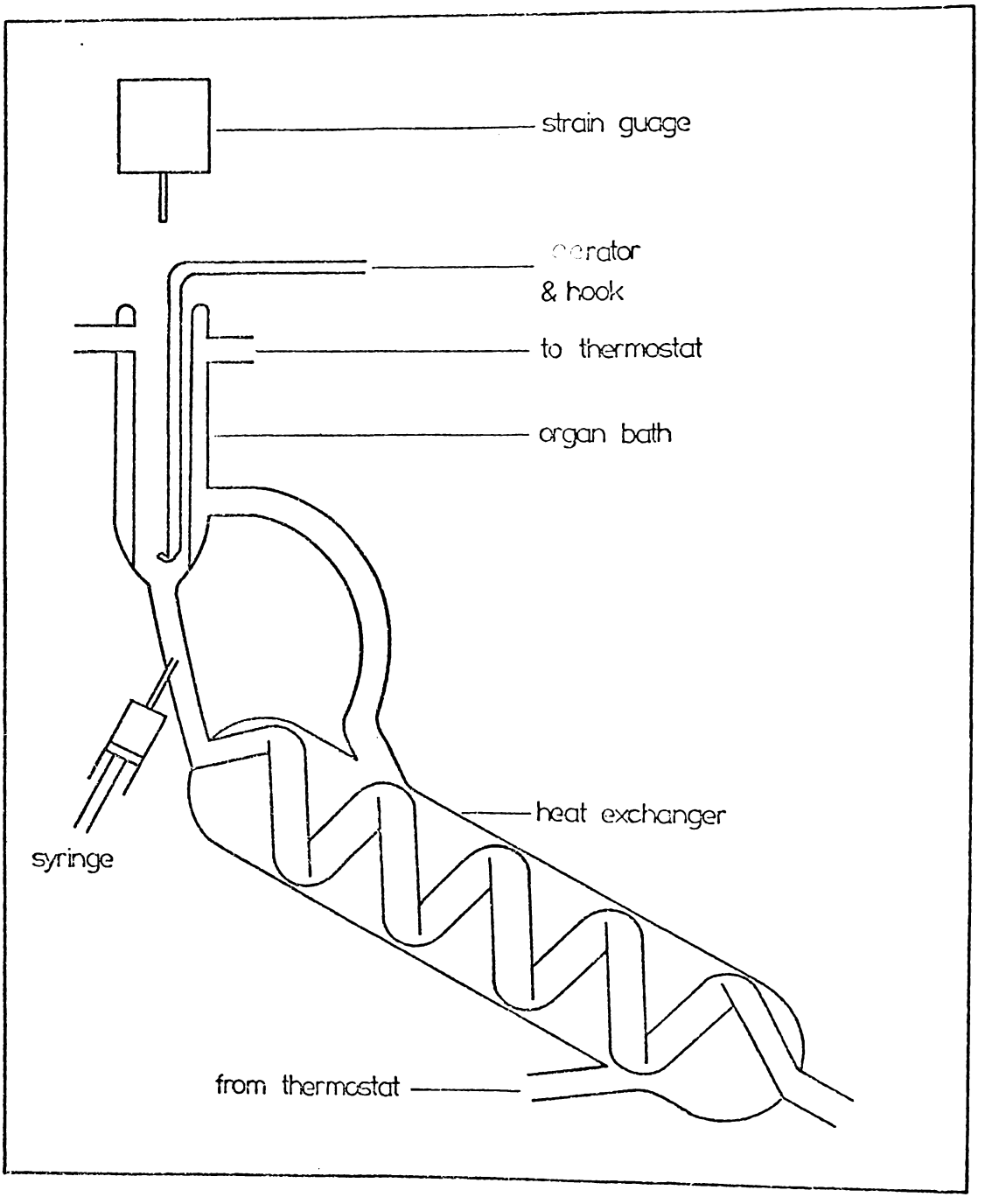

Fig. 7. Diagram of organ bath arrangement used for histamine assay.

All gastrin extracts were stored at -20°C, and on the day of assay were dissolved in physiologically normal saline (0.9%, wt. vol^{-1}) to give a concentration of 1 mg. ml^{-1}.

Histamine Assay: All extracts were tested for the presence of histamine using a modification of the technique of Adam, Hardwick and Spencer (1954) utilising the isolated guinea-pig ileum. Adult guinea-pigs were killed by delivering a blow to the head. A short length of ileum was excised and then suspended in an organ bath, care being taken not to occlude the lumen. The preparation was perfused with Tyrode Ringer solution (see Appendix 2) at a rate of 3 ml. min^{-1} and aerated with carbogen (95% oxygen and 5% carbon dioxide). The temperature was maintained at 37°C. Histamine or test substances were introduced via the silastic rubber coupling at the base of the bath using a hypodermic needle and 1 ml syringe. This arrangement is shown in Fig. 7.

Method of Assay: Responses of the isolated guinea-pig ileum to high doses of test extract (1×10^{-4}g) and low doses of histamine (1×10^{-7}g) (Ethicals Ltd., Auckland) were directly compared.

Assay of Ovine Gastrin Extracts: Gastrin extracts were assayed using the perfused rat stomach technique described by Smith et al. (1970) as discussed in the previous chapter.

All assays of the gastrin extracts were designed as a (2 + 2) dose assay comparison of the gastrin activity of the extracts against pentagastrin (Peptavlon, I.C.I.,England). The (2 + 2) assay procedure consisted of high and low doses of pentagastrin and test extracts in the range of 2.0×10^{-5}g to 4.0×10^{-5}g per rat (approximately 4.0×10^{-5}g to 8.0×10^{-5}g. kilogram $^{-1}$ body weight) so that acid secretory responses fell on the linear part of the dose response curve. The relative potency of the test extracts and pentagastrin was calculated from $R = y_+ - y_+' / y_s - y_s'$, where R = relative potency, y_+ = extract response, y_s = pentagastrin response (Wood and Finney, 1946).

Loss of Activity with Time: A single freeze dried antral extract, stored under air at $-4^{o}C$, was assayed at three weekly intervals over twenty four weeks to assess any loss of activity with time.

Comparison of Activities of Freeze Dried and Air Dried Extracts: Extractions of three groups of 40 duodena were prepared. The pooled ammonuim bicarbonate fractions were halved: one half was freeze dried while the other was air dried. The activities of the freeze dried and air dried extracts were compared by bioassay of each extract in 3 rats. Similarly the activities of 3 freeze dried antral and fundic extracts were compared to the activities of air dried antral and fundic extracts reported by Jury (1974).

Extracts from the Ovine Gastrointestinal Tract: Six gastrin extracts were prepared from batches of 3 rumen, reticulum and omasa, 6 abomasa and 6 caeca. Extracts were also prepared from six batches of 20 duodena, each duodenum being divided into four successive 20 cm segments from the pylorus. A single extract of 6 oesophagi was prepared. Extracts were prepared from the separated muscle and mucosa of 6 antra and abomasal bodies, and 3 rumen, reticulum and omasa.

All extracts were each assayed in 3 different rats, giving a total of 18 assays for each region of the gastrointestinal tract investigated. From these results the relative potencies of the extracts were calculated. Gastrin-like activity present in the extracts has been expressed as the equivalent weight of pentagastrin (µg).

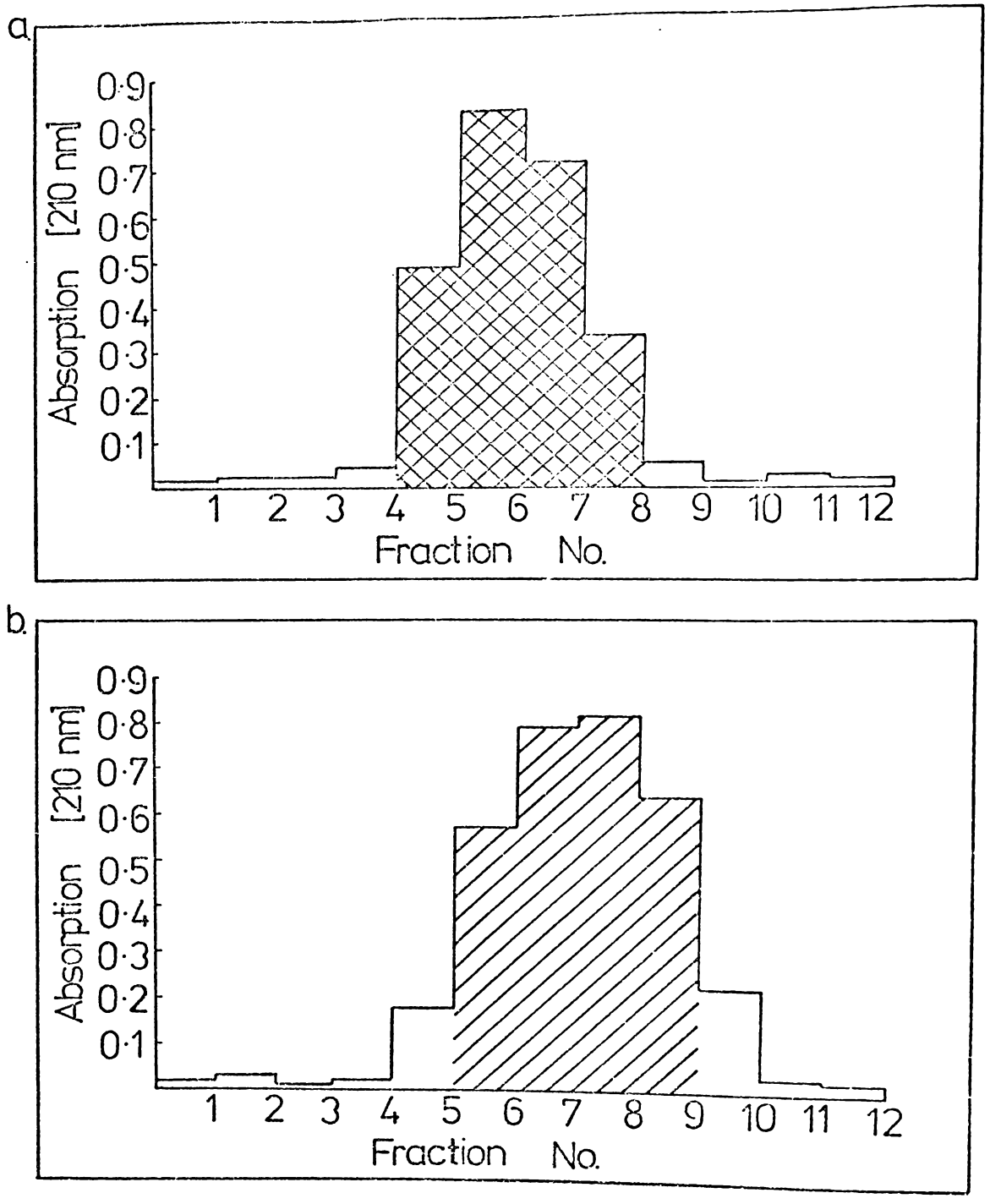

Fig. 8. Fractionation of gastrin extract on diethylaminoethyl cellulose.

(a) Distilled water eluant; shaded peak contained a gelatinous pigment.

(b) 0.1M NH_4HCO_3 eluant; shaded peak contained gastrin activity.

RESULTS

Extraction and Purification: After initial purification of the aqueous mucosal extract, by absorption onto diethylaminoethyl cellulose floc, solvent extraction and reprecipitations, the still heavily pigmented extract was fractionated on a column of diethylaminoethyl cellulose. Figure 8 (a and b) shows the absorption (at 210 nm) of the eluant as the column was initially washed with distilled water and then with 0.1 M ammonium bicarbonate. Distilled water fractions showing strong absorption appeared to contain large amounts of brown pigment, whilst the major absorption peak of the ammonium bicarbonate eluant contained gastrin activity.

Histamine Assay: Following the method of Adam et al. (1954), the number of doses of histamine required to stabilise the ileum varied between preparations; however most required approximately 20 applications of histamine (1×10^{-5}g) after which the base line was steady, and there was a rapid response to histamine. The contraction in response to histamine began within 4 seconds of the dose commencing to flow over the ileum, and reached its maximum in 10 - 12 seconds. The preparation was usually completely relaxed within 30 - 45 seconds after application of the stimulant. Histamine and test extracts were applied at intervals of one minute.

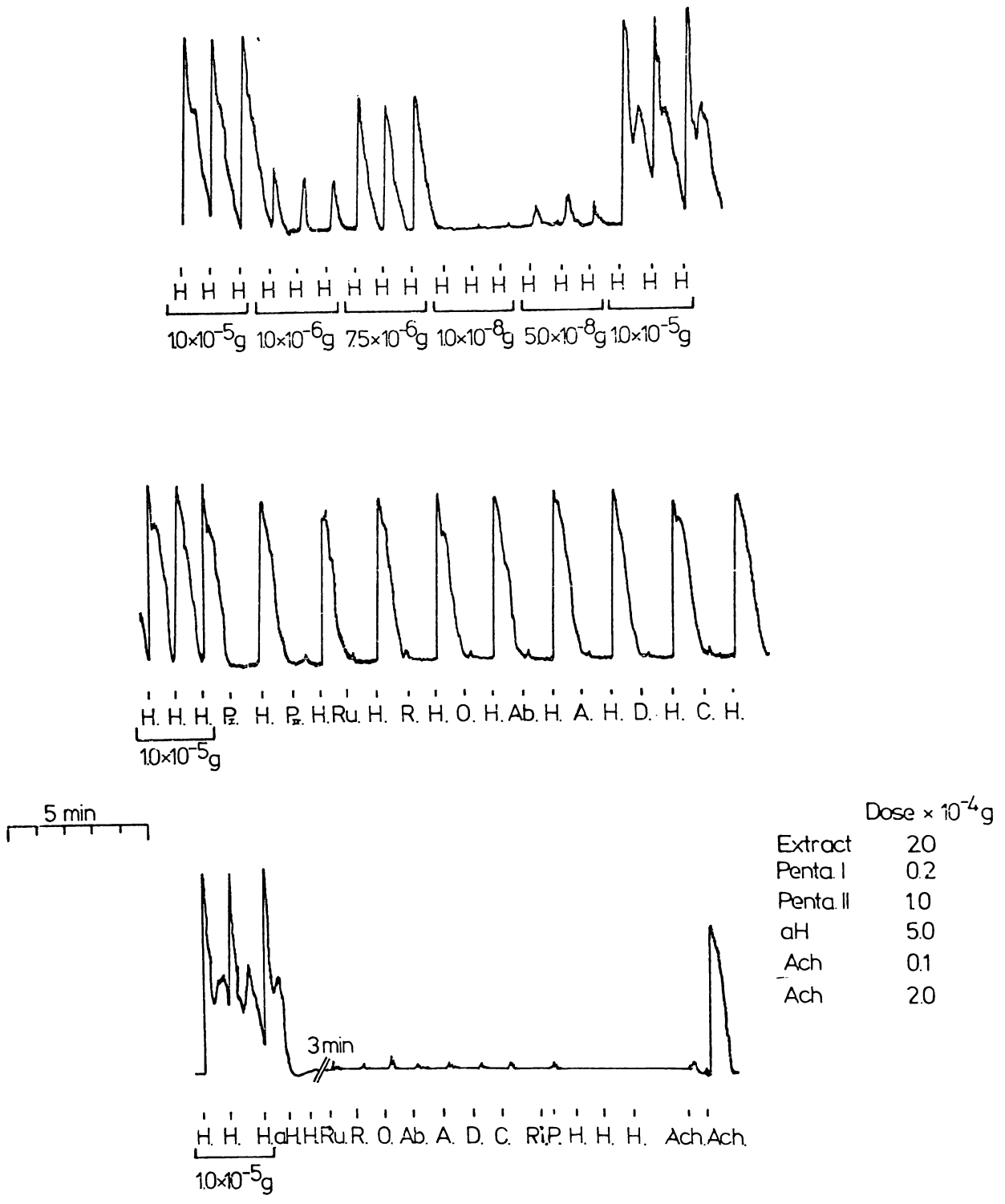

Fig. 9. Responses of isolated guinea-pig ileum to histamine, gastrin extracts and pentagastrin.

(a) Responses to graded doses histamine.
(b) Responses to histamine and gastrin extract.
(c) Effect of antihistamine.

(H, histamine; aH, antihistamine; P, pentagastrin; Ru, rumenal; R, reticular; Ri, Ringer; O, omasal; Ab, abomasal body; A, antral; D, duodenal; C, caecal; Ach, acetylcholine).

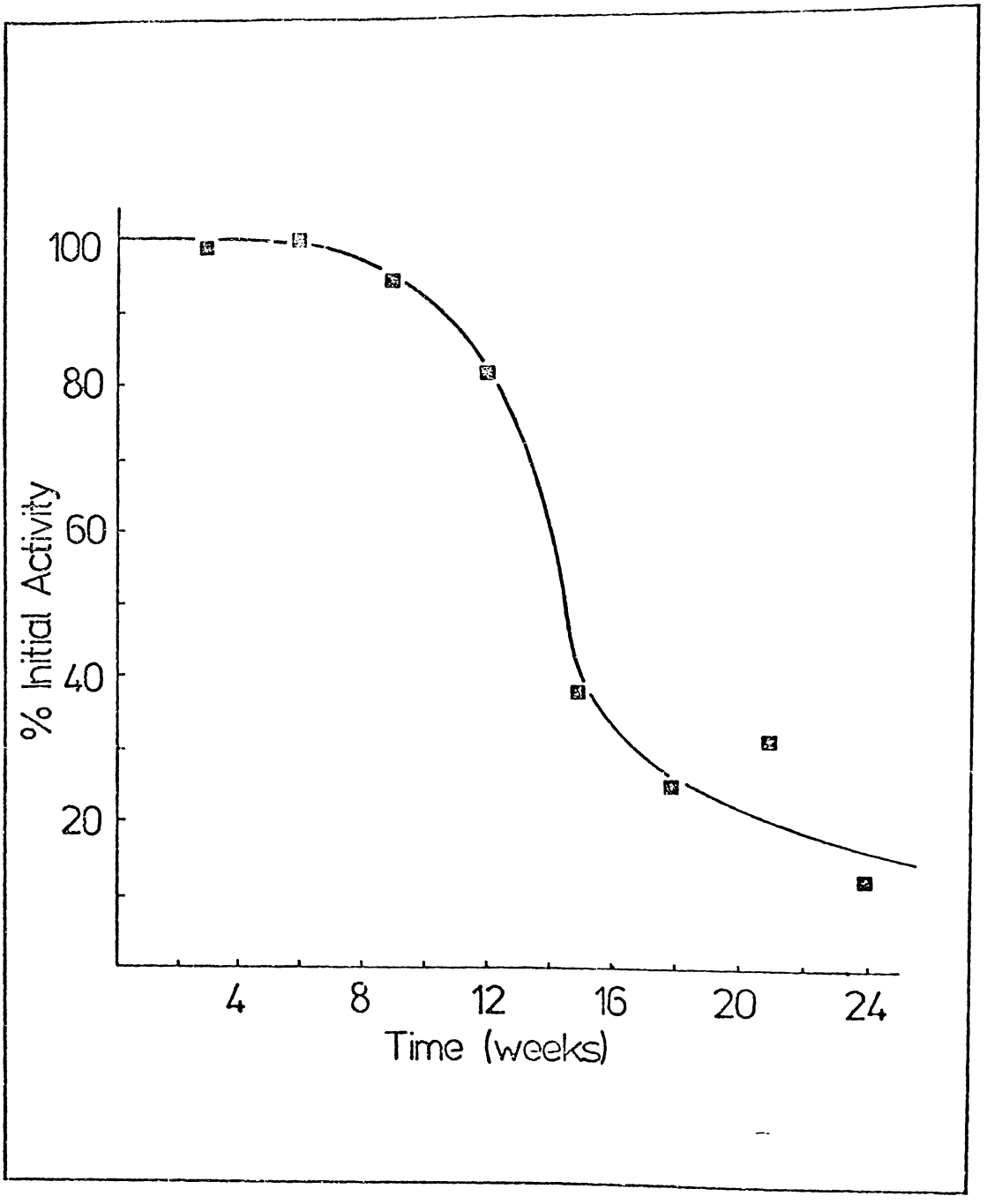

Fig. 10. Activity displayed by an antral extract when assayed at 3 weekly intervals after preparation. Graph shows the loss of activity as a percentage of the initial activity.

Strips of guinea-pig ileum gave graded responses to histamine (1.0×10^{-8}g, 5.0×10^{-8}g, 1.0×10^{-6}g, 7.5×10^{-6}g and 1.0×10^{-5}g). Typical responses may be seen in Fig. 9a. Freeze dried gastrin extracts from all regions of the ovine gastrointestinal tract 2.0×10^{-4}g and pentagastrin (Peptavlon, I.C.I. England) 2.5×10^{-5}g resulted in a small contraction of the ileum (Fig. 9b).

Contractions in response to histamine were abolished by the anti-histamine mepyramine maleate (Antihisan, May and Baker Ltd., New Zealand) but the responses to the extracts and pentagastrin were unaffected (Fig. 9c).

Assay of Ovine Gastrin Extracts: Although rats varied in their sensitivity to the gastric secretory stimulants employed, doses of gastrin extracts in the range 1×10^{-5}g to 8×10^{-5}g.Kg^{-1} body weight constantly produced increases in the rate of gastric acid secretion.

Loss of Activity with Time: A single antral extract (10.4×10^{-3}g) was assayed at 3 weekly intervals, each assay consisting of a comparison of the response to doses of 2.0×10^{-5}g and 4.0×10^{-5}g of extract and pentagastrin. When stored at -4^{o}C under air the gastrin activity was preserved for about six weeks. After this period the activity gradually declined until approximately twelve weeks, after which there was a rapid drop in activity (Fig. 10; Appendix IV).

Table 5. Wet weight, weight of freeze dried and air dried gastrin extracts (after halving initial aqueous extract), and weight of extracts expressed as ug extract per g wet weight duodena for 40 duodena. The means and standard error of the means are shown for 3 extractions.

		Duodenal Wet Wt. (g)	Wt. of Extract ($g \times 10^{-3}$)	μg ext. g^{-1} duodena
Extract 1	F.D.	786	21.1	58.4
	A.D.		20.1	53.4
Extract 2	F.D.	802	19.8	49.3
	A.D.		20.2	50.3
Extract 3	F.D.	690	19.2	55.6
	A.D.		18.3	52.2
Mean	F.D.	759	20.0	54.4
	A.D.		19.5	51.9
S.E.M.	F.D.	2.6	0.34	0.72
	A.D.		0.34	0.42

Table 6. Wet weight of abomasa, weight of freeze dried extracts, and weight of extracts expressed as μg extract $.g^{-1}$ wet weight abomasa for extracts from the antrum and abomasal body. Means and standard error of the means for 3 freeze dried extracts are shown.

	ANTRAL			ABOMASAL BODY		
	Wet Weight (g)	Wt. ext. ($gx10^{-3}$)	μg ext/g tissue	Wet Weight (g)	Wt.ext. ($gx10^{-3}$)	μg ext/g tissue
EXTRACT 1	182.5	9.6	52.6	590.4	14.7	24.9
EXTRACT 2	208.4	10.4	49.9	695.8	15.1	21.7
EXTRACT 3	177.6	8.7	49.0	593.3	12.4	20.9
MEAN	189.5	9.6	50.5	626.5	14.1	22.5
S.E.M.	6.1	0.35	0.76	24.5	0.59	0.89

Table 7. The relative HCl - stimulating activities of pentagastrin compared to that of freeze dried and air dried extracts from the abomasum and the duodenum. The ratios in all cases have been obtained by comparing the secretory activity of equal weights of pentagastrin and extract.

*From Jury (1974)

	FREEZE DRIED			AIR DRIED		
	Duodenal/ Penta.	Antral/ Penta.	Ab. body/ Penta.	Duodenal/ Penta.	Antral/ Penta.	Ab. body Penta.
EXTRACT 1	0.59	0.96	0.38	0.21	-	-
EXTRACT 2	0.56	0.99	0.36	0.22	-	-
EXTRACT 3	0.54	0.95	0.42	0.21	-	-
MEAN	0.56	0.97	0.39	0.21	*0.49	*0.18
S.E.M.	0.02	0.01	0.007	0.01	*0.02	*0.01

Table 8. Gastrin activity /g wet tissue (expressed as the equivalent weight pentagastrin, µg) of freeze dried and air dried abomasal and duodenal extracts. Final column shows relation between activity of freeze dried and air dried extracts.

* From Jury (1974).

	Gastrin Activity (µg penta.g^{-1} tissue)		
	F.D. ext.	A.D. ext.	Air dried ext./ F.D. ext.
ANTRAL:			
EXTRACT 1	50.73		0.56
EXTRACT 2	48.92	28.4*	0.58
EXTRACT 3	46.63		0.60
AB. BODY:			
EXTRACT 1	9.52		0.54
EXTRACT 2	7.86	5.22*	0.66
EXTRACT 3	8.85		0.59
DUODENAL:			
EXTRACT 1	34.97	11.39	0.33
EXTRACT 2	27.70	10.48	0.38
EXTRACT 3	30.38	10.88	0.36
MEAN	-	-	0.52
S.E.M.	-	-	0.04

Comparison of Activities of Freeze Dried and Air Dried Extracts: A summary of the weights of duodenal freeze dried and air dried extracts and the yield of extract expressed as a function of duodenal weight, resulting from the extraction of three batches, each of 40 duodena, are shown in Table 5. One half of the aqueous extract was freeze dried while the other was air dried. Similarly Table 6 compares the weights of 3 antral and 3 abomasal body freeze dried extracts.

When administered to anaethetized rats, both air dried and freeze dried extracts were stimulants of gastric acid secretion. However (2 + 2) assays of each extract in 3 rats showed there to be a considerable difference in activity between the air dried and the freeze dried extracts. Table 7 shows the relative HCl - stimulating activities of pentagastrin compared to that of freeze dried and air dried extracts from the duodenum and freeze dried extracts from the abomasum. The activities of air dried abomasal extracts reported by Jury (1974) are also included for comparison. The gastrin activity.g^{-1} wet weight tissue (expressed as the equivalent weight of pentagastrin in μg) of freeze dried and air dried (air dried abomasal extracts from Jury (1974)) extracts are shown in Table 8. By a comparison of their activities (μg pentagastrin), the air dried extracts were 0.52 times as active as the freeze dried extracts.

Assay of Extracts from the Ovine Gastrointestinal Tract: A

Table 9. Means and standard error of the means of wet weight tissue, weight of extracts, and weight of extracts as a function of tissue weight for 6 extracts of rumen, reticulum, omasa, caeca and oesophagi, 3 extracts of abomasa and extracts of 4 successive 20 cm segments of duodenum.

	Wet Weight Tissue (g)		Weight Extract ($gx10^{-3}$)		μg ext.g^{-1} wet tissue	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Oesophagus	178.2 ±	15.1	0	-	-	-
Rumen	509.5 ±	69.7	25.3 ±	3.6	49.8 ±	2.2
Reticulum	152.5 ±	24.1	8.2 ±	1.5	54.2 ±	3.2
Omasum	215.0 ±	12.5	12.0 ±	1.0	55.6 ±	2.0
Antrum	189.5 ±	6.1	9.6 ±	0.4	50.5 ±	0.9
Abomasal Body	626.5 ±	24.5	14.1 ±	0.6	22.5 ±	0.8
Duodenum 1	292.8 ±	28.7	19.8 ±	2.6	67.6 ±	5.7
2	169.5 ±	15.3	11.4 ±	1.1	67.2 ±	3.0
3	175.3 ±	10.4	10.9 ±	0.8	62.2 ±	2.8
4	145.6 ±	7.8	8.5 ±	3.5	58.4 ±	3.0
Caecum	298.0 ±	14.8	15.4 ±	1.0	51.8 ±	2.2

Table 10. The relative HCl - stimulating activities of pentagastrin compared to those of rumenal, reticular, omasal, abomasal body, duodenal and caecal freeze dried gastrin extracts. The ratios in all cases have been obtained by comparing the secretory activity of equal weights of pentagastrin to that of extract.

Extract	Rumenal: Penta.	Reticular: Penta.	Omasal: Penta.	Ab. Body: Penta.	Antral: Penta.	Duodenal: Penta. 1	2	3	4	Caecal: Penta.
1	0.33	0.36	0.33	0.38	0.96	0.43	0.41	0.30	0.21	0.40
2	0.39	0.37	0.35	0.36	0.99	0.42	0.16	0.12	0.08	0.43
3	0.38	0.40	0.38	0.42	0.95	0.35	0.30	0.28	0.10	0.43
4	0.34	0.36	0.37	-	-	0.29	0.27	0.24	0.08	0.54
5	0.34	0.35	0.47	-	-	0.33	0.22	0.22	0.09	0.39
6	0.33	0.35	0.35	-	-	0.40	0.22	0.16	0.12	0.45
Mean	0.36	0.36	0.37	0.39	0.97	0.36	0.24	0.20	0.10	0.44
S.E.M.	0.01	0.008	0.008	0.007	0.01	0.01	0.02	0.02	0.008	0.03

Table 11. The gastrin activity as μg pentagastrin $.g^{-1}$ wet weight rumen, reticulum, omasum abomasal body, antrum, duodenum and caecum. The means and standard error of the means, for 6 extracts from all regions except the abomasal body and antrum where 3 extracts were considered, are shown.

Extract	Rumenal	Reticular	Omasum	Abomasal body	Antral	Duodenal Segment 1	2	3	4	Caecal
1	17.73	19.85	19.96	9.52	50.73	22.77	30.44	20.01	13.28	19.02
2	21.11	22.32	21.20	7.87	48.93	31.90	10.65	8.88	5.85	22.73
3	19.38	24.57	21.22	8.86	46.63	29.87	22.25	19.80	6.24	23.60
4	15.21	16.13	19.36	-	-	14.73	12.73	9.30	3.97	24.40
5	19.15	21.95	23.09	-	-	23.00	15.32	13.06	6.30	23.93
6	18.74	21.65	21.17	-	-	30.35	16.16	11.03	6.22	24.20
Mean	18.55	21.09	20.99	8.74	48.76	25.44	18.26	13.68	6.98	22.98
S.E.M.	0.39	0.59	0.40	0.19	0.45	1.5	1.6	1.4	0.8	

Table 12. The total gastrin activity (as pentagastrin equivalents) in regions of the ovine gastrointestinal tract. These estimations are based on the data presented in tables 9 and 11.

	Total Gastrin Activity (penta. equivalents)	
	Mean	S.E.M.
Rumen	3147	136
Reticulum	1068	58
Omasum	1504	38
Abomasal body	911	53
Antrum	1539	26
Duodenum 1	372	39
2	155	25
3	120	18
4	50	9
Caecum	1140	40

summary of wet weight tissue, weight freeze dried gastrin extracts and the yield of extract expressed as a function of tissue weight is shown in Table 9. The means and standard error of the means have been calculated from extractions of batches of 3 rumen, reticulum, omasa; 6 oesophagi; abomasa and caeca; and batches of 20 duodena, each duodenum being divided into 4 successively more caudal 20 cm segments. When administered to anaesthetized rats, these extracts caused increases in the rates of gastric acid secretion.

A (2 + 2) dose assay of each extract was performed in 3 rats. Table 10 shows the relation between the acid stimulating activity of pentagastrin and of gastrin extracts from the ovine rumen, reticulum, omasum, abomasal body, antrum, duodenum and caecum. The gastrin activity (as μg pentagastrin .g wet tissue $^{-1}$) of each of the extracts is summarised in Table 11 where the mean and standard error of the mean for six extracts from each region are shown. The total gastrin activity of each of the regions of the ovine gastro-intestinal tract investigated (as the equivalent weight of pentagastrin (μg)) is shown in Table 12. The equivalent gastrin-like activity has been derived by comparing the secretory activity of equal weights of pentagastrin and extract.

The distribution of gastrin activity in the ovine gastro-intestinal tract, as a percentage of antral gastrin

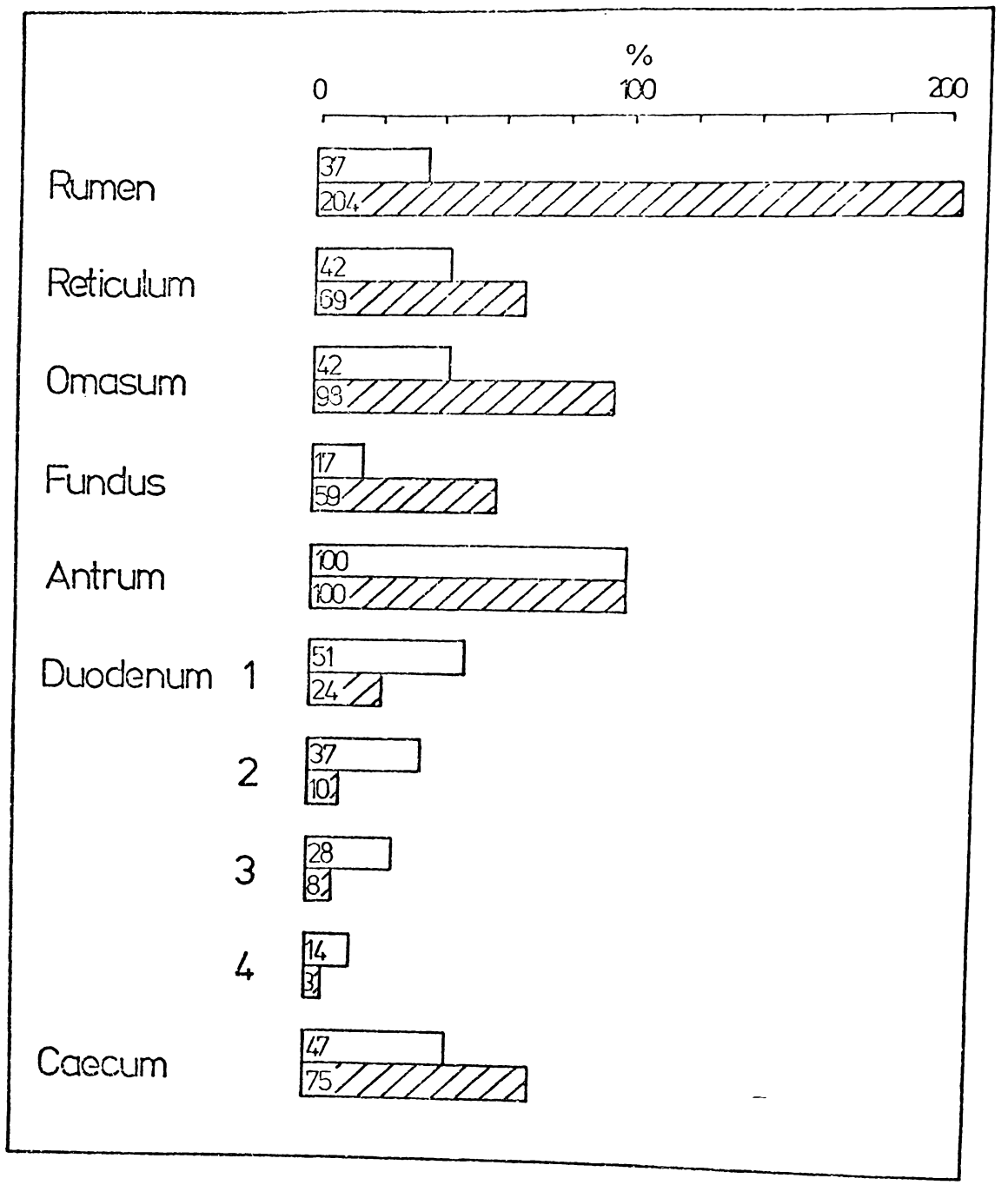

Fig. 11. The distribution of gastrin activity in the ovine gastrointestinal tract, as a percentage of antral gastrin activity.

☐ Concentration: pentagastrin equivalents per g wet tissue.

▨ Content: Total gastrin activity per region (pentagastrin equivalents).

Table 13. Wet weight tissue, weight of extracts and weight of extracts as a function of tissue weight, for extracts of separated muscle and mucosa from the rumen, reticulum, omasum, abomasal body and antrum.

mus. ≡ muscle

muc. ≡ mucosa

	Weight (g)		Wt. Ext. ($g \times 10^{-3}$)		μg Ext.g^{-1} tissue	
	mus.	muc.	mus.	muc.	mus.	muc.
Abomasal Antrum	212	102.7	0.3	10.1	1.4	98.3
Abomasal Body	675.2	192.3	0.5	13.6	0.7	70.7
Rumen	560.7	186.9	0.4	21.1	0.7	112.8
Reticulum	145.4	94.5	0.2	8.9	1.3	94.1
Omasum	225.1	143.2	0.2	12.8	0.8	89.3

Table 14. The relative stimulating activities of rumenal, reticular, omasal, abomasal body and antral mucosal extracts compared to that of pentagastrin. The gastrin concentration (in pentagastrin equivalents) is also shown.

		Extract/Penta.	µg penta.g^{-1}muc.	Mean (SEM)
Antral Extact	1	0.96	94.4	96.6 (2.4)
	2	0.98	96.3	
	3	1.01	99.3	
Ab. Body Extract	1	0.40	28.3	28.5 (0.3)
	2	0.41	28.9	
	3	0.40	28.3	
Rumenal Extract	1	0.36	40.6	42.1 (2.6)
	2	0.36	40.6	
	3	0.40	45.1	
Reticular Extract	1	0.37	34.8	34.5 (1.4)
	2	0.38	35.7	
	3	0.35	32.9	
Omasal Extract	1	0.39	34.8	33.3 (2.5)
	2	0.35	31.3	
	3	0.38	33.9	

activity is shown in Fig. 11.

Extraction of Separated Muscle and Mucosa: A summary of weight of tissue, weight of gastrin extract, and the yield of extract as a function of tissue weight for extracts of separated muscle and mucosa is shown in Table 13. Rumenal, reticular, omasal, abomasal body and antral mucosal extracts were assayed in three rats by comparing directly the secretory activity of equal doses (4.0×10^{-5}g) of extract and pentagastrin. The quantity of extract obtained from the muscle was insufficient for bioassay. Table 14 shows the relative stimulating activity of the mucosal extracts compared to that of pentagastrin, and the gastrin concentration as pentagastrin equivalents.

A more complete summary of the results, showing the wet weight tissue, weight of extract, μg extract $.g^{-1}$ wet tissue, the pH change and acid secreted during each bioassay of all freeze dried extracts from each region of the ovine gastrointestinal tract investigated, are tabulated in Appendices V - VI.

DISCUSSION

The present results have demonstrated gastrin activity to be present in the rumen, reticulum, omasum, fundus, antrum, duodenum and caecum of the sheep. The presence of gastrin activity in the ruminant antrum and fundus confirms reports of Anderson et al. (1961, 1962), Agarwal et al. (1968) and Jury and McLeay (1974).

To my knowledge this is the first study of gastrin distribution in the ovine gastrointestinal tract involving the forestomach, duodenum and caecum.

Recent reports indicate that the more simple methods for the extraction of gastrin are not satisfactory for the extraction of ovine gastrin as they fail to separate an unknown gelatinous substance from the fraction containing gastrin activity (Agarwal et al., 1968; Jury, 1974). In the present work the modification reported by Jury (1974) was used for the extraction of ovine gastrin. Although the method did not give highly purified preparations of gastrin, the water soluble extracts were potent stimulants of gastric acid secretion when injected into anaesthetized rats.

During the extraction procedure it was unlikely that histamine would have been absorbed onto the diethylaminoethyl cellulose floc with gastrin. Hence it was doubtful whether the final gastrin extracts contained appreciable amounts of

histamine. However, since gastrin-like activity was located in regions of the gastrointestinal tract not previously investigated, all extracts were tested for the presence of histamine using the bioassay method described by Adam et al. (1954). A (2 + 2) dose assay comparison of the histamine activity in the gastrin extracts with that of a commercial preparation of histamine was envisaged. However, since only very small amounts of histamine were present in the extracts it was considered sufficient to compare directly, the responses of the guinea pig ileum to high doses of extract (1×10^{-4}g) and lower doses of histamine (1×10^{-7}g). All extracts and pentagastrin had only a slight effect on guinea pig ileum, on which basis it was estimated that the extracts contained less than 1.0×10^{-6}g histamine (estimated as the hydrochloride) per milligram gastrin extract.

The anaesthetized rat is relatively insensitive to histamine (see Table 4; Smith, 1973), and in this work good gastric secretory responses were obtained after intravenous injection of solutions containing 5×10^{-6}g of extract (approximately 1×10^{-5}g.Kg^{-1} body weight). This amount of extract would have contained about 5×10^{-9}g histamine hydrochloride which is only a two thousandth of the smallest dose of histamine (2×10^{-5}g.Kg^{-1} body weight in the present work) that produced a secretory response in the rat perfused stomach preparation.

It has been reported that the isolated guinea pig ileum is sensitive to gastrin and pentagastrin (Bennett, 1965; Mikos and Vane, 1967). That the slight excitatory effects of the extracts on guinea pig ileum were due to gastrin is indicated by the abolition of the response to histamine, but not the extracts, by antihistamine preparations.

The perfused rat stomach preparation described by Smith et al. (1970) was used for the assay of gastrin extracts and was sensitive to doses of ovine gastrin extracts in the range 5×10^{-6}g to 4×10^{-5}g (1×10^{-5} to 8×10^{-5}g.Kg^{-1} body weight). This is a lower dose than that used by Amure and Omole (1971) who reported that doses of gastrin extract from various mammals (including cattle) in the range 1×10^{-4}g to 8×10^{-4}g (approximately 5×10^{-4} to 4×10^{-3}g.Kg^{-1} body weight) were required to elicit a secretory response from the rat perfused stomach preparation; an observation possibly reflecting the crude nature and low gastrin content of their extracts which were obtained by the method of Blair et al. (1961).

As discussed in the previous chapter, the first response of the perfused rat stomach preparation was variable and was hence rejected. Otherwise the variability with successive doses was low, thus allowing a (2 + 2) dose assay comparison of the activity of two extracts and pentagastrin in the one animal. Pentagastrin was used as the bioassay standard

because it is considerably cheaper, and more easily obtainable than commercial preparations of pure gastrin. Such a choice was justified as Smith et al. (1970) have reported that pentagastrin has approximately the same activity as synthetic human gastrin I over the range of sensitivity of the perfused rat stomach assay.

Although not as sensitive as radio-immunoassay techniques (McGuigan, 1968; McGuigan et al. 1968; Berson and Yalow, 1972), methods involving bioassay of gastrin activity have the advantage of measuring the physiologically active component of the extract, and were quite sufficient for the detection of gastrin in extracts of the ovine gastrointestinal tract.

In the present work no extended study was undertaken to quantitatively measure the loss of gastrin like activity with time, and under varying storage conditions. It was of interest to assess whether gastrin activity was maintained during the time in which the three bioassays were carried out. As there was little change in the activity of the extracts during the first three weeks after preparation of the extract, loss of activity would not have influenced the calculation of relative potencies of the extracts from the three bioassays, which were usually completed within one week of preparation. The extracts were not stored under vacuum or in an inert gas and thus it seems likely that the loss of activity was caused by oxidation. However the effects of

bacterial activity cannot be discounted.

Variation of the activities of initial duodenal extracts lead to an investigation of the difference in activity between freeze dried and air dried extracts. Subsequently it was discovered that freeze dried extracts were approximately 50% more active than air dried extracts. This difference in activity may be attributable to:

(1) The air dried extracts having a higher water content than freeze dried extracts, so that solutions of freeze dried extracts would have contained more active material.

(2) The final precipitation and air drying, by decreasing the pH, and drying at room temperature, denaturing some of the active components.

(3) The air dried extracts containing residual quantities of ammonium bicarbonate so that greater amounts of inert material were present than in the freeze dried extracts. Being volatile this salt would have been sublimed during freeze drying.

The results of the biological assay of ovine gastrin extracts have been expressed as the equivalent weight of pentagastrin (µg) per gram wet tissue extracted. Rumenal, reticular, omasal and caecal tissue contained approximately

equal amounts of gastrin activity; 18.55 μg, 21.09 μg, 20.99 μg and 22.98 μg $.g^{-1}$ wet weight respectively. The proximal duodenal segment contained 25.44 μg pentagastrin per gram wet weight, but in more distal segments the activity dropped markedly, the fourth 20 cm segment containing only 6.98 μg pentagastrin per gram wet weight. On this basis the antrum contained most gastrin activity at 48.76 μg, and the fundus contained 8.74 μg pentagastrin per gram wet weight.

It should be noted that material was collected from sheep varying in age from 1 - 4 years, and that all regions studied were not necessarily collected from the same animal. However, estimates of the relative total content of gastrin in the respective tissues designating the antrum 100% are about; 50% in the abomasal body, 50% in the proximal 800 mm of intestine and 400% in the rumen, reticulum and omasum collectively. Thus taking into account any variation in relative weights of the respective regions, it is apparent that when the total gastrin activity in each region is considered the amount of extra-antral gastrin is substantial.

It has been reported that gastrin activity is present in the antral mucosa of the human, dog, cat and hog (Nilsson et al., 1973). In the present investigation, extraction of separated rumenal, reticular, omasal, abomasal body and antral mucosa yielded freeze dried extracts in comparable

quantities to that by extraction of the whole organs. The small quantity of material extracted from the separated muscle probably originated from residual mucosa, but the yield was insufficient to allow bioassay. However, bioassay of the mucosal extracts showed them to contain gastrin activity similar to that of extracts obtained from whole organs. The concentration of gastrin activity (in pentagastrin equivalents) was 96, 28.5, 42.1, 34.5 and 33.3 μg penta $.g^{-1}$ mucosa in the antrum, abomasal body, rumen, reticulum and omasum respectively. In bovine antral mucosa Amure and Omole (1971) have reported a gastrin concentration of 28.5 ug per gram of mucosa, when mucosal extracts were bioassayed against porcine gastrin II. This may reflect a real difference in gastrin content between sheep and cattle antral mucosa, but the lower figure reported for cattle is probably due to the unpurified nature of their extracts which were obtained by the relatively simple method of Blair et al. (1961).

The reason for variation of the specific activity of extracts from different regions (that is, the degree of stimulation per unit weight of extract) is not known. It has been suggested that the greater mass of tissue associated with the fundus may be a contributing factor (Jury, 1974). This does not, however, account for the lower specific activity of reticular, omasal and duodenal extracts. Similarly, Anderson et al. (1961, 1962) observed a difference in specific activity of their antral and fundic

extracts.

Anderson et al. (1961, 1962) reported that their bovine gastrin extracts had a higher specific activity than their ovine extracts, but their ruminant gastrins were less potent as stimulants of acid secretion when injected into dogs provided with denervated pouches, than porcine gastrin prepared by the same method. However, as their extracts were relatively crude, these differences may have been due to differing amounts of inert material present in the extracts, as highly purified ovine and bovine gastrins have been reported to show activity identical in form and degree to porcine gastrin (Agarwal et al., 1968). In the present work, comparisons of the activity of ovine gastrins with gastrins of non-ruminant origin have not been attempted.

Using the present methods, gastrin activity was not extractable from the ovine oesophagus. Nilsson et al. (1973) have reported the presence of minute quantities of gastrin activity in the oesophagus of the cat, dog, hog and human, a result possibly reflecting the greater sensitivity of radioimmunoassay of the initial aqueous extracts of tissue compared with the relatively insensitive bioassay system used in the present experiments.

The presence of gastrin-like activity in the ovine antrum confirms the reports of Anderson et al., (1962),

Agarwal et al. (1968) and Jury (1974). Gastrin activity has also been located in the bovine antrum (Anderson et al., 1961; Agarwal et al., 1968; Amure and Omole, 1971). In a comparative study Amure and Omole (1971) investigated the presence of gastrin-like activity in the antral mucosa of the goat, rabbit, cattle, cat, dog, man and pig, and reported that the amounts of gastrin activity per gram wet mucosa from the herbivores (goat, rabbit and cattle) were greater than those from the non-herbivores. However, Agarwal et al. (1968) have reported that lower yields of gastrin extract were obtained from the ovine and bovine antrum than from the hog antrum. These conflicting reports are probably due to the different extraction procedures that have been used. Nevertheless, it is apparent that ruminants are similar to other mammals in having considerable amounts of gastrin activity in their antral mucosa. The presence of gastrin activity in the body region (confirming the reports of Anderson et al., 1961, 1962; Jury, 1974) is in contrast to animals with a more simple stomach where gastrin activity is largely confined to the antrum, although, using bioassay methods, a small amount has been detected in the cardiac region of the hog (Edkins, 1905; Gregory et al., 1961) and cat (Lim, 1922; Emås and Fyrö, 1968) stomach. Using radioimmunoassay techniques, Nilsson et al. (1973) have reported that in the human, dog and cat, the remaining portion of the stomach contained 1.3%, 4.3% and 1.1% respectively, of the gastrin activity detectable in the antrum.

It has been reported that duodena of the cat, dog, hog and human contain gastrin activity; the concentration being highest in the proximal portion, and decreasing in more caudal regions (Emås et al., 1968; Nilsson et al., 1973). Some workers have been unable to detect gastrin activity in the duodenum of the hog (Uvnäs, 1945 a; Gregory and Tracy, 1961) and the human (Elliott et al., 1963) and only slight activity in 1 of 4 extracts of the cat duodenum (Uvnäs, 1943). Since a concentration gradient of gastrin exists in the duodenum of the human, dog, cat and hog (Nilsson et al., 1973) the gastrin concentration of extracts of the whole duodenum may have been too low to detect, thus accounting for the variable reports. Lia (1964) has reported gastrin-like activity to be present in the human duodenum in a ratio of unit activity of 10, compared to 13.5 for that of the antrum. Radioimmunoassay studies (Nilsson et al., 1973) have shown that the proximal duodenum of the human, dog, cat and hog contained 38%, 1.0%, 1.6% and 0.06% respectively, of the gastrin activity detectable in the antrum, but that only the whole human duodenum contained quantities of gastrin nearly equal to those present in the antrum. Thus, from the present work (where the proximal 800 mm of the ovine duodenum contained about 50% of the gastrin activity present in the antrum) it is apparent that the sheep is similar to the human in having substantial quantities of gastrin activity present in the duodenum.

Small quantities of gastrin activity have been detected

in the human (Lia, 1964; Nilsson et al., 1973) cat, dog and hog (Nilsson et al., 1973) jejunum and ileum. The presence of gastrin in the caecum has not previously been reported. Likewise the presence of gastrin activity in the ruminant rumen, reticulum and omasum has not been reported.

Fig. 12. Forestomach, mainstomach, pyloric stomach, duodenal ampulla and duodenum of the dolphin Delphinus delphis.

CHAPTER IV: ISOLATION OF GASTRIN-LIKE ACTIVITY FROM THE DOLPHIN (DELPHINUS DELPHIS)

INTRODUCTION

Our laboratory received a young female dolphin, Delphinus delphis which had died in a fishing net. As we had not read of previous attempts on the extraction of gastrin from the gut of dolphins, an extraction from certain parts of the complex stomach of this animal was attempted.

The complex stomach of the dolphin consists of forestomach, mainstomach, pyloric stomach, duodenal ampulla and intestines (Fig. 12). The forestomach serves both as a storage organ for ingested whole fish, and aids digestion by exerting a mechanical action and breaking its contents, probably aided by ingested stones or sand. The whole of the mainstomach is lined by mucous cells, chief cells and parietal cells, and corresponds to the body of the stomach in most mammals, from which acid and pepsin secretion occurs. The pyloric stomach contains only mucous secreting cells, and has the form of a simple tube of uniform diameter. The presence of a sphincter between the pylorus and duodenum implies that the products of digestion are retained for varying periods of time (Harrison, Johnson and Young, 1970). The function of the duodenal ampulla has not been described.

In the present study the extraction of gastrin-like material from the forestomach, mainstomach, pyloric stomach, duodenal ampulla, intestine and skeletal muscle of Delphinus delphis was attempted. All extracts were bioassayed for histamine and gastrin activity.

METHODS

The animal was in good condition on arrival at the laboratory within an estimated 24 hours of death, and was deep frozen for 14 days before an extraction of gastrin was attempted.

Extractions were made separately on skeletal muscle, forestomach, mainstomach, pyloric stomach, duodenal ampulla and a segment of duodenum extending 30 cm candal to the termination of the duodenal ampulla. The extraction procedure was that described previously for the extraction of gastrin-like material of ruminant origin.

All extracts were assayed for the presence of histamine using an isolated guinea-pig ileum. The procedure was the modification of the method of Adam, Hardwick and Spencer (1954) described previously; responses of the ileum to high doses of test extract (2×10^{-4}g) and low doses of histamine (1×10^{-6}g) (Ethicals Ltd., Auckland) were directly compared. The effects of the antihistamine chlorpheniramine maleate (Piriton, Allen and Hanbury's, England) were studied.

The extracts were assayed for gastrin-like activity using the perfused rat stomach technique described by Smith et al. (1970) as discussed in the previous chapter. Each extract was assayed in two rats except that of the duodenal ampulla which was assayed in one rat. In each assay, the

secretory response to doses (4×10^{-5}g) of extract and pentagastrin were compared.

Table 15. Weights of tissues and extracts and their gastrin activity as μg pentagastrin for each assay, for regions of the Dolphin gastro-intestinal tract.

Region	Wet Weight tissue (g)	Weight of Extract ($g \times 10^{-3}$)	μg penta. g^{-1} tissue
Forestomach	547	202	119
			92
Mainstomach	235	121	77
			113
Pyloric Stomach	136	25	34
			28
Duodenal Ampulla	68	5	10
Intestine	81	14	26
			24
Skeletal Muscle	434	0	-

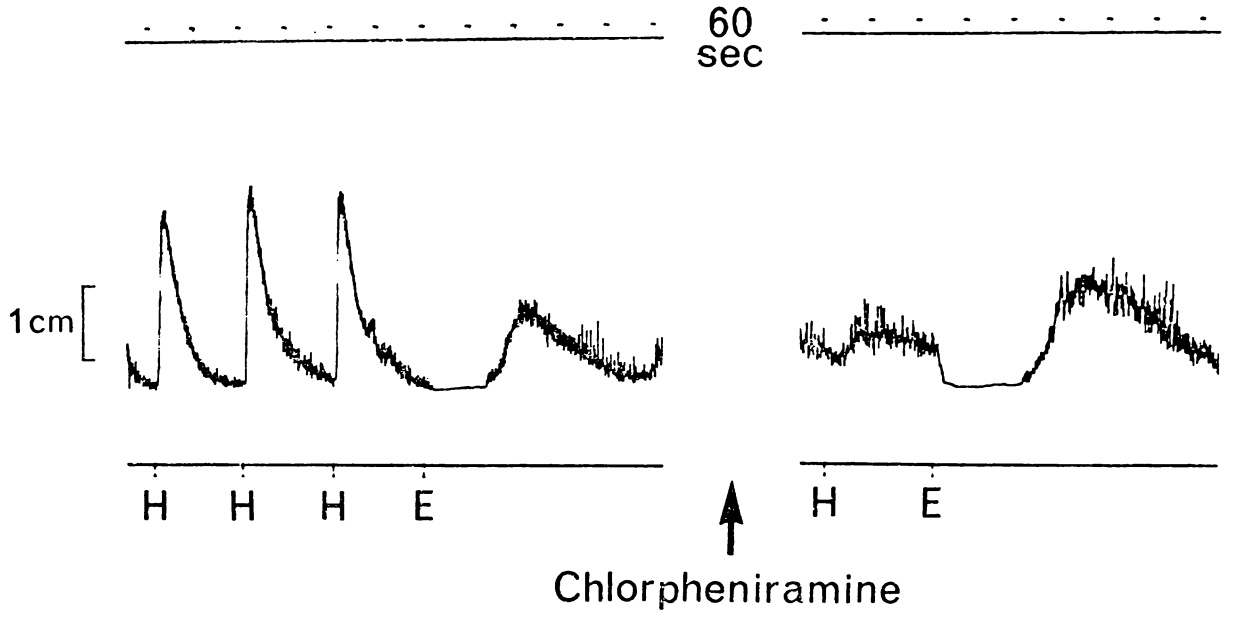

Fig. 13. Responses of isolated guinea pig ileum to histamine - - - and the dolphin pyloric stomach extract.

RESULTS

Extracts were obtained from all tissues except that of skeletal muscle. Table 15 shows the weight of material extracted, and the weight of the extract.

Histamine Assay: The isolated guinea-pig ileum was stabilised by applying a number of doses of histamine (see Chapter III), after which it contracted in response to the extracts (2×10^{-4}g) with a characteristic latency of 60 - 120 sec. (Fig. 13). In contrast a standard dose of histamine (1×10^{-6}g) or pentagastrin (2.5×10^{-5}g) caused an immediate response. Contractions in response to histamine were abolished by the anti-histamine chlorpheniramine maleate but the responses to the extracts and to pentagastrin remained (Fig. 13).

Assay of Gastrin Activity: All extracts stimulated HCl secretion from the anaesthetized rat's perfused stomach when administered in doses of 4×10^{-5}g. In particular substantial activity was obtained from the forestomach and mainstomach. Table 15 shows the gastrin activity as pentagastrin equivalents per gram of tissue extracted.

DISCUSSION

These preliminary experiments show that gastrin activity may be extracted from all regions of the complex stomach and from the duodenum of the dolphin. Delphinus delphis. In particular appreciable activity was obtained from the forestomach and the mainstomach, with smaller amounts in the pyloric stomach and intestine. It is possible that the activity extractable from the duodenal ampulla may be influenced by the difficulties encountered in harvesting such small amounts of extract. Judged on the responses of the guinea-pig ileum, as discussed for the extracts of ruminant origin, it appears unlikely that the active principle is histamine.

Due to the relatively small amounts of material harvested from separate regions of the stomach and intestine of the one dolphin, and the necessity of testing the extracts for histamine activity, bioassays could only be undertaken in two rats for all extracts, except that of the duodenal ampulla which was assayed in one rat. The number of assays performed was insufficient to allow a quantitative assessment of activity, but they do, however, provide qualitative information. By a comparison to previous reports concerning other animals (Nilsson et al., 1973) it was expected that gastrin-like activity would be found in the duodenum as well as in the pyloric stomach. More surprising, however, were the substantial amounts of gastrin

activity present in the mainstomach and forestomach. However, the present study has indicated the presence of gastrin activity in the forestomach and the acid secreting part of the sheep stomach, and thus Delphinus delphis and the ruminant are similar in having gastrin-like activity distributed throughout their complex stomachs.

Previous reports on the distribution of gastrin in vertebrates other than the mammal appear to be limited to a few conflicting studies in birds. Blair, Sherratt and Wood (1967) have reported that extracts of chicken duodenum had gastrin-like actions on gastric secretion in the cat, while Olowo-Okorum and Amure (1973) found that extracts of chicken proventriculus but not duodenum had gastrin-like actions on acid secretion in the rat. In contrast it has been reported that gastrin-like activity was not present in extracts of chicken gizzard, proventriculus or duodenum using bioassays in chicken and rat, or using radioimmunoassay (Barrington and Dockray, 1976).

CHAPTER V: CHARACTERISATION OF THE EXTRACTS

INTRODUCTION

In the present work the extracts have been characterised by determining the molecular weight of their active components, and an estimation of their isoelectric point.

Gastrin Heterogeneity

Gregory and Tracy (1964), who succeeded in purifying gastrin from hog antral mucosa, found gastrin to be a pair of 17 amino acid peptides with a molecular weight of approximately 2,100. These they named gastrin I and gastrin II, (GI and GII). The only difference between the two was a sulphate group in the tyrosine residue of gastrin II. It was generally assumed that these peptides represented some form of the gastrin molecule.

Subsequent reports, however, have indicated the existance of gastrins with varying molecular weights. Tauber and Madison (1965) reported gastrin to be a molecule of molecular weight 12,500. It has been claimed that the antrum, in addition to gastrin 17 contains gastrin components with molecular weights between 4,000 and 10,000 (Dyce, Bundy, Stubrin, Andersson, Borisoff and Haverback, 1964; Wilding, Dyce, Rinderknecht and Haverback, 1966a and b).

Using radioimmunoassay techniques, Yalow and Berson (1970a and b) have reported that immunoreactive gastrin circulates as two components. One component having size and charge characteristics similar to 17-amino acid gastrin, and the other, the prominent component, a larger and more basic molecule consisting of 34 amino acids with a molecular weight of around 7,000. They named the new component BG or 'basic gastrin' or 'big gastrin', and the heptadecapeptide-like gastrin component H-LG. Incubation with trypsin converted all the immunoreactivity in the big component to the gastrin-17-like component (Yalow and Berson, 1971). Peptides with properties identical to those of plasma BG and H-LG have also been demonstrated to be present in tissue extracts from mucosa of human antrum, duodenum and jejunum (Yalow and Berson, 1971). The proportion of BG present in the extracts increased from antrum to duodenum to jejunum. A pair of gastrin peptides having the size and charge characteristics of BG have also been isolated from gastrinoma tissue (Gregory and Tracy, 1974). Like the heptadecapeptides, the pair of BG peptides contained the same amino acids, differing only in the presence or absence of a sulphated tyrosine. The peptides have hence been named BGI (without sulphate) and BGII (with sulphate).

Yalow and Berson (1972) reported a small quantity of gastrin activity which was eluted in the void volume of Sephadex G50 columns. It had a molecular weight in excess

of 30,000, and has been termed BBG or 'big big gastrin'. BBG is the predominant form of gastrin found in the serum of fasting man, hog and dog but it does not increase on feeding. A BBG component is present in extracts of Zollinger-Ellison (Z.E.) tumour and hog antral extracts (Yalow and Wu, 1973). The chemical nature of BBG has not been determined. On Sephadex G50 columns, emerging just before the BG peak is a small peak which has been named component I. It is an unpaired peptide (Rehfeld, 1973). A small component consisting of the C-terminal tridecapeptide amide of gastrin-17, in both sulphated and non-sulphated form, is present in extracts of Z.E. tumour. It is known as 'mini-gastrin' or MG (Gregory and Tracy, 1974).

Using antibodies with selective specificity for the N-terminal region of G-17, Dockray and Walsh (1975) have discovered a 13-amino acid fragment corresponding to the N-terminal fragment of heptadecapeptide gastrin, in serum. A pair of 1-13 N-terminal fragments (sulphated and non-sulphated) have been isolated from hog antral mucosa (Gregory, 1974). It is unlikely that these fragments have any effect on gastric secretion as they lack the biologically active C-terminal tetrapeptide of G-17. The isolation of the C-terminal tetrapeptide, corresponding to these N-terminal fragments has not yet been reported.

Walsh, Debas and Grossman (1974) have reported that

pure human BG has a circulation half life of 15.8 min compared to that for H-LG of 3.2 min. Hence BG causes a greater and more prolonged acid secretory response (Gregory and Tracy, 1973). However, when secretory responses are related to circulating gastrin levels it is found that a nearly 5 times greater molar increment of circulating BG is required compared with H-LG (Walsh et al., 1974). The 'mini gastrin' peptides have about half the potency of heptadecapeptide gastrin, but have only a slightly shorter half life. The activity and structures of BBG and component I have not yet been determined.

Cholecystokinin-like activity has been isolated from the ruminant duodenum (Reynolds and McLeay, 1976). Cholecystokinin is a peptide hormone present in the small intestine which acts as a stimulant of the entry of bile into the small intestine, by virtue of its actions on the gallbladder and sphincter of oddi (Harvey, 1975). Cholecystokinin has an effect on gastric acid secretion. In man and dog cholecystokinin acts only as a partial agonist for gastric acid secretion, and thus acts as a competitive inhibitor for gastrin (Grossman, 1970). However, in the rat and cat it is a full agonist for gastric acid secretion, and therefore does not inhibit the action of gastrin (Harvey, 1975). To ensure that the secretory activity displayed by the extracts was not attributable to the presence of cholecystokinin the isoelectric points of the crude gastrin extracts have been determined.

Cholecystokinin (from the duodenum) and ovine antral gastrin have isoelectric points of 5.0-5.5 (Grossman, 1950) and 3.5-4.0 (Anderson et al.,1962) respectively.

Crude gastrin extracts from all regions of the ovine gastrointestinal tract, investigated in the present work, have been fractionated on Sephadex G25 SF and G50 SF columns. Peaks showing absorption at 210 nm were freeze dried and tested for gastrin-like activity in the anaesthetized rat. Isoelectric points of the crude extracts were determined using a technique of isoelectric focussing on polyacrylamide gels.

To rule out the possibility that the higher molecular weight fractions of gastrin are aggregations of smaller molecules a preliminary investigation has been carried out. This has included fractionations of crude extracts on columns of Sephadex at high pH to deter hydrogen bonding, and the treatment of extracts with sodium dodecyl sulphate (S.D.S.) to prevent molecular aggregation. S.D.S. forms complexes with individual protein chains, which have a rod-like configuration. The length of these complexes is a function of the molecular weight. The individual differences between electrophoretic mobility of the S.D.S.-protein complexes is small. Therefore the relationship between the migration rate on polyacrylamide gels during electrophoresis, and molecular weight is derived from the proportionality between molecular weight and effective

complex size (Banker and Cotman, 1972).

METHODS

Molecular Weight Studies

Two crude freeze dried extracts from each of the rumen, reticulum, omasum, abomasum, duodenum and caecum were fractionated on Sephadex G25 and G50 columns (26 mm diameter and 400 mm long) at room temperature packed in 0.1 molar $NH_4 HCO_3$ (pH 8.0) buffer. The columns were perfused by gravity at a rate of 1 ml. min^{-1}.

All extracts were initially fractionated on a Sephadex G25 column. Any material eluted in the void volume of this column (i.e. having a molecular weight in excess of 5,000) was freeze dried and then fractionated on a Sephadex G50 column.

Between 5.0×10^{-3}g and 1.0×10^{-2}g of the extract was dissolved in a minimum amount of buffer, usually about 2 ml. This solution was applied to the column, and the absorption of the eluant measured at 210 nm in a spectrophotometer equipped with a flow cell (Shimadzu uv-200) and recorded (Heathkit, model 112-18 m, chart recorder). The eluant was collected in 5 ml fractions (LKB fraction collector). Fractions showing absorption at 210 nm were pooled and then freeze dried, before being assayed for gastrin activity in the anaesthetized rat.

Calibration of Sephadex Columns: The Sephadex columns were

calibrated using substances of known molecular weight so that estimations of the molecular weights of the components of the crude extracts could be made. Sephadex G25 columns were calibrated with insulin (A-chain, M.W. 2,300; B-chain, M.W., 3,400; total insulin M.W., 5,700) and pentagastrin (Peptavlon, I.C.I. England) (M.W., 768). Sephadex G50 columns were calibrated using insulin (insulin M.W. 5,700; A chain M.W. 2,300; B chain M.W. 3,400) and cytochrome C (M.W. 12,384). The elution volume of each standard was plotted against the log molecular weight.

Isoelectric Focussing

Isoelectric points of the extracts were determined using a technique of isoelectric focussing, modified from that reported by Wrigley (1972). Disc electrophoresis apparatus was used with gel tubes of 5 mm internal diameter, and 80 mm long. Eight such tubes could be fitted into the apparatus.

Polyacrylamide Gel Preparation: The acrylamide solution used for gel formation contained 30g acrylamide monomer and 1g N.N-methylene bisacrylamide (the cross linking dimer) dissolved in water to 100 ml.

For each gel tube 1.4 ml water, containing 2.0×10^{-4}g of sample extract, was mixed with 0.05 ml Ampholine carrier ampholytes (40% solution, pH range 3-10; LKB Produkter - AB Stockholm, Sweden). To this was then added 0.5 ml of

the acrylamide solution and 0.1 ml fresh 1% ammonium persulphate. The gel tubes, closed off at the lower end by means of a rubber bung, were three quarters filled with this mixture. A little water was carefully layered on top of the mixture to prevent aerobic inhibition of polymerization. The gels usually set within one hour at room temperature.

Electrophoresis: The bungs were removed from the gel tubes. The lower ends of the tubes were placed in 0.4% ethanolamine solution in contact with the cathode, and the upper end in contact with 0.2% sulphuric acid and the anode of the electrophoresis apparatus. The voltage was slowly increased and a maximum of about 3mA per tube was maintained. The proteins were left to focus in the gels overnight.

Staining: The gels were removed from the tube by rimming between the gel and tube with a fine needle well lubricated with water. The gel was sliced longitudinally, and one half stained for about one hour in a solution of 0.2% bromophenol blue in ethanol: water: acetic acid (50:45:5). After staining, the gel was destained overnight in a solution of ethanol: water: acetic acid (30:65:5). With this procedure the protein bands stained an intense yellow/ green colour. These gels were scanned in a densitometer (Helena Laboratories Ltd. "Quick Scan") and then the isoelectric point of each band was determined.

Determination of Isoelectric Point: The stained and unstained halves of the original gel were compared and the zones corresponding to protein bands were cut from the unstained portion of the gel. These sections were soaked in 1 ml distilled water (pH 5.5) for one hour, and then the pH of the extract was measured (scale expanded on recorder so that full scale deflection was equivalent to 1.0 pH unit). This pH was taken to be the isoelectric point of the test protein.

Investigation of Interelationships between M.W. Fractions

Fractionation at Higher pH: Two crude freeze dried extracts from the reticulum were fractionated on a column of Sephadex G25 packed in 0.01M KOH (pH 12.0) buffer.

S.D.S. Electrophoresis: Glass disc electrophoresis tubes, 10 cm long and 6 mm internal diameter were soaked in chromic acid, rinsed and oven dried before use. For an experiment with 8 gels, 15 ml of gel buffer (Appendix VI) was deaerated and mixed with 13.5 ml acrylamide solution. After further deaeration, 15 ml of fresh ammonium persulphate solution (15×10^{-6} g ml^{-1}) and 0.045 ml N, N, N^1, N^1 - tetramethylethylene diamine, were added. After mixing, each was filled with 2 ml of the solution. A few drops of water were layered on the surface of the gel to prevent aerobic inhibition of polymerisation. The gels usually set within 30 min. The molecular weight standards used were pentagastrin, cytochrome C, myoglobin, soybean trypsin

inhibitor, ovalbumin and bovine serum albumin. These standards, and crude extracts from the reticulum and the abomasal body at a concentration of $1.0 \times 10^{-3} g.ml^{-1}$., were incubated at $37^{o}C$ for 2 hr with 0.01 molar sodium phosphate buffer (pH 7.0) (Appendix VII) with 1% S.D.S., and 1% B-mercaptoethanol. For each gel tube a solution comprising, 3ul of tracking dye (0.05% Bromphenol blue in water), 1 drop glycerol, 5µl of mercaptoethanol and 50µl of phosphate buffer was prepared in a small test tube. Then 10ul of the protein solution was added. After mixing, the solutions were applied to the gels. Gel buffer (Appendix IX) was then carefully layered on each sample to fill the tubes, and the two compartments of the disc electrophoresis apparatus were filled with gel buffer diluted 1:1 with water.

After electrophoresis the gels were removed from the tubes by rimming between the gel and tube with a fine needle well lubricated with water, and the position of the bromphenol blue tracer marked by puncturing the gel with Indian ink.

After placing in small slotted tubes, the gels were stained by leaving overnight in a staining solution prepared by dissolving 1.25 g of Comassie brilliant blue in a mixture of 454 ml of 50% methanol and 46 ml glacial acetic acid. After rinsing in distilled water the gels were destained in a solution of 75 ml acetic acid, 50 ml of

methanol, and 875 ml of water, for 1 day. Positions of the bromphenol blue tracer and the blue protein bands were recorded, and the mobility of the proteins calculated as:

$$\text{Mobility} = \frac{\text{distance of protein migration}}{\text{distance of dye migration}}$$

The log mobilities were plotted against the known molecular weights of the standards.

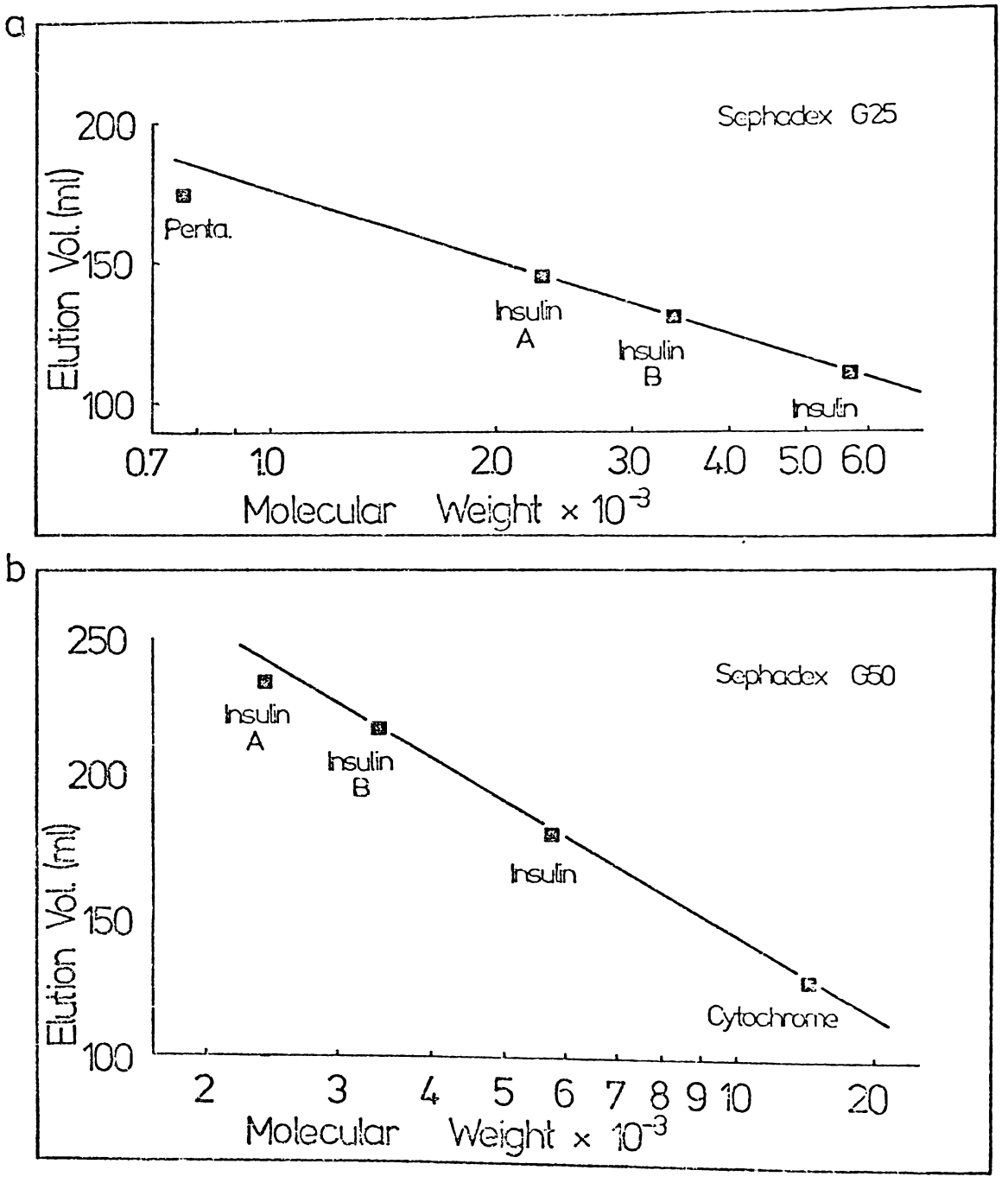

Fig. 14. The elution volume verus log molecular weight plots for the calibration of Sephadex columns.

(a) Calibration of Sephadex G25 with Insulin and pentagastrin.

(b) Calibration of Sephadex G50 with Insulin and cytochrome C.

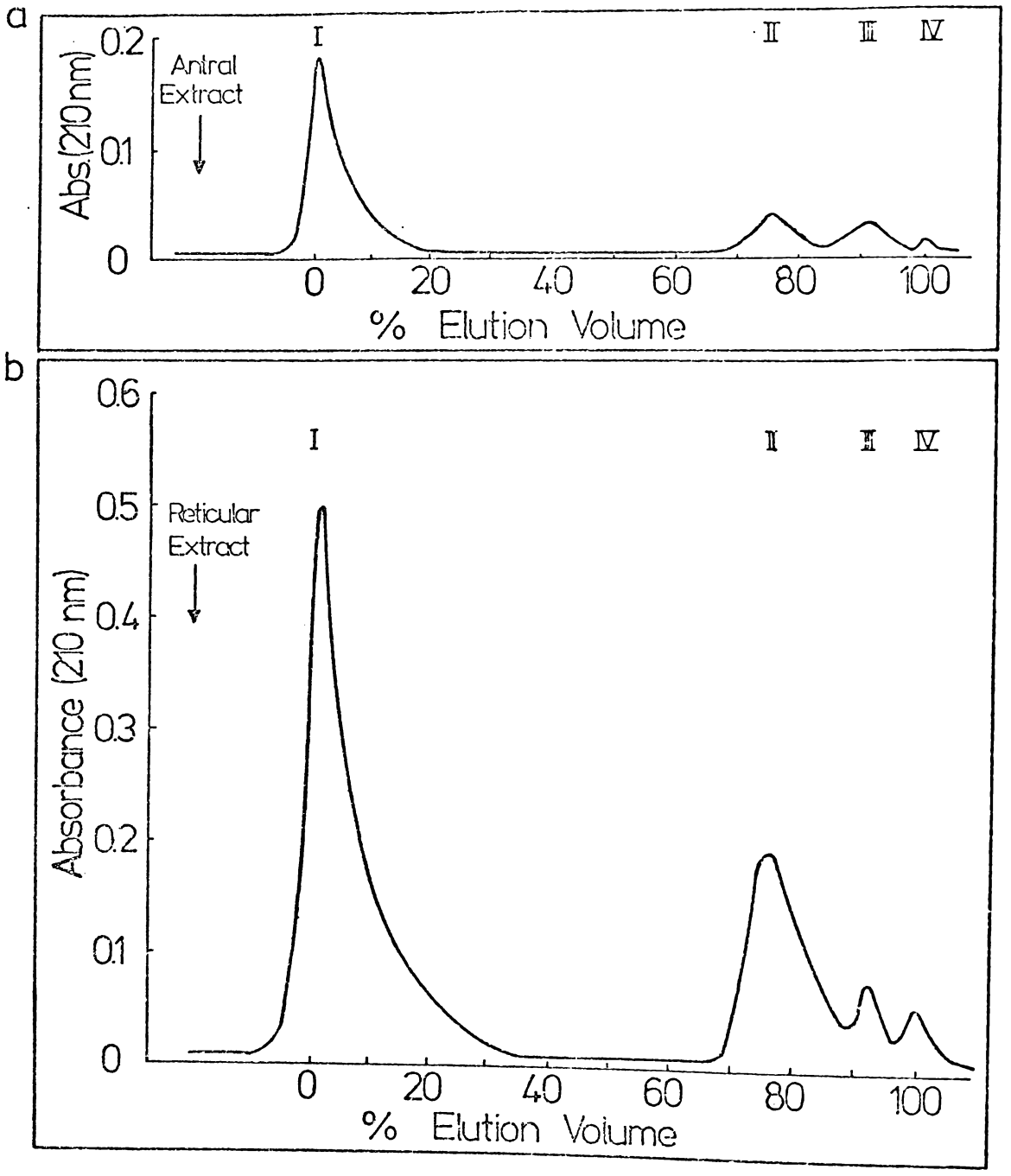

Fig. 15. Fractionation of a gastrin extract on a column of Sephadex G25. Peaks I - IV are referred to in the text.

(a) 4.5×10^{-3}g crude freeze dried antral extract.

(b) 8.0×10^{-3}g crude freeze dried reticular extract.

RESULTS

Molecular Weight Studies

Molecular Weight Calibration of the Columns: Initially the columns of Sephadex G25 and G50 were calibrated with substances of known molecular weight. Figs. 14a and 14b show the elution volume versus log molecular weight plot for insulin and pentagastrin fractionated on a column of Sephadex G25 and insulin and cytochrome C on a column of Sephadex G50.

Fractionation of Extracts

Consistancy of Extract Behaviour on Sephadex: Two crude freeze dried extracts of the ovine rumen, reticulum, omasum, abomasal body, antrum, duodenum and caecum were fractionated on columns of Sephadex G25 and G50. Each pair of extracts behaved in an identical manner when fractionated on both of these columns.

Fraction of Crude Extracts on Columns of Sephadex G25: Extracts from the different regions of the ovine gastro-intestinal tract behaved similarly when fractionated on columns of Sephadex G25, although there was some variation in the relative size of the peaks with extracts from different regions. Approximately 60% appeared as a single peak in the void volume of the column (peak I) followed by two smaller peaks (peaks II and III) at a position corresponding to 75% and 91% of the elution volume of the

Table 16. The relative gastrin activities (as extract activity/pentagastrin activity) on an equal weight basis of material taken from peaks I - IV obtained after fractionation of crude freeze dried extract on a column of Sephadex G25.

Peak	Rumen	Reticulum	Omasum	Ab. body	Antral	Duodenal	Caecal
I	1.09	0.95	0.97	1.00	1.01	1.03	1.12
II	0.14	0.19	0.16	0.15	0.13	0.15	0.18
III	0.06	0.06	0.06	0.06	0.05	0.06	0.05
IV	0	0	0	0	0	0	0

Table 17. The relative gastrin activities (as extract activity/pentagastrin activity) on an equal weight basis of material taken from peaks V - VIII obtained after fractionation of peak I (from Sephadex G25) on a column of Sephadex G50.

Peak	Rumen	Reticulum	Omasum	Ab. body	Antral	Duodenal	Caecal
V	1.05	1.33	1.26	1.18	1.11	1.26	1.23
VI	0	0	0	0	0	0	0
VII	1.11	1.23	1.09	1.05	1.01	1.44	1.31
VIII	0	0	0	0	0	0	0

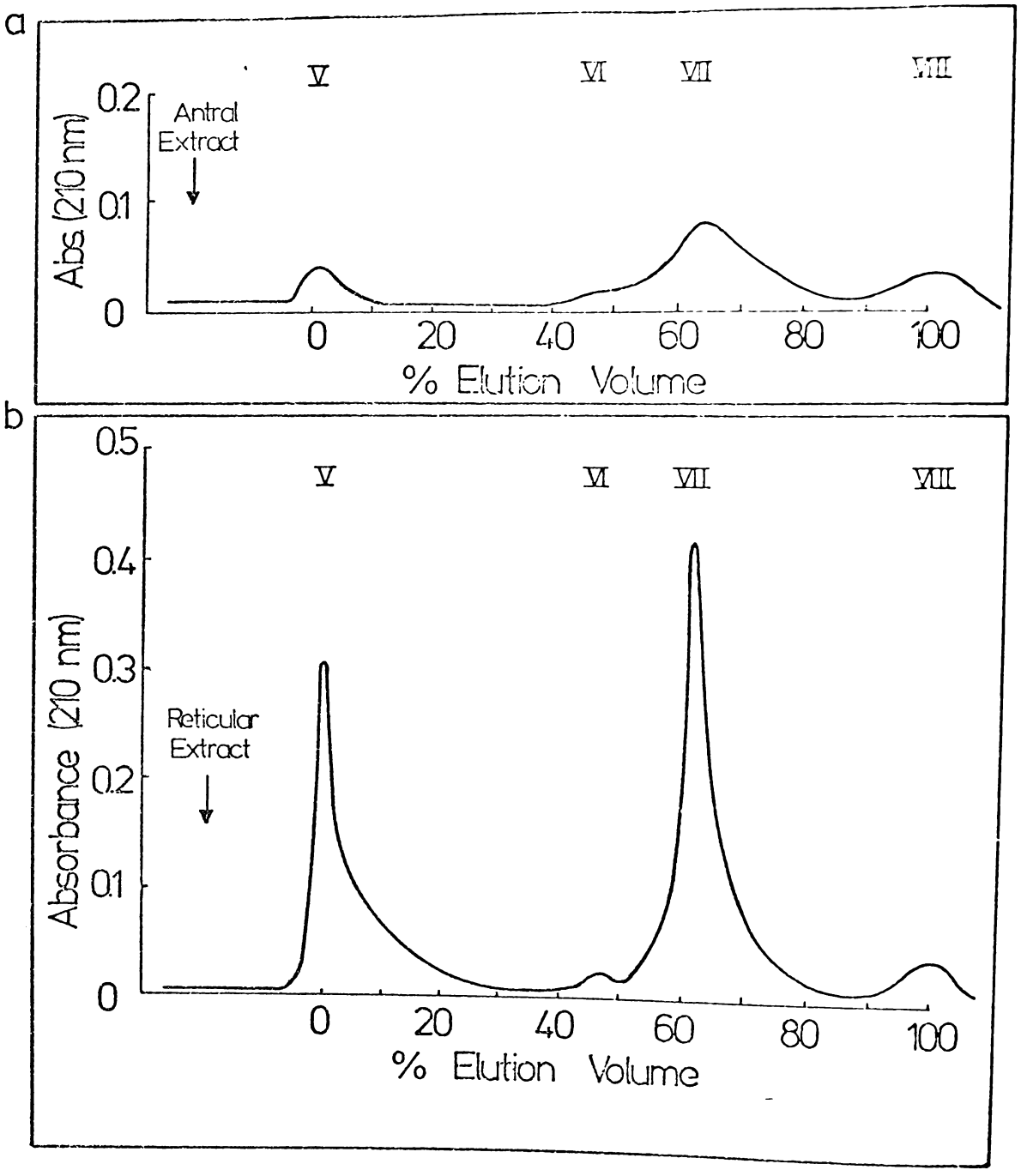

Fig. 16. Fractionation of peak I (fractionated from crude extract on columns of Sephadex G25) on a column of Sephadex G50.

(a) 2.0×10^{-3} g peak I antral extract.

(b) 6.0×10^{-3} g peak I reticular extract.

column. A small peak was present in the bed volume of the column(peak IV) (Fig. 15).

Distribution of Gastrin Activity: Freeze dried protein from peak I, on a unit weight basis, was the most potent stimulant of gastric acid secretion in the anaesthetized rat, followed in potency by peaks II and III. Peak IV showed no secretory activity. Table 16 shows the relative activity (as a fraction of pentagastrin activity) of the four peaks obtained by fractionation of crude gastrin extracts on Sephadex G25.

Fractionation of Peak I on Columns of Sephadex G50: After passage through columns of Sephadex G50, peak I was consistently eluted as four fractions. Most of the protein appeared as a single large peak (VII) in a position corresponding to 60% of the elution volume. This peak was preceded by a small peak (VI) (45% of the elution volume) and followed by a peak in the bed volume of the column (peak VIII). A distinct peak (V) consistently appeared in the void volume of the column. Fig. 16 shows the elution pattern of antral and reticular peak I on columns of Sephadex G50.

Distribution of Gastrin Activity: Freeze dried protein from peaks V and VII showed similar secretory activity when tested in the anesthetized rat. Peaks VI and VIII were inactive (Table 17).

Table 18. The apparent molecular weight (estimated from the calibration plots of the Sephadex columns) of each peak eluted from columns of Sephadex G25 and G50.

	PEAK							
	I	II	III	IV	V	VI	VII	VIII
M.W. ($\times 10^3$)	5.0	2.15	1.34	<1.0	30.0	12.0	6.95	1.5

Table 19. The percentage of each molecular weight fraction present in extracts of the ovine gastro-intestinal tract are shown. The relative amounts of each fraction have been calculated from the area under each peak eluted from columns of Sephadex.

The possible designation of some components is also shown.

Com. 1 ≡ Component 1; MG ≡ mini gastrin; H-LG ≡ heptadecapeptide like gastrin; BG ≡ big gastrin; BBG ≡ big big gastrin.

M.W. x 10^3	<1.0	1.34	2.15	6.95	12.0	30.0
Peak	IV	III	II	VII	VI	V
		?MG	?H-LG	?BG	?Com.1	?BBG
Rumen	1.0	20.0	25.0	29.0	2.0	23.0
Reticulum	9.0	13.0	26.0	29.0	2.0	21.0
Omasum	8.0	16.0	22.0	29.0	4.0	21.0
Ab. Body	0.5	11.5	17.0	57.0	4.0	10.0
Antrum	1.0	13.0	15.0	55.0	2.0	14.0
Duodenum	6.0	6.0	21.0	44.0	1.0	22.0
Caecum	3.0	4.0	13.0	44.0	1.0	35.0

Molecular Weight Determination: The elution volumes of each peak were compared to the standards, and the molecular weights estimated from the log molecular weight versus elution volume plot (Fig. 14). Table 18 shows the estimated molecular weight of each peak.

Distribution of Molecular Weight Fractions: The area under each peak was calculated so that the relative amounts of each molecular weight fraction could be estimated. Table 19 shows the relative amounts of each fraction expressed as a percentage.

Isoelectric Point Determination

After electrophoresis and staining of the gels for each crude freeze dried extract, three protein bands were clearly discernable.

The isoelectric points of the three bands are shown in Table 20. To characterise these three bands, freeze dried samples of peaks II, III, V and VII (as obtained by fractionation on columns of Sephadex) were subjected to isoelectric focussing. Isoelectric point pH's of 4.73, 4.74, 4.82 and 4.98 were obtained from individual determinations of peaks III, II, V and VII respectively. Thus peaks II and III combined, peak V, and peak VII, would appear to correspond to the three bands obtained by isoelectric focussing of the crude extracts.

Table 20. Using a technique of isoelectric focussing the crude extracts could be separated into three distinct bands, of different isoelectric point, on polyacrylamide gel. The isoelectric points of each of the three bands, for each of the extracts investigated, is shown.

Band	Rumen	Reticulum	Omasum	Ab. Body	Antrum	Duodenum	Caecum	Mean
a	5.05	4.99	4.99	4.97	4.97	4.94	4.89	4.97
b	4.80	4.81	4.85	4.80	4.84	4.85	4.79	4.82
c	4.71	4.75	4.73	4.76	4.75	4.70	4.75	4.73

Table 21. Estimated molecular weights of the three bands obtained by S.D.S. electrophoresis of crude extracts of the reticulum and abomasal body.

	Reticular Extract			Abomasal Body Extract		
	Band a	Band b	Band c	Band a	Band b	Band c
M.W. ($\times 10^3$)	2.0	3.75	34.5	2.0	3.80	34.5

Investigation of Interelationships between M.W. Fractions

Gel filtration of peak VII (?BG) on a column of Sephadex resulted in the separation of two components corresponding to peaks II and III. Similarly peak V (?BBG) separated into components corresponding to peaks VI and VII.

Fractionation at pH 12.0: Fractionation of crude extract on columns of Sephadex G25 packed in 0.01 M KOH caused no alteration of the elution pattern of the molecular weight fractions.

S.D.S. Electrophoresis: S.D.S. electrophoresis of crude gastrin extracts from the abomasal body and reticulum produced three fractions. Two fractions had molecular weights of 2,000 and 34,500 which probably correspond to peaks II and V obtained by gel filtration on columns of Sephadex. The third fraction, with a molecular weight of 3,800, was not comparable to the gel filtration studies. The molecular fractions separated from reticular and abomasal body extracts by S.D.S. electrophoresis are shown in Table 21.

DISCUSSION

The results presented indicate that the crude gastrin extracts obtained from different regions of the ovine gastrointestinal tract were not homogenous but consisted of a number of components of different molecular weight. These components could be separated by gel filtration with columns of Sephadex and by electrophoresis on polyacrylamide gel. Similarly porcine and human gastrin can be separated into a number of molecular weight fractions (Walsh, 1975).

The molecular weights referred to are estimations based on elution volumes from columns of Sephadex. However, as peptide compounds were used to calibrate the columns there should be good agreement between the estimated and actual molecular weights of the extracts. The reported molecular weights for the gastrin components have been obtained by gel filtration on columns of Sephadex (reviewed by Walsh, 1975) and are therefore comparable to the results obtained in the present work.

Gel filtration of crude extracts from the ovine rumen, reticulum, omasum, abomasal body, antrum, duodenum and caecum on columns of Sephadex G25 separated four fractions. The two middle fractions (peaks II and III) had molecular weights, calculated from their elution volume on the column of 2,150 and 1,340 respectively. Both fractions showed gastrin activity in the anaesthetized rat, although the

2,150 M.W. fraction was approximately 3 times more active than the 1,340 M.W. fraction. It seems likely that the fraction with a molecular weight of 2,150 corresponds to the heptadecapeptide gastrins isolated by Gregory and Tracy, (1964). In the sheep H-LG I and II have molecular weights of 2,024 and 2,140 respectively (Kenner and Sheppard, 1973). It has been reported that a small gastrin component containing the 13 C-terminal amino acids of H-LG can be isolated from gastrinoma tissue (Gregory, 1974) and human serum (Rehfeld and Stadil, 1973). This component has been named 'mini gastrin' (MG) and is a stimulant of gastric acid secretion having about half the potency of H-LG, and a slightly less half life in the circulation. It is suggested that the 1,340 M.W. fraction obtained in the present work corresponds to the MG reported by these authors. The fourth fraction was present in the elution volume of Sephadex G25 columns and would contain substances of molecular weight less than 1,000, presumably amino acids and small peptides. The N-terminal 1-13 fragment of gastrin, which does not show gastrin activity as it lacks the active C-terminal has been found in the serum (Walsh, 1975) and hog antral extracts (Gregory, 1974). If such fragments were present in ovine extracts, it is likely that they would be present in this fraction.

The first fraction eluted from columns of Sephadex G25 contained a mixture of proteins and peptides with molecular weights in excess of 5,000. This material was

freeze dried and then divided into four fractions by gel filtration on columns of Sephadex G50. The major fraction was eluted from the column in a position corresponding to 60% of the elution volume and was a potent stimulant of gastric acid secretion in the anaesthetized rat. Such fractions had an apparent molecular weight of 6,950. This fraction appears to correspond to a component which has been isolated from human serum (Yalow and Berson, 1970b) and extracts of hog antrum (Gregory, 1974) named 'big gastrin' or BG. On Sephadex, BG has an apparent molecular weight of 7,000 (Yalow and Berson, 1970b), and a minimal molecular weight of 3,870 calculated from its amino acid composition (Gregory, 1974). It has been suggested that this difference is either due to the anomalous behaviour of Sephadex in some situations or that in solution the BG peptides exist as dimers (Gregory, 1974).

On columns of Sephadex G50, emerging immediately before the BG peak, was a small peak with molecular weight of approximately 12,000. Freeze dried fractions of this component were not stimulants of acid secretion. A peak eluted in this position after gel filtration of human serum, showing gastrin immunoreactivity, but not biological activity, has been named Component I (Rehfeld, 1972).

Yalow and Berson (1972) found an immunoreactive gastrin component which was eluted in the void volume of Sephadex G50 columns which would have a molecular weight in excess

of 30,000. This material was named 'big-big-gastrin' or BBG. In the present work a fraction was eluted in the void volume of columns of Sephadex G50 and was a potent stimulant of gastric acid secretion in the anaesthestized rat. It is likely that this fraction corresponded to Yalow and Berson's BBG.

The small peak in the elution volume of Sephadex G50 columns probably contained amino acids and break down peptides of larger molecules with molecular weights less than 1,500. These fractions did not display gastrin-like activity.

By comparisons of the apparent molecular weights of the fractions obtained by gel filtration of gastrin extracts in the present work with those reported for the gastrin components of human serum and extracts of hog antrum, it seems likely that the gastrin extracts from all regions of the ovine gastrointestinal tract contained varying amounts of BBG, BG, H-LG, MG and Component I.

The apparent low activity of the H-LG fraction was unexpected as pure human H-LG I and pentagastrin have been reported to have similar activity in the anaesthetized rat (Smith et al., 1970). However, it has been reported that with equal doses, the increase in acid secretion in dogs was about 20% higher with human BG than with H-LG (Walsh et al., 1974). In the present work the difference

in activity was greater than this, and the apparent low activity of H-LG may be the result of the longer circulation half-life of BG (Walsh et al., 1974), or of the use of weight doses rather than molar doses of extract. However, this is unlikely as pentagastrin and BBG were not similarly affected. Therefore it is possible that this is a real difference in activity between ovine H-LG and the larger ovine gastrins, although the presence of impurities in the H-LG extracts cannot be discounted.

Gastrin components, except Component I, isolated from the human and the pig, exist as pairs of sulphated and non-sulphated peptides (Gregory, 1974). Similarly ovine H-LG exists as a pair of peptides (Agarwal et al., 1968), and thus it would be reasonable to expect the same of ovine BBG, BG and MG. However, the ion exchange column used in the present work, owing to its restricted length, lacked sufficient resolution to separate the sulphated and non-sulphated forms of the gastrin peptides.

The relative amounts of each component present in the different extracts has been determined and the relative abundance of BG increases, while that of H-LG and MG decreases in the more caudal parts of the ovine gastro-intestinal tract (see Table 19). In the human BG accounted for 10-35% of the gastrin extracted from antra obtained at surgery, and up to 50% of that extract from those taken at autopsy. The relative abundance of BG

increased caudally, with the proximal jejunum containing this component almost exclusively (Yalow and Berson, 1973). An interesting feature in the present work is that the abomasum contains predominantly BG, and that the extra-abomasal regions appear to be the richest sources of BBG. In the serum of a patient with a previous antral resection, BBG was the only form of gastrin detected, thus suggesting an extra-antral source for BBG (Yalow and Wu, 1973).

Rehfeld (1973) has reported that in human serum 5-25%, 40-95%, 0-40% and 0-13% of the immunoreactive gastrin was Component I, BG, H-LG and MG respectively. Yalow and Wu (1973) have found that BBG accounted for 40% of the immunoreactive serum gastrin in a normal patient. The distribution of gastrin components in ovine serum has not yet been documented.

Isoelectric focussing fractionated crude freeze dried extracts from all regions, into three components with isoelectric points of 4.98, 4.82 and 4.72. These values corresponding to the isoelectric points of the three fractions were comparable to BG, BBG and H-LG respectively. The fraction comparable to MG having an isoelectric point of 4.73 (undistingishable from that of the H-LG fraction) was probably not separated from the H-LG fraction. Using this technique, the isoelectric points may not be absolute, but the relative values are comparable to those reported

for the human gastrin peptides. Human BG is a more basic peptide than human H-LG (Yalow and Berson, 1970b), and human BBG is slightly more acidic than BG, although actual figures have not been given (Yalow and Wu, 1973). The isoelectric point of MG has not previously been documented. A similar pattern of isoelectric points was observed for ovine extract fractions comparable to BBG, BG and H-LG.

Cholecystokinin is a more basic molecule than the gastrin peptides, having an isoelectric point of 5.0 - 5.5. (species not cited) (Grossman, 1950). From their observed isoelectric points it is apparent that the extracts discussed in the present work were of a more acidic nature than this. It is therefore unlikely that the active principle in the extracts was cholecystokinin.

Anderson et al. (1962) found that the most active ovine gastrin was obtained when precipitated from aqueous solution at a pH of 3.0. Similarly the most active bovine and porcine gastrins were obtained when precipitated at pH's of 3.0 - 4.0 and 4.5 - 5.5. respectively. These pH's, at which precipitation occurred, would be close approximations of the extracts isoelectric points. The difference between the isoelectric points of the extracts in the present work and those above, possibly reflect the unpurified nature of previous extracts.

Gel filtration of the BG component on columns of

Sephadex resulted in the separation of H-LG and MG in the same proportions as in the crude extracts. Similarly BBG separated into Component I and BG. Such interchange of the gastrin components has not been documented previously and would suggest that the larger gastrin components occur as aggregations of molecules in equilibrium with the smaller components. However, these larger components are apparently aggregated by chemical bonding as fractionation at high pH's, thus preventing hydrogen bonding, and S.D.S. electrophoresis failed to dissociate the larger components. Yalow and Berson (1973) have suggested that BG is composed of H-LG covalently linked to a more basic molecule of about 5,000 M.W.

Just prior to completion of this work Rehfeld et al. (1977) reported that BBG in blood and gastrointestinal mucosa is not true macromolecular gastrin. Gel and affinity chromatography monitored radioimmunochemically indicated that apparent BBG results from assay interference by albumin and other plasma proteins, or-from non-covalent binding mainly of gastrin-17 to tissue proteins.

S.D.S. electrophoresis separated three bands of different molecular weight. Two of these, 2,100 and 34,500 M.W. correspond to H-LG and BBG. The third band, with an estimated molecular weight of 3,800 is likely to correspond to BG; a value close to the calculated molecular weight of 3,870 for BG (Gregory, 1974). However, this is

approximately half the molecular weight for BG estimated from its elution pattern on Sephadex and it has been suggested that the BG peptides exist as dimers in solution (Gregory, 1974). Such a view is supported by the present work where treatment with S.D.S. would have dissociated dimers. It is likely that MG and Component I were not present in sufficient quantities to form distinct bands in the gel.

CHAPTER VI: EFFECTS OF OVINE EXTRACTS ON GASTRIC ACID SECRETION FROM FUNDIC POUCHES OF THE ABOMASAL BODY IN CONCIOUS SHEEP

INTRODUCTION

Gregory and Tracy (1964) described a method for obtaining pure extracts of the hormone gastrin. Subsequent studies using these extracts have shown that many of the effects which the earlier crude extracts had in addition to being stimulants of gastric acid secretion were also displayed by purified gastrin. These additional effects of gastrin have been discussed in Chapter I; the stimulatory and inhibitory effects of gastrin on gastric acid secretion only being of interest in the present work.

Single subcutaneous injections of GI or GII in the range 2.5×10^{-7}g - 2.5×10^{-6}g.Kg^{-1} b.w. stimulate acid and volume flow of gastric juice in concious dogs provided with denervated fundic pouches (Gregory and Tracy, 1964). Maximal response of dogs with gastric fistulas to gastrin is effected by subcutaneous injections of 8.0×10^{-6}g.Kg^{-1} b.w., and to intravenous infusions of 5.0×10^{-6}g.Kg^{-1} b.w. .hr^{-1} (Grossman, 1967). In man, maximal responses are effected by a single subcutaneous dose of gastrin of 2.0×10^{-6}g.Kg^{-1} b.w. (Markhlouf, McManus and Card, 1964a) and intravenous infusion of gastrin at the rate of 8.0×10^{-7}g.Kg^{-1} b.w. .hr^{-1} (Markhlouf et al., 1964b).

The inhibitory effect of gastrin on acid secretion from a denervated fundic pouch was first reported by Uvnäs (1943) who observed that in dogs the maximal acid response to injections of gastrin was lower than that to injections of histamine. These observations were confirmed by Gillespie and Grossman (1963) who found that in dogs the rapid intravenous injection of gastrin extract would inhibit secretion from a Heidenhain pouch secreting in response to the continuous infusion of gastrin extract. Gregory and Tracy (1964) also reported that large doses of their pure gastrin would inhibit acid secretion in dogs. However, maximal endogenous release of gastrin does not inhibit gastric acid secretion (Quintana, de la Rosa and Dragstedt, 1965). Although studies have suggested that large doses of gastrin may inhibit gastric secretion in rats, it seems likely that the phenomenon is limited to dogs (Thompson, 1969).

There have been few reports dealing with the effects of gastrin on abomasal secretion in the ruminant. McLeay and Titchen (1970b) have reported that infusions of pentagastrin in doses of $1.2 \times 10^{-6} - 1.86 \times 10^{-5}$ g.Kg^{-1} .hr^{-1} inhibited both acid and pepsin secretion in fed sheep. In contrast secretion was stimulated by similar doses of pentagastrin in sheep which had been fasted 20 - 48 hr.

In the present work the effect of infusions of pentagastrin and ovine gastrin extracts on secretion from

innervated fundic abomasal pouches in sheep has been studied.

METHODS

Two ewes of the New Zealand Dorset Horn breed with body weights of 32 Kg (sheep 2) and 35 Kg (sheep 3) were used in the present experiments. The animals were kept in individual metabolism crates in an animal house at the Ruakura Agricultural Research Centre, Nutrition Division, during their recovery from operative procedures, and during the course of the experiments. When not being used the animals were held in the crates by means of a leather collar and chain thus allowing easy access to food and water. To restrain movement during the experiments the animals were placed in head stocks.

Food and water were normally available to the animals ad libitum. The food provided was chaffed pasture hay, fresh supplies of which were provided between 8.30 and 9.00 a.m. daily, when the residue from the previous day was removed. Fresh water, and a salt block were always available to the animals.

Operative Procedure: The anaesthetic procedure and the preparation of fundic pouches were as described by McLeay and Titchen (1970a). In dwelling venous catheters (Portex, England; 1 mm internal diameter) placed in the jugular vein at least 24 hr. before experiments, were used to make intravenous infusions.

Collection of Pouch Secretion: A light polythene tube about 20 mm in diameter was attached to the shaft of the cannula by a rubber ring. The tube was lead over the side of the metabolism crate into a 300 mm length of 50 mm diameter plastic tube. The secretion was collected, by means of a funnel, into test tubes for 15 min intervals with the aid of an automatic fraction collector (L.K.B., 17000 minirac). On the day of collection the volume of each 15 min sample was measured, and an estimate of total acidity was made by titrating a 1 ml aliquote to pH 7.0 using 0.1M NaOH and an automatic titrator (Radiometer, Titrator 11, Autoburette ABU 11).

Experimental Procedure: During the experiments food and water were available ad libitum, or the animal was fasted 12 hr, and in later experiments 24 hr preceding the experiment. Each experiment consisted of an infusion of pentagastrin (1.2×10^{-6}g Kg^{-1} $.hr^{-1}$) or gastrin extract (the equivalent activity of 1.0 or 1.4×10^{-6}g pentagastrin $.Kg^{-1}$ $.hr^{-1}$; as determined by rat bioassay) for one hour. The period of gastrin infusion was preceded by a saline infusion (0.9% saline, May and Baker Ltd., England) for 1.5 hours, and followed by a similar saline infusion for 1.5 or 2 hours. These results were compared to those obtained with infusions of saline over 5 hr periods.

All infusions, by means of a peristaltic pump (Watson-Marlow, HR Flow Inducer, MHRE 7) set to deliver 40 $ml.hr^{-1}$,

were made via the jugular catheter.

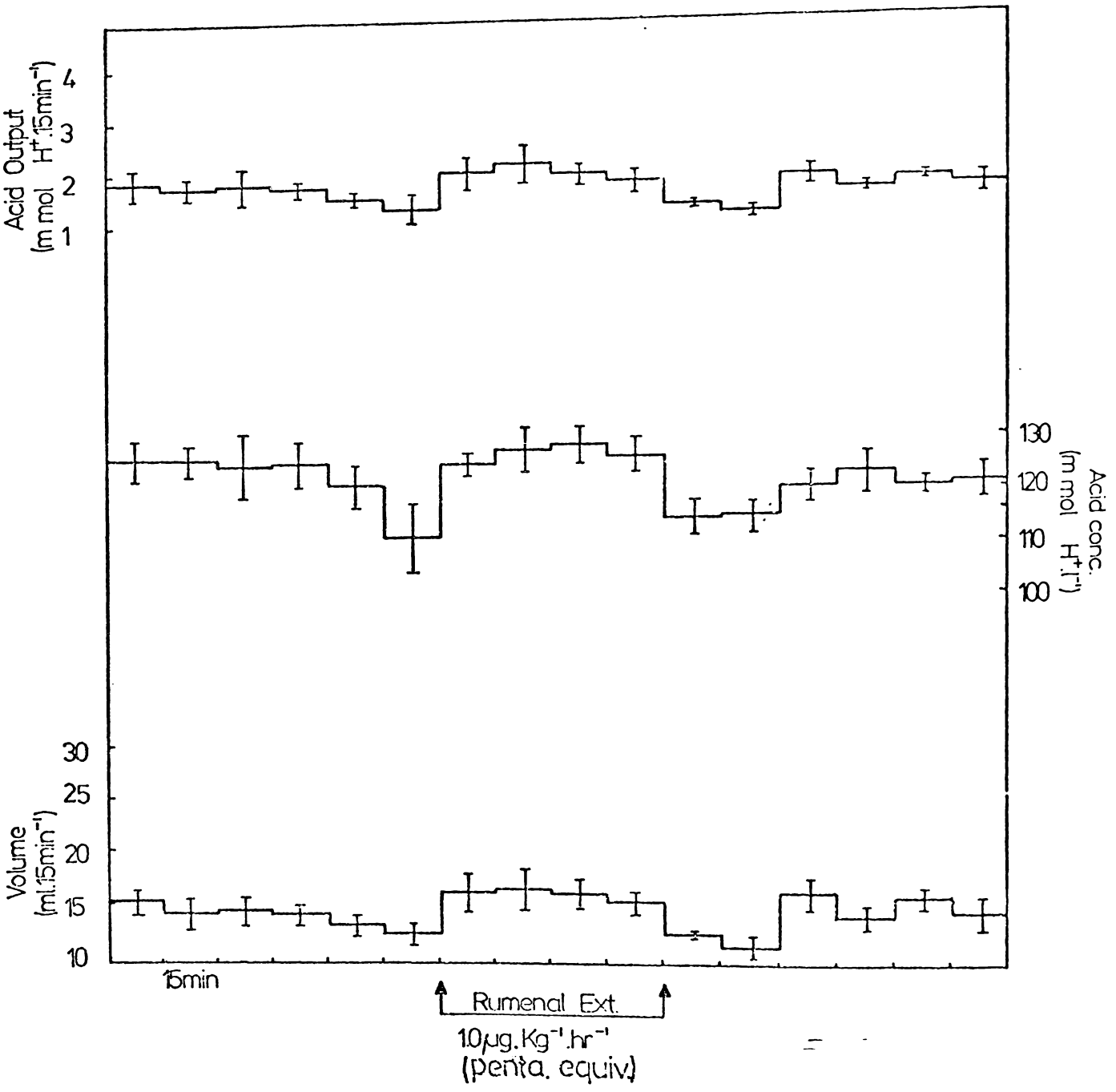

Fig. 17. The response of an abomasal body pouch to intravenous infusion of rumenal extract when the animal had food freely available. The means, and standard error of the means for three experiments in sheep 3 are shown.

RESULTS

Basal Secretion

Continuous secretion was observed from the fundic pouches of both sheep. The rate of secretion was not constant, the highest rate being from the pouches of sheep that were feeding, or from those that had recently fed. The average acid output during feeding was 3.2m mol.15 min^{-1} compared to 0.15m mol.15 min^{-1} for sheep fasted 24 hr.

Intravenous Infusions of Saline

Infusions of 0.9% saline for collection periods of 4 hr caused little change in fundic pouch secretion. Secretion during saline infusions was used as a base to measure the response to infusions of gastrin extract or pentagastrin.

Intravenous Infusions of Gastrin Extract

Fed Sheep: In animals with food and water freely available infusions of rumenal and reticular gastrin caused small increases in the volume and acidity of pouch secretion. Although there was variation between experiments, increases in secretion usually occurred within 30 min of the beginning of infusion and lasted up to 60 min. Fig. 17 shows the average volume and acid concentration, and acid output of a fundic pouch for 3 experiments during the infusion of rumenal gastrin in sheep 3.

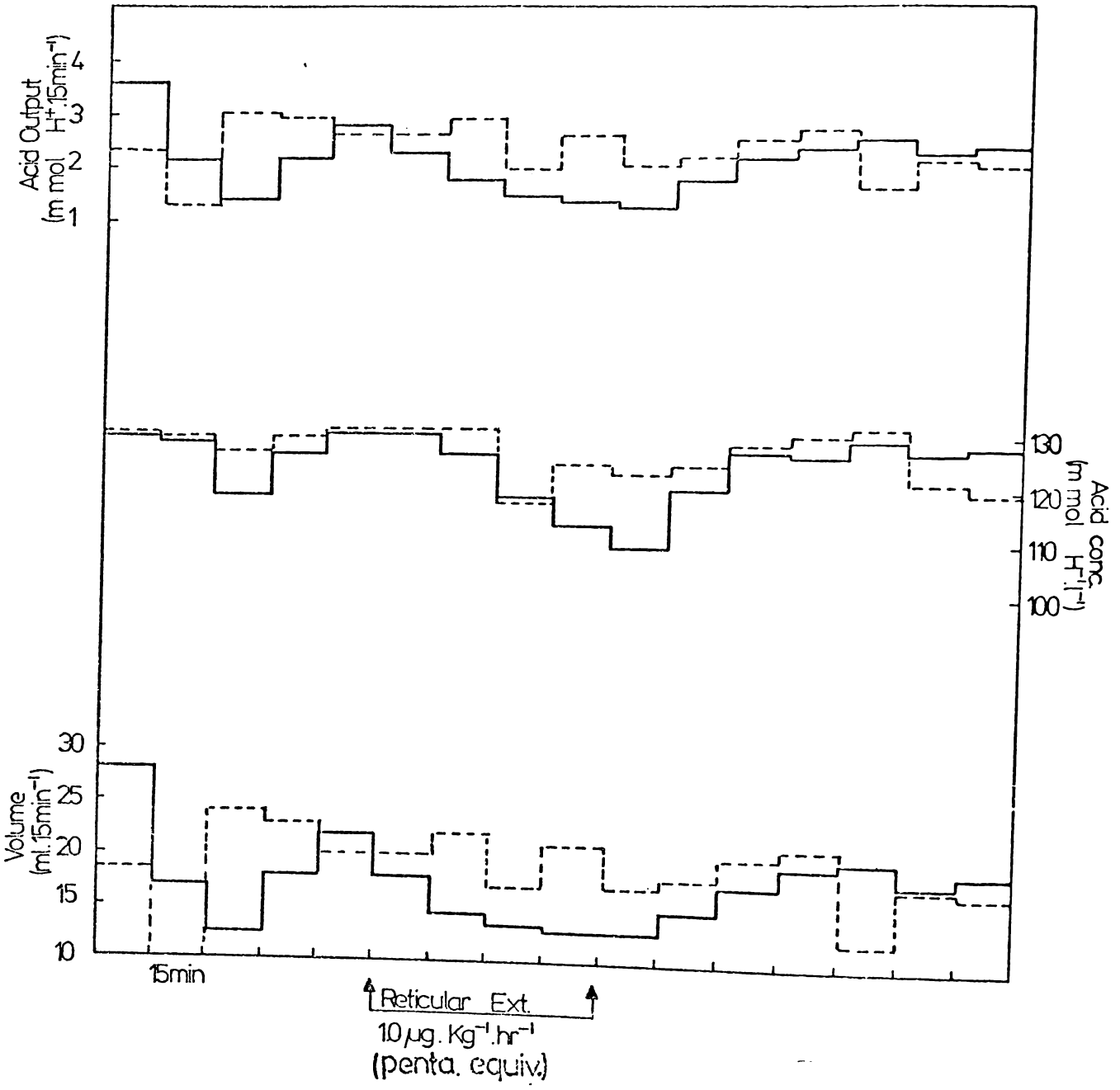

Fig. 18. An apparant inhibition of abomasal body pouch secretion during an infusion of reticular gastrin in sheep 3 when food was freely available. Pouch secretion during an intravenous infusion of saline is shown by the dashed line (single experiment).

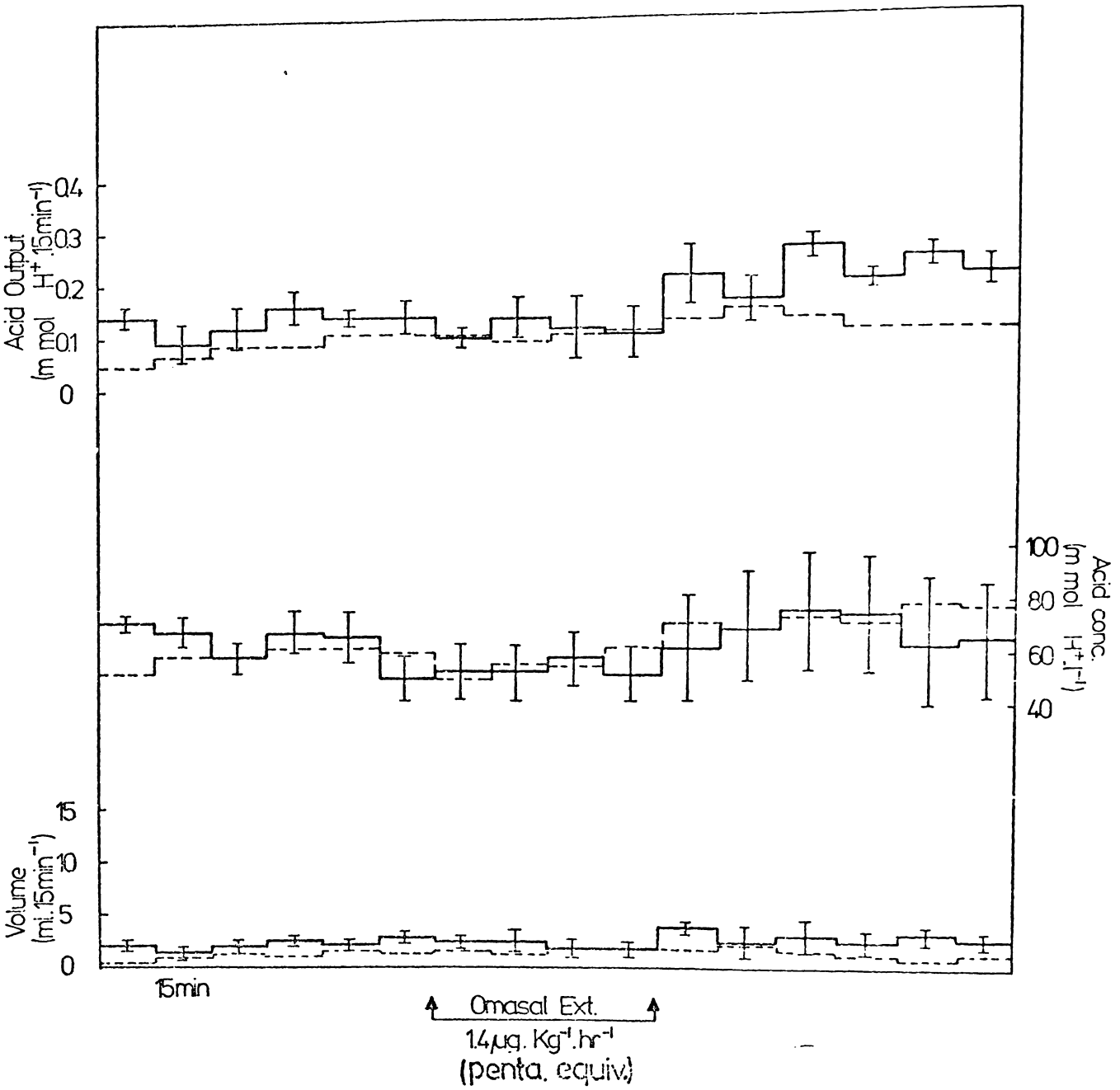

Fig. 19. A small but prolonged increase in secretion from an abomasal body pouch after a latent period of 60 min in response to an infusion of omasal gastrin in sheep 2 after being fasted 24 hours. The means and standard error of the means for three experiments are shown. Pouch secretion during intravenous infusion of saline is shown by the dashed line (means of three experiments).

In one experiment, when the level of unstimulated secretion was higher than normal, an infusion of reticular gastrin had no effect. Under similar conditions, in another experiment, a 1 hr infusion of reticular gastrin appeared to cause a reduction in the volume of secretion, and a reduction of acid concentration within 15 min after commencement of the infusion. This apparent inhibition lasted 75 min (Fig. 18).

Fasted Sheep: For 24 hours preceding the experiment, food was removed, but the animal had access to water and a salt block. In both experimental animals an infusion of gastrin extract caused a small but prolonged increase in the volume and acidity of the fundic pouch secretion after a latent period of up to one hour. For example, 60 min after the commencement of an infusion of omasal gastrin the volume of the pouch secretion increased from 2 ml to 4 ml. The acid concentration of the secretion began to increase 30 min after commencement of the infusion, and rose from 51 m mol $H^+ . l^{-1}$ to 76 m mol $H^+ . l^{-1}$. An increased acid output was maintained for a further 90 min (Fig. 19).

Between individual experiments, and with gastrins obtained from different regions of the ovine gastrointestinal tract there was some variation in the latency of the response. However, the magnitude, and the duration of the response in both sheep was similar for all gastrins. Figs. 20 and 21 show the average volume and

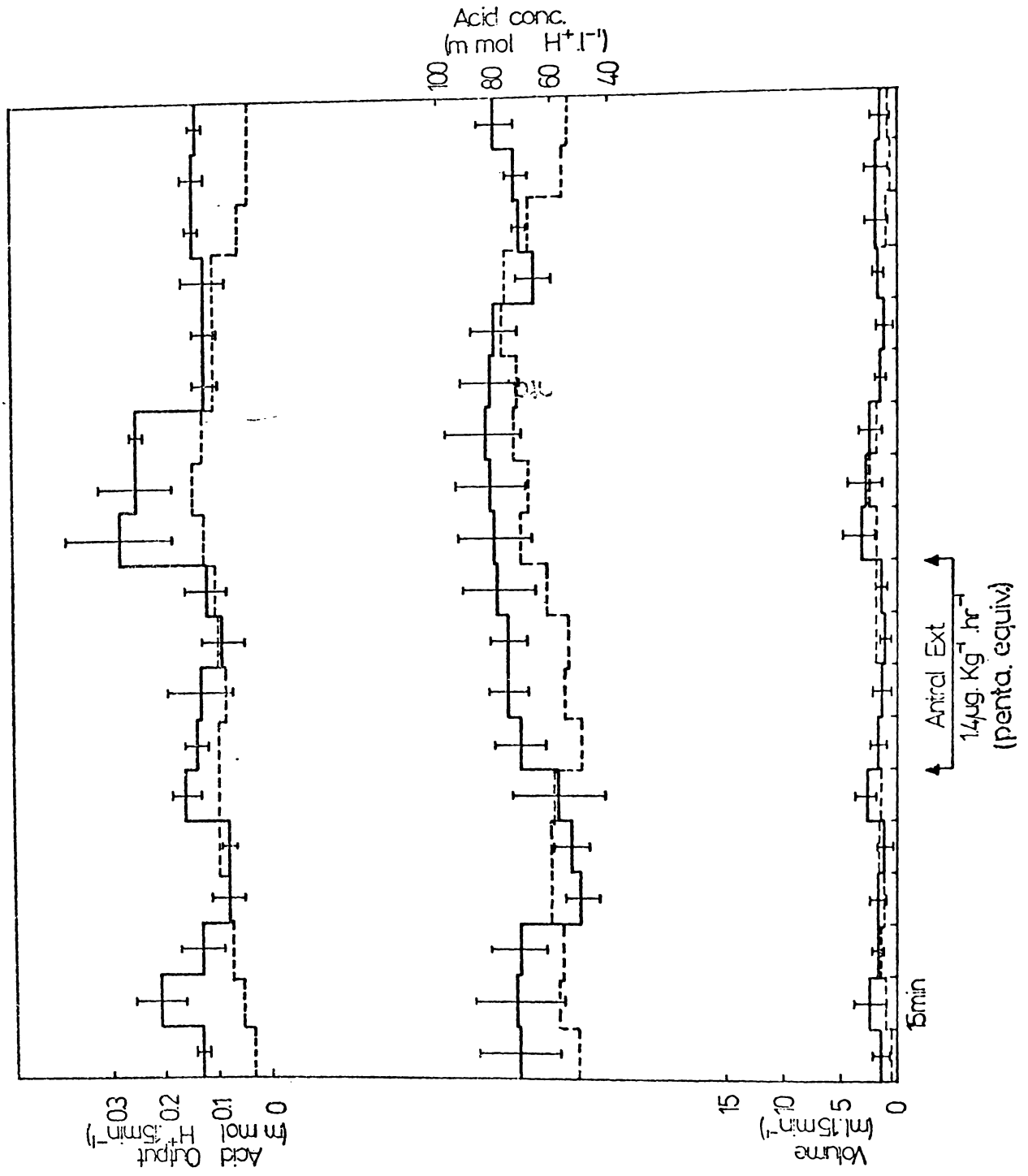

Fig. 20. The increase in acid output from an abomasal body pouch after a latent period of 60 min, in response to an infusion of antral gastrin in sheep 2 after being fasted 24 hours. The means and standard error of the means for three experiments are shown. Pouch secretion during intravenous infusion of saline is shown by the dashed line (means of three experiments).

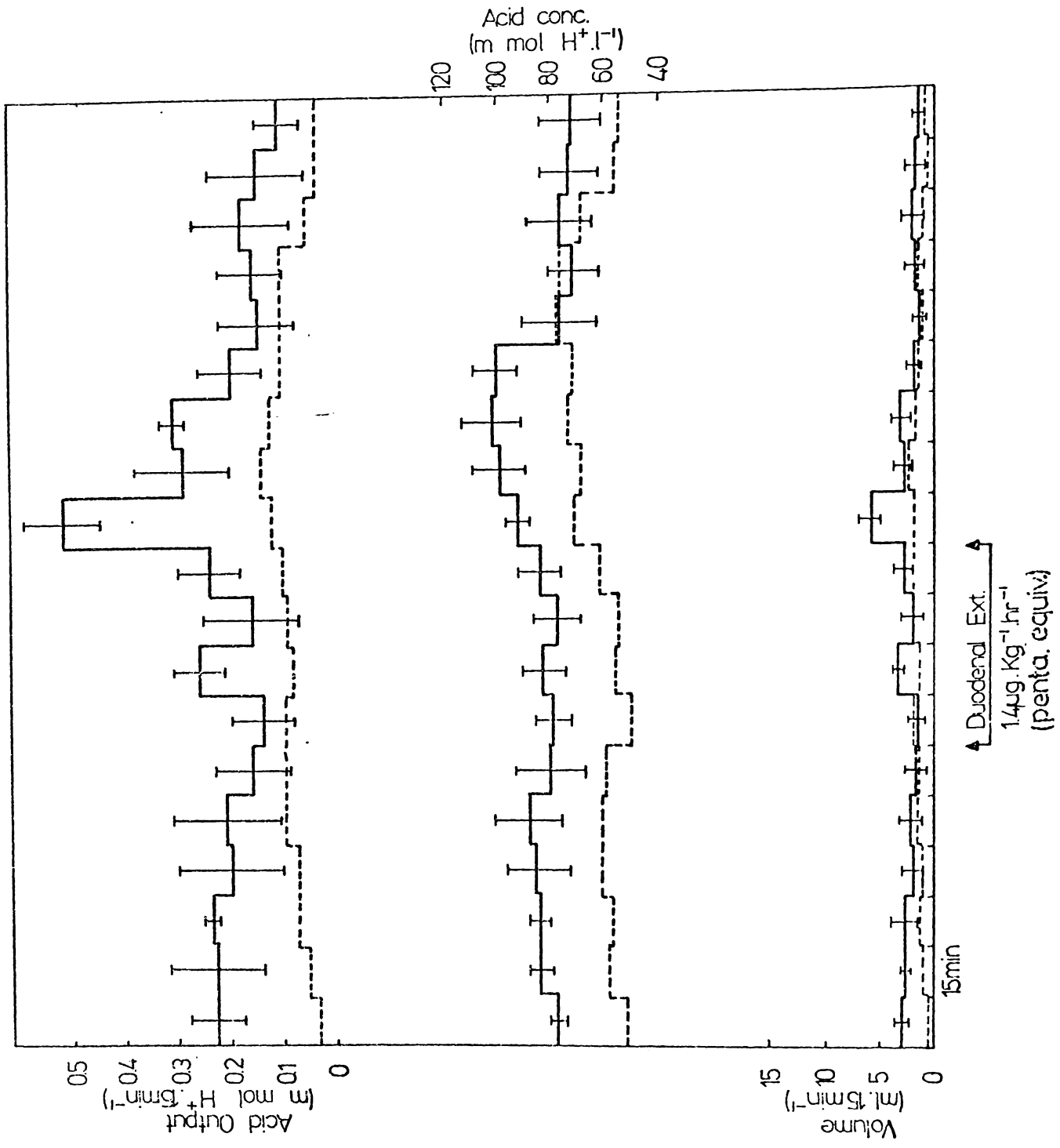

Fig. 21. An increase in abomasal body pouch secretion after a latent period of 15 min, in response to an infusion of duodenal gastrin in sheep 2 after being fasted 24 hours. The means and standard error of the means for three experiments are shown. Pouch secretion during intravenous infusion of saline is shown by the dashed line (means of three experiments).

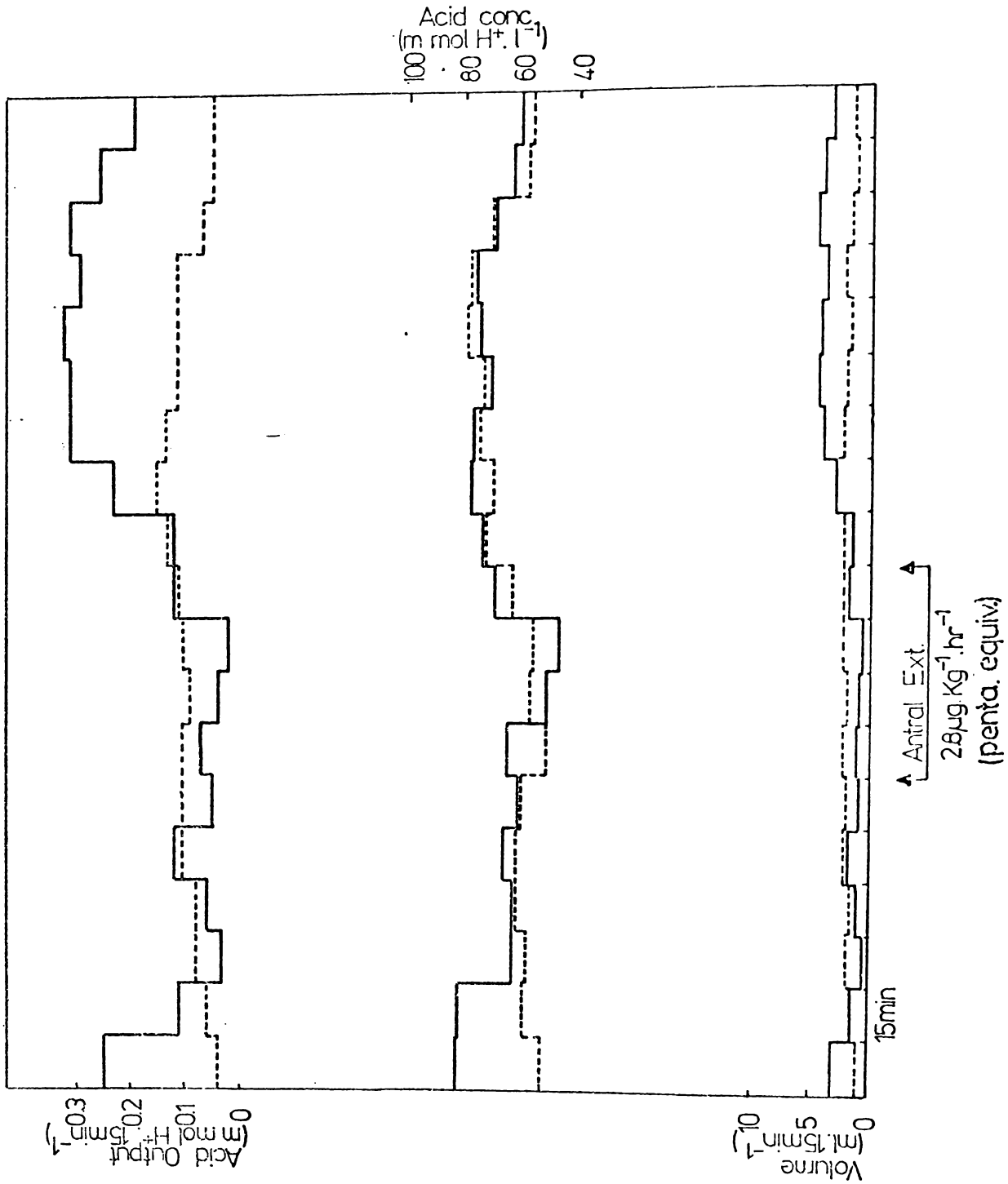

Fig. 22. The response to an infusion of increased dose of antral gastrin in sheep 2 after being fasted 24 hours. Pouch secretion during intravenous infusion of saline is shown by the dashed line (means of three experiments).

acid output of the fundic pouch in response to the infusion of antral and duodenal gastrin.

In response to an infusion of antral gastrin of 2.8×10^{-6} g (twice the usual dose) the acid output increased from 0.12 m mol.15 min^{-1} to 0.3 m mol.15 min^{-1} after a latent period of 75 minutes. An increased acid output was maintained for a further 90 minutes (Fig. 22).

Intravenous Infusions of Pentagastrin

Each experiment consisted of an infusion of pentagastrin, 1.4×10^{-6} g .Kg b.w.$^{-1}$.hr^{-1}, in both fed and fasted sheep.

Fed Sheep: In an animal with food and water freely available a single infusion of pentagastrin caused an increase in the volume of the secretion from 9.5 ml.15 min^{-1} to 19.0 ml.15 min^{-1} and in acid output of the pouch from 1.2 m mol H^+.15 min^{-1} to 2.5 m mol H^+.15 min^{-1} within 15 min of the beginning of the infusion. However, after 15 min the acid output rapidly dropped to 1.4 m mol H^+.15 min^{-1}, and a reduced acid secretion was continued for 75 min (Fig. 23).

Fasted Sheep: In both sheep, when fasted 24 hr, 1 hr infusions of pentagastrin caused increases in the volume of the secretion and in acid output within 15 min, which persisted for up to a further 60 min; the acid output

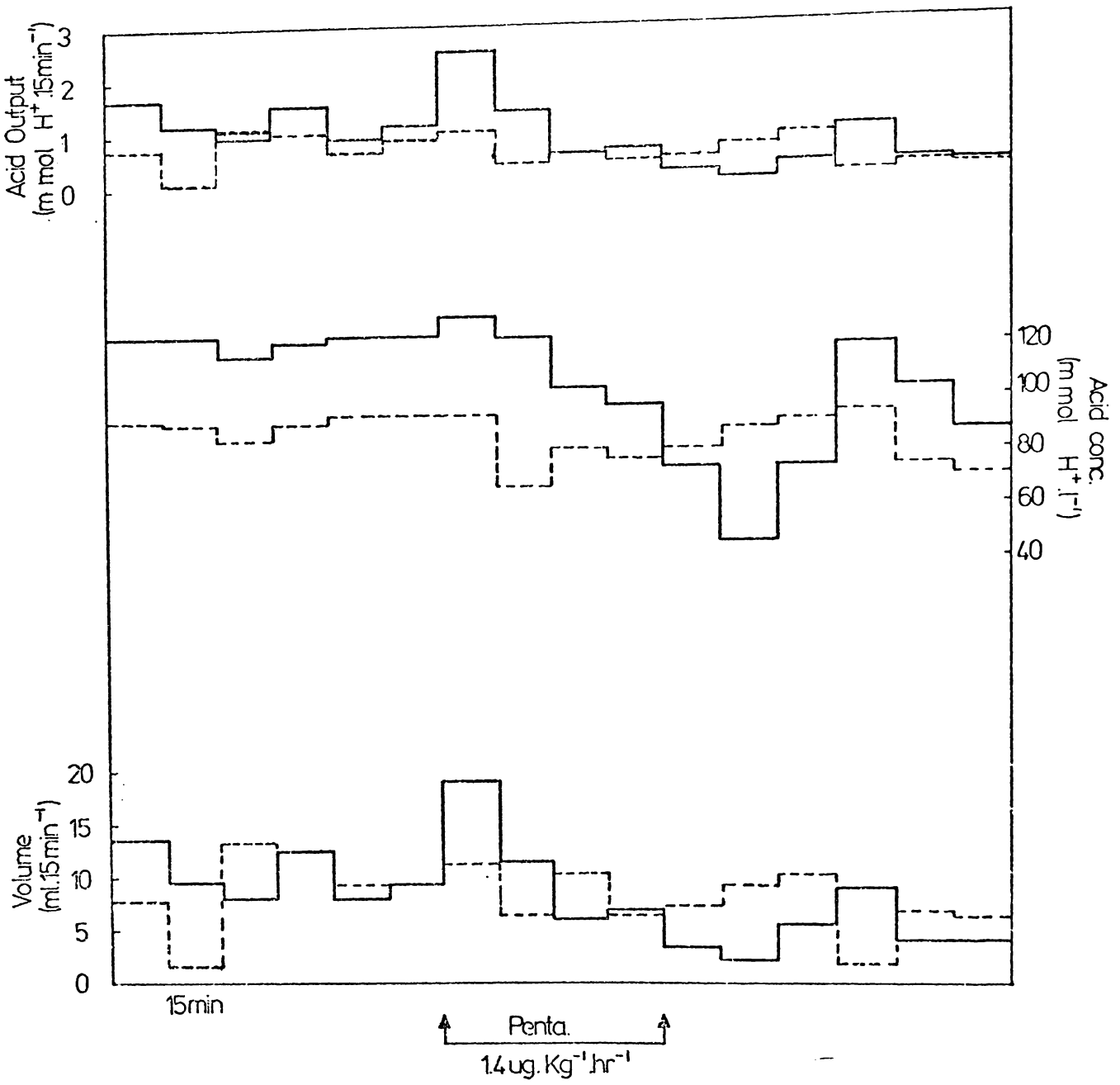

Fig. 23. When food was freely available to sheep 2, an infusion of pentagastrin caused an initial increase in abomasal body pouch secretion, followed by an inhibition which lasted 75 min. Pouch secretion during an intravenous infusion of saline is shown by the dashed line (single experiment).

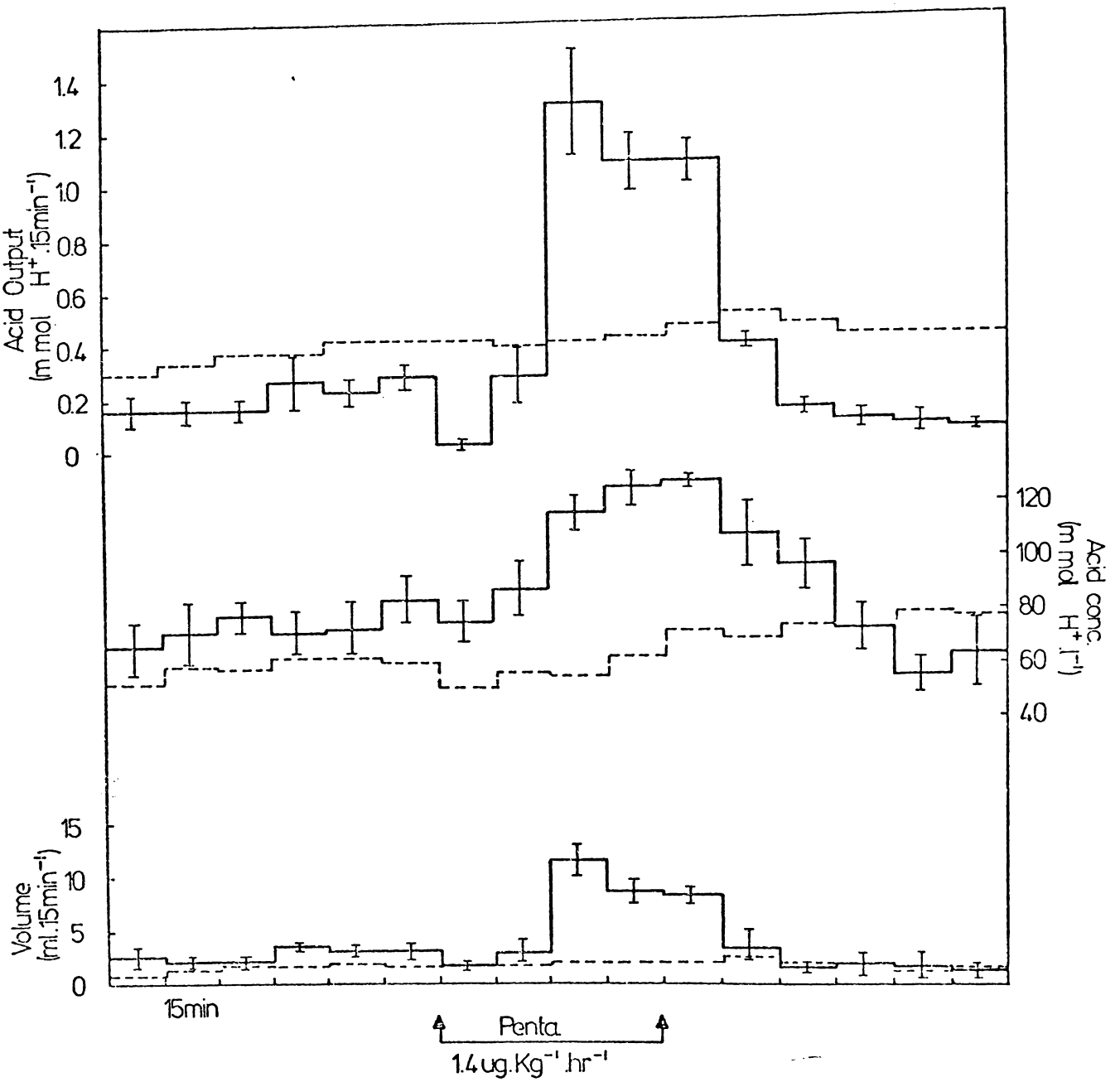

Fig. 24. After a latent period of 15 min an infusion of pentagastrin caused a marked increase in acid secretion from an abomasal body pouch in sheep 2 after being fasted 24 hours. The means and standard error of the means from three experiments are shown. Pouch secretion during intravenous infusion of saline is shown by the dashed line (means of three experiments).

increased from 0.2 m mol H^+.15 min^{-1} to 1.3 m mol H^+.15 min^{-1} in sheep 2 and 0.3 m mol H^+.15 min^{-1} to 1.4 m mol H^+.15 min^{-1} in sheep 3. Fig. 24 shows the volume of secretion, acid concentration and acid output of sheep 2 when fasted during an infusion of pentagastrin and should be compared with Fig. 27 (response in the fed animal).

DISCUSSION

The results presented demonstrate that infusions of gastrin extract, after a latent period of up to 60 min caused a small but prolonged elevation of acid output from the pouch of sheep fasted 24 hr. Infusions of pentagastrin in fasted sheep, after a relatively short latent period, resulted in a marked increase of acid output which rapidly returned to basal levels after the infusion was stopped. In contrast single injections and infusions of porcine gastrin and pentagastrin have been reported to be potent stimulants of acid secretion in the dog (Konturek and Grossman, 1965; Gregory and Tracy, 1964), in man (Markhlouf et al., 1964; Markhlouf et al., 1966; Konturek and Lankosz, 1967; Aagaard and Schmidt, 1967). In conscious dogs gastrin isolated from the ovine and bovine antrum showed activity identical in form and degree with those described for porcine gastrin (Agarwal et al., 1968). Since the extracts used in the present work were active in the anaesthetized rat, the observations of low activity in the conscious sheep were unexpected. The experiment where an infusion of double the usual dose of antral gastrin extract resulted in a response of similar magnitude and slightly more prolonged than infusions of smaller amounts of extract would indicate that this relative inactivity of the extracts was not an effect of the dose rates used.

Variation in activity of the extracts between the

anaesthetized rat and conscious sheep could be accounted for by one of the following possibilities: a difference in the gastric secretory behaviour of the conscious and anaesthetized animal; the gastrin activity of pentagastrin is of a different form from that contained in the extracts.

An extract of hog duodenal mucosa made by the method of Gregory and Tracy (1961) was very active when assayed on the anaesthetized rat, but showed no gastrin-like activity when given to a conscious dog (Gregory, cited by Lia, 1964). This difference was attributed to a species specificity of some gastrins or a difference in the behaviour of conscious and anaesthetized animals. It has been reported that in the rat general anaesthesia results in a reduced gastric acid secretion (Maitrya, 1967; Barrett et al., 1966). However, such considerations do not explain the different response to gastrin extracts by the anaesthetized rat and conscious sheep, observed in the present work.

The rat displays the same sensitivity to pentagastrin and porcine gastrin (Smith et al., 1970), and porcine gastrin and ruminant gastrins have been reported to have the same activity (Agarwal et al., 1968). Since both the sheep and the rat were sensitive to pentagastrin it seems unlikely that species specificity would explain the variation in activity.

Differences in the time course of the response to gastrin extracts, namely the long latent period and prolonged response, compared to pentagastrin would indicate a difference in the active principle of pentagastrin and the gastrin extracts. This difference cannot be attributed to the presence of histamine in the extracts as bioassay has shown this substance to be absent.

Fractionation of gastrin extracts on Sephadex columns, and radioimmunoassay have shown that together with heptadecapeptide gastrin there exist other forms of the molecule with higher and lower molecular weights (Walsh, 1975). In the dog, Walsh et al., (1974) have shown that the larger molecular weight forms of gastrin are less potent as stimulants of acid secretion, but have a longer half life in the circulation than heptadecapeptide gastrin. It has been shown (Chapter V) that the gastrin extracts used in the present work contained a gastrin component of greater molecular weight than the pentapeptide pentagastrin. It is suggested that under the experimental conditions employed, the anaesthetized rat does not display a marked difference in sensitivity to the different forms of gastrin, and that the presence of a higher molecular weight gastrin in the extracts caused the long latent period and small and prolonged secretory responses observed in the sheep.

Observed secretory responses in the fed animal, where

pouch secretion was at a higher level, were different. Infusions of rumenal gastrin may have caused a small increase in acid output, but appeared to be of shorter duration when compared to the fasted animal. In one experiment the infusion of reticular gastrin resulted in a suspected inhibition of secretion. In another experiment, under similar conditions reticular gastrin had no effect. Infusions of pentagastrin resulted in an initial increase in acid output, followed by an inhibition in the fed animal. The variable effect of pentagastrin, either stimulatory or inhibitory, depending on the level of pouch secretion is similar to that reported by McLeay and Titchen (1970b and 1975).

It has been reported that acidification of the antrum suppresses gastric acid secretion (Oberhelman, et al., 1952). It is well documented that this inhibition is at least caused in part by the prevention of the release of gastrin (Anderson, 1967), but the mechanism involved is still unclear. Similarly acid conditions in the abomasum inhibit gastric acid secretion in the ruminant (Ash, 1961; Hill, 1960; McLeay and Titchen, 1970a, 1971). However, it seems unlikely that acid inhibition of gastrin release would account for the inhibitory responses caused by the infusion of pentagastrin and reticular gastrin. That this inhibitory response occurred rapidly and was maintained only for the duration of the infusion would indicate that pentagastrin and the extract contained an inhibitory fraction, or the

concentrations of the hormone used were inhibitory when infused against a high level of gastric acid secretion. As the extracts were potent stimulants of gastric acid secretion in the anaesthetized rat the existence of an inhibitory fraction is doubtful, although the presence of a specific inhibitor of acid secretion in the sheep cannot be discounted.

In the conscious dog acid secretion can be maintained in response to slow intravenous infusion or repeated subcutaneous injections of gastrin. If during this time there is superimposed a rapid intravenous injection of gastrin a complete inhibition of acid secretion will occur (Gregory and Tracy, 1964). These inhibitory properties of gastrin in the dog have also been reported by Gillespie and Grossman (1963). McLeay and Titchen (1970b and 1975) have reported the inhibitory effects of pentagastrin on fundic pouch secretion. As these inhbitory effects occurred without preceding stimulation and hence could not be attributed to acid inhibition of acid secretion, McLeay and Titchen (1975) have suggested that the exogenous pentagastrin superimposed on the circulating endogenous gastrin may have produced inhibitory blood levels of the hormone.

CHAPTER VII: GENERAL DISCUSSION

Some previous attempts to isolate the gastrin peptides from gut material of ruminant origin were hindered by the presence of an unidentified brown pigment which was not easily separable from the hormone fraction (Agarwal et al., 1968; Jury, 1974). In the present work gastrin-like material has been isolated from certain regions of the ovine gastrointestinal tract using the modification of the method of Gregory and Tracy (1964) reported by Jury (1974), which involved the separation of the pigment on an ion exchange resin. Bioassays, using a perfused rat stomach preparation, have shown that extracts of the ovine rumen, reticulum, omasum, abomasal body, antrum, duodenum and caecum contain varying amounts of gastrin-like activity. The antrum showed the highest concentration of gastrin but substantial concentrations were found in the rumen, reticulum, omasum, caecum and proximal duodenum declining progressively with distance from the pylorus. A lesser concentration occurred in the abomasal body. Consideration of the relative total content of gastrin in the respective tissues showed that the amount of extra-antral gastrin was substantial.

In the present study no amino-acid analysis of the extracts was undertaken, so unequivocal proof that the secretory activity of the extracts was due to the presence

of gastrin is lacking. However, the behaviour on ion exchange columns and the pH's at which the extracts could be precipitated from aqueous solution were similar to those reported by Gregory and Tracy (1964) for gastrin. As the perfused rat stomach preparation is relatively insensitive to histamine (Smith, 1973), and as the histamine assay using an isolated guinea-pig ileum indicated its absence, it is clear that the secretory activity displayed by the extracts cannot be attributed to histamine.

Cholecystokinin, a hormone present in the small intestine which effects the entry of bile into the small intestine from the gall bladder, can also act as a stimulant of gastric acid secretion. However, determination of the isoelectric points showed that the components of the extracts were of a more acidic nature than cholecystokinin (pH 5.0 - 5.5; cited by Grossman, 1950).

Further evidence that the active principle in the extracts was gastrin was provided by fractionation studies using columns of Sephadex. These studies have shown that extracts from all regions of the ovine gastrointestinal tract consisted of several components of different molecular weight. By a comparison of their respective molecular weights to those reported for components separated from extracts of non-ruminant origin, it seems likely that components corresponding to MG, HLG, BG BBG and component I (Walsh, 1975) were present in varying amounts in the

extracts such that the abomasum contained predominantly BG, and the extra-abomasal regions were the richest sources of BBG. The precise physiological function of these gastrin components, or whether the larger components represent some form of precursor molecule in the synthesis of gastrin, has not yet been determined.

Intravenous infusions of gastrin at the rate of $5.0 \times 10^{-6} g.Kg^{-1}.hr^{-1}$, and $8.0 \times 10^{-7} g.Kg^{-1}.hr^{-1}$, cause a maximal secretory response in the dog (Gregory and Tracy, 1964) and man (Markhlouf et al., 1966) respectively. Similar studies for the sheep are lacking, but pentagastrin has been reported either to stimulate or to reduce acid secretion in sheep (McLeay and Titchen, 1970b; 1975). In the present work, infusion of gastrin in sheep fasted 24 hr caused, after a relatively long latent period, a small but prolonged increase in acid output from a fundic pouch. This was in contrast to pentagastrin which caused an immediate and pronounced response.

It has been reported that pentagastrin is markedly inactivated after a single passage through the liver whereas larger gastrin peptides, such as HLG are affected little or not at all (Strunz, Thompson, and Grossman, 1976). As a large portion of the extracts used, existed in large molecular weight forms, such differences in deactivation time could account for the differences in response observed in the present work. Similarly, when

infused intravenously the BG peptides were potent stimulants of gastric acid secretion in pouch dogs, but the response began more slowly and persisted longer than the response to a similar infusion of heptadecapeptide gastrin (Gregory and Tracy, 1972). In agreement with these observations, a recent report has indicated circulation half lives in the dog of 2.7 min, 3.1 min and 14.7 min for pentagastrin, HLG and BG respectively (Walsh, Debas and Grossman, 1974). The same authors have reported that under steady-state conditions approximately five times higher molar concentrations of BG than HLG in the circulation are required to induce equal rates of acid secretion. Hence it seems likely that the apparent low activity of the gastrin-extracts in the sheep was a reflection of the nature of the active components.

In fed sheep, where the basal secretion was at higher levels, gastrin extracts appeared to be inhibitory, or had little effect on acid secretion. The response to pentagastrin was short lived. In similar experiments McLeay and Titchen (1970b, 1975) have reported inhibitory effects when using pentagastrin, and have suggested that in these animals it was possible that the exogenous gastrin, together with endogenous gastrin, reached inhibitory levels.

The part played by gastrin in the overall control of abomasal secretion in the ruminant is not well documented. The similarity of the stimuli involved in abomasal secretion

(Hill, 1960, 1968; Ash, 1961a, 1961b; McLeay and Titchen, 1970a) to the mechanisms involved in the control of gastric secretion in the non-ruminants, and the presence of gastrin activity in antral extracts of the abomasum, would suggest the importance of gastrin in the control of abomasal secretory activity. Such a presumption is supported by Hill (1968) who reviewed the factors influencing abomasal secretory activity. McLeay and Titchen (1971; 1977) have reported that in the sheep, perfusion of innervated antral pouches with acetylcholine and carbachol stimulated acid output (volume concentration) from innervated fundic pouches of the abomasal body. Similar perfusions with atropine and hexamethonium reduced resting secretion and blocked secretory responses to feeding, and lignocaine reduced pouch resting secretion bud did not block secretory responses to feeding. These results draw attention to a locally operating mechanism in the antrum, and the authors have concluded that in the sheep antral secretion of gastrin contributes to both the maintenance of resting acid secretion and to the increase in acid secretion on feeding.

Direct evidence for the release of gastrin from the extra antral regions investigated in the present work is not yet available, but several reports direct attention to the function of these regions in the control of gastric acid secretion in the ruminant. Hill (1960) reported that distension of the main body of the abomasum alone stimulated acid secretion. In animals with a more simple stomach such

responses are mediated via vago-vagal and local reflexes (Grossman, 1967). In the ruminant it is possible that in addition to these reflexes, the secretory response caused by distension of the fundus was mediated in part by the release of gastrin which is present in this region.

McLeay and Titchen (1975) studied fundic secretion of acid in sheep with both innervated antral and fundic pouches. Of interest in relation to the present work was the observation of continuous acid secretion from the fundic pouch after antrectomy by removal of the antral pouch. Antrectomized animals, although having a reduced acid output, continued to show secretory responses to teasing with food and insulin hypoglycaemia. The authors have suggested that gastrin present in the fundic region may have contributed to this continuing secretion. Their report draws attention to a possible function of the considerable amounts of extra-antral gastrin observed in the present work, but the continuing decline in fundic pouch secretion following antrectomy seems to indicate that gastrin from these sources is not secreted in sufficient amounts to compensate for the loss of antral gastrin. However, the present observations have indicated that collectively greater amounts of gastrin are present in the extra-antral regions than in the antrum and the suggestion of McLeay and Titchen (1975) that fundic gastrin was unable to maintain the parietal cell population of the antral pouches would also evidently apply to the other sources of extra-

antral gastrin in the ruminant.

Studies using animals with a more simple stomach than the ruminant have provided evidence of the significance of duodenal gastrin. Millar, Jackson and Thompson (1970) have shown that in dogs with a gastrectomy, feeding produces a significant rise in serum gastrin levels. Similarly in man, Stern and Walsh (1973) have reported that serum gastrin levels increase in response to feeding in patients with gastrectomies and gastroduodenal anastomosis. In contrast, little or no change was noted in the serum gastrin levels of patients with gastrojejunal anastomosis. There is no evidence to suggest that such considerations cannot be applied to the ruminant.

The ruminant stomach, as in animals with a more simple stomach, develops from the embryonic gastric spindle. The abomasum is derived from the area which in non-ruminants becomes the pyloric antrum (Hill, 1968), and the three parts of the forestomach are outgrowths from areas which correspond to the cardia and fundus of the simple stomach (Nickel, Chummer and Seiferle, 1973). As it has been documented that extracts of the cardia (Gregory and Tracy, 1961) as well as the antrum of some non-ruminants contain gastrin, it could be argued that gastrin present in the ovine fore-stomachs and fundus is an indication of the embryonic development pattern and is not of physiological significance. However, a recent publication indicates that the amount of

gastrin in the cardiac region is extremely low (Nilsson et al., 1973). When this is considered with the relatively high amounts of gastrin material found in the forestomachs and the fundus, and the available physiological evidence outlined above, it seems likely that these extra-antral sources of gastrin may play a part in the control of abomasal acid secretion in the sheep.

Although gastrin activity has been demonstrated in these experiments to exist throughout the stomach and intestines of Delphinus delphis, further experiments are required, preferably on fresh specimens, to quantify this activity. However, the presence of gastrin activity in the forestomach of Delphinus delphis supports the view that despite its structural similarity to the lower end of the oesophagus (Harrison et al., 1970) the forestomach probably arises from the embryonic gastric spindle and not the embryonic oesophagus. A similar situation exists in the ruminant.

The present study has shown there to be considerable gastrin activity distributed throughout the ovine gastrointestinal tract. Gel filtration studies showed the extracts to be composed of several fractions of differing molecular weight. Although similar components have been isolated from other mammals, their exact significance has yet to be elucidated. It seems likely that

further physiological studies will provide evidence on the part played by these gastrin components in the control of gastrointestinal function in the sheep.

APPENDIX I: Composition of the stock propionic-succinic buffer used for perfusion of the rat stomach. Final pH of the buffer was adjusted to 5.5 using 1.0M sodium hydroxide or 1.0M hydrochloric acid. Stock buffer was diluted 5 ml to 1 l distilled water for each experiment.

Propionic Acid	0.5M
(Analar, B.D.H. Chemicals Ltd., England)	
Succinic Acid	0.5M
(Analar, B.D.H. Chemicals Ltd., England)	
Sodium Hydroxide	1.0M
(Analar, B.D.H. Chemicals Ltd., England)	

APPENDIX II: Composition of Tyrode Ringer used to perfuse isolated guinea-pig ileum during histamine assay's.

	$g.100\ ml^{-1}$
Sodium Chloride (Lab. Chemicals, May and Baker, England)	0.80
Calcium Chloride (Analar, B.D.H. Chemicals Ltd., England)	0.02
Potassium Chloride (Analar, B.D.H. Chemicals Ltd., England)	0.02
Sodium Bicarbonate (Analar, B.D.H. Chemicals Ltd., England)	0.10
Magnesium Chloride (Lab. Chemicals, May and Baker, England)	0.01
Sodium di-Hydrogen Phosphate (Analar, B.D.H. Chemicals Ltd., England)	0.005
Glucose (Analar, B.D.H. Chemicals Ltd., England)	0.10

APPENDIX III: Dose of pentagastrin, pH change and acid secreted by two groups of rats for preparation of dose response curves.

Group 1 (n=3)

pH Change

Rat	Dose Pentagastrin			
	5.0×10^{-8} g	5.0×10^{-7} g	1.0×10^{-6} g	5.0×10^{-5} g
1	1.07	0.10	0.05	0.03
2	1.04	0.11	0.06	0.04
3	1.16	0.12	0.05	0.03

u mol H^+.30 min^{-1}

1	36.30	3.30	1.67	1.14
2	34.97	3.10	2.20	1.30
3	38.63	3.66	1.67	1.00

Group 2 (n=6)

pH Change

Rat	Dose Pentagastrin			
	5.0×10^{-6} g	1.0×10^{-5} g	2.0×10^{-5} g	4.0×10^{-5} g
1	0.15	0.45	0.71	1.23
2	0.17	0.26	0.75	0.94
3	0.17	0.29	0.40	0.77
4	0.12	0.14	0.38	0.94
5	0.12	0.28	0.43	0.55
6	0.13	0.22	0.47	1.02

u mol H^+.30 min^{-1}				
1	4.99	14.90	23.70	40.90
2	5.60	8.65	24.90	31.30
3	5.60	9.65	13.30	25.90
4	3.60	4.60	12.60	31.30
5	3.60	9.32	14.30	18.30
6	3.90	7.30	15.60	33.90

APPENDIX IV: Loss of gastrin-like activity displayed by an antral extract. Activity of the extract (10.4×10^{-6}g) is expressed as u-equiv pentagastrin .g weight wet tissue^{-1}, and as a percentage of the initial activity when assayed at 3 weekly intervals.

Time (weeks)	u-equiv Penta.	% Initial Activity
0	30.90	100
3	30.40	98.4
6	30.81	99.7
9	29.14	94.3
12	25.15	81.4
15	11.95	38.6
18	7.43	24.0
21	8.96	28.9
24	3.84	12.4

APPENDIX V: Wet weight tissue (g), weight of freeze dried extract (mg) and ug extract per gram wet tissue for all extracts of ovine rumen, reticulum, omasum, abomasal body antrum, duodenum and caecum, are shown on the next 10 pages.

RUMENAL EXTRACTS

(3 rumen per extract)

	Wet Weight Rumen (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	172.1	9.0	52.2
Ext. 2	516.5	27.5	53.2
Ext. 3	556.2	28.1	50.5
Ext. 4	602.0	26.8	44.5
Ext. 5	567.0	24.0	42.3
Ext. 6	643.4	36.1	56.1
Mean	509.5	25.3	49.8
S.E.M.	69.7	3.6	2.2

RETICULAR EXTRACTS

(3 reticulum per extract)

	Wet Weight Reticulum (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	123.4	7.0	56.7
Ext. 2	86.0	5.1	59.2
Ext. 3	131.4	7.9	60.1
Ext. 4	129.1	5.8	44.9
Ext. 5	195.5	8.5	43.5
Ext. 6	249.4	15.2	60.9
Mean	152.5	8.2	54.2
S.E.M.	24.1	1.5	3.2

OMASAL EXTRACTS

(3 omosa per extract)

	Wet Weight Omasum (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	190.6	11.3	59.6
Ext. 2	240.4	14.5	60.3
Ext. 3	237.3	13.2	55.6
Ext. 4	182.7	9.4	51.5
Ext. 5	188.7	9.0	47.8
Ext. 6	250.1	14.7	58.8
Mean	214.0	12.0	55.6
S.E.M.	12.5	1.0	2.0

ABOMASAL BODY EXTRACTS

(6 abomasa per extract)

	Wet Weight Fundus (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	590.4	14.7	24.9
Ext. 2	695.8	15.1	21.7
Ext. 3	593.3	12.4	20.9
Mean	626.5	14.1	22.5
S.E.M.	24.5	0.6	0.9

ANTRAL EXTRACTS

(6 antra per extract)

	Wet Weight Antrum (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	182.5	9.6	52.6
Ext. 2	208.4	10.4	49.9
Ext. 3	177.5	8.7	49.0
Mean	189.5	9.6	50.5
S.E.M.	6.1	0.3	0.8

DUODENAL EXTRACT (0-20 cm)

(6 Duodena per extract)

	Wet Weight Duodenum 1 (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	365.4	19.0	51.9
Ext. 2	314.4	24.0	76.3
Ext. 3	363.3	31.0	85.3
Ext. 4	203.4	14.1	69.3
Ext. 5	295.2	15.0	50.8
Ext. 6	215.3	16.1	74.7
Mean	292.8	19.8	67.6
S.E.M.	28.7	2.6	5.7

DUODENAL EXTRACT (20-40 cm)

(6 Duodena per extract)

	Wet Weight Duodenum 2 (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	228.7	16.1	51.9
Ext. 2	174.3	12.5	76.3
Ext. 3	167.7	12.0	85.3
Ext. 4	130.0	9.0	69.3
Ext. 5	186.9	9.8	50.8
Ext. 6	129.4	9.0	74.7
Mean	169.5	11.4	67.2
S.E.M.	15.3	1.1	3.0

DUODENAL EXTRACT (40-60 cm)

(6 Duodena per extract)

	Wet Weight Duodenum 3 (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	219.8	14.0	63.6
Ext. 2	183.5	12.0	65.3
Ext. 3	158.5	11.0	69.4
Ext. 4	147.3	8.5	57.7
Ext. 5	178.7	9.1	50.9
Ext. 6	164.2	11.0	61.0
Mean	175.3	10.9	62.2
S.E.M.	10.4	0.8	2.9

DUODENAL EXTRACT (60-80 cm)

(6 Duodena per extract)

	Wet Weight Duodenum 4 (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	168.3	10.2	59.4
Ext. 2	153.9	10.5	68.2
Ext. 3	126.7	8.1	63.9
Ext. 4	121.7	7.0	57.5
Ext. 5	162.2	8.1	49.9
Ext. 6	140.7	7.0	49.7
Mean	145.6	8.5	58.4
S.E.M.	7.8	3.5	3.0

CAECAL EXTRACT

(6 caeca per extract)

	Wet Weight Caecum (g)	Wt. Ext. (mg)	ug ext.g^{-1} Tissue
Ext. 1	310.1	14.4	46.5
Ext. 2	340.6	17.8	52.4
Ext. 3	280.9	15.2	54.2
Ext. 4	299.6	13.3	44.4
Ext. 5	320.6	19.0	59.3
Ext. 6	236.1	12.7	53.9
Mean	297.9	15.4	51.8
S.E.M.	13.3	1.1	1.9

APPENDIX VI: pH change and acid secreted by the anaesthetized rats stomach during bioassay of rumenal, reticular, omasal, abomasal body, antral, duodenal and caecal extracts are shown on the next 30 pages.

RUMENAL EXTRACTS

	Dose (µg)	pH Change	µ mol H^+.30 min^{-1}
EXTRACT 1:			
Assay 1			
Rumenal	40	0.10	3.31
	20	0.04	1.33
Penta.	40	0.28	9.28
	20	0.15	4.97
Assay 2			
Rumenal	40	0.19	6.29
	20	0.09	2.98
Penta.	40	0.53	17.56
	20	0.26	8.61
Assay 3			
Rumenal	40	0.14	4.64
	20	0.07	2.32
Penta.	40	0.39	12.92
	20	0.20	6.63
EXTRACT 2:			
Assay 1			
Rumenal	40	0.11	3.64
	20	0.06	1.99
Penta.	40	0.31	10.27
	20	0.15	5.3

Assay 2			
Rumenal	40	0.14	4.64
	20	0.08	2.65
Penta.	40	0.33	10.93
	20	0.17	5.63
Assay 3			
Rumenal	40	0.21	6.96
	20	0.11	3.64
Penta.	40	0.58	19.22
	20	0.28	9.28

EXTRACT 3:

Assay 1			
Rumenal	40	0.16	5.30
	20	0.09	2.98
Penta.	40	0.45	14.91
	20	0.23	7.62
Assay 2			
Rumenal	40	0.14	4.64
	20	0.70	2.48
Penta.	40	0.38	12.59
	20	0.19	6.29
Assay 3			
Rumenal	40	0.31	10.27
	20	0.16	5.30
Penta.	40	0.81	26.84
	20	0.39	12.92

EXTRACT 4:

Assay 1

Rumenal	40	0.39	12.92
	20	0.20	6.63
Penta.	40	1.11	36.77
	20	0.55	18.22

Assay 2

Rumenal	40	0.28	9.28
	20	0.14	4.64
Penta.	40	0.85	28.16
	20	0.48	14.25

Assay 3

Rumenal	40	0.22	7.29
	20	0.11	3.18
Penta.	40	0.61	20.21
	20	0.30	9.94

EXTRACT 5:

Assay 1

Rumenal	40	0.48	15.90
	20	0.22	7.29
Penta.	40	1.32	43.0
	20	0.70	23.19

Assay 2

Rumenal	40	0.53	17.56
	20	0.29	9.61
Penta.	40	1.62	53.01
	20	0.88	29.15

Assay 3

Rumenal	40	0.60	19.55
	20	0.32	10.60
Penta.	40	1.74	57.45
	20	0.90	29.82

EXTRACT 6:

Assay 1

Rumenal	40	0.16	5.30
	20	0.09	2.98
Penta.	40	0.48	15.90
	20	0.25	8.28

Assay 2

Rumenal	40	0.34	11.26
	20	0.16	5.30
Penta.	40	0.95	31.91
	20	0.45	14.91

Assay 3

Rumenal	40	0.35	11.60
	20	0.18	5.96
Penta.	40	1.20	39.76
	20	0.59	19.55

RETICULAR EXTRACTS

	Dose (μg)	pH Change	μ mol H^+.30 min^{-1}
EXTRACT 1:			
Assay 1			
Reticular	40	0.11	3.64
	20	0.05	1.66
Penta.	40	0.28	9.28
	20	0.15	4.97
Assay 2			
Reticular	40	0.18	5.96
	20	0.09	2.98
Penta.	40	0.53	17.56
	20	0.26	8.61
Assay 3			
Reticular	40	0.16	5.30
	20	0.07	2.32
Penta.	40	0.39	12.92
	20	0.20	6.63
EXTRACT 2:			
Assay 1			
Reticular	40	0.12	3.98
	20	0.06	1.99
Penta.	40	0.31	10.27
	20	0.15	5.30

Assay 2			
Reticular	40	0.15	4.97
	20	0.07	2.32
Penta.	40	0.33	10.93
	20	0.17	5.63
Assay 3			
Reticular	40	0.18	5.96
	20	0.09	2.98
Penta.	40	0.58	19.22
	20	0.28	9.28

EXTRACT 3:

Assay 1			
Reticular	40	0.17	5.63
	20	0.09	2.98
Penta.	40	0.45	14.91
	20	0.23	7.62
Assay 2			
Reticular	40	0.16	5.30
	20	0.08	2.65
Penta.	40	0.38	12.59
	20	0.19	6.29
Assay 3			
Reticular	40	0.33	10.93
	20	0.17	5.63
Penta.	40	0.81	26.84
	20	0.39	12.94

EXTRACT 4:

Assay 1

Reticular	40	0.43	14.25
	20	0.22	7.29
Penta.	40	1.11	36.77
	20	0.55	18.22

Assay 2

Reticular	40	0.29	9.61
	20	0.15	4.97
Penta.	40	0.85	28.16
	20	0.48	14.25

Assay 3

Reticular	40	0.21	6.96
	20	0.10	3.31
Penta.	40	0.61	20.21
	20	0.30	9.94

EXTRACT 5:

Assay 1

Reticular	40	0.42	16.13
	20	0.20	6.63
Penta.	40	1.32	43.0
	20	0.70	23.19

Assay 2

Reticular	40	0.53	17.56
	20	0.25	8.28
Penta.	40	1.62	53.01
	20	0.88	29.15

Assay 3

Reticular	40	0.75	24.85
	20	0.41	13.58
Penta.	40	1.74	57.45
	20	0.90	29.82

EXTRACT 6:

Assay 1

Reticular	40	0.19	6.29
	20	0.10	3.31
Penta.	40	0.48	15.90
	20	0.25	8.28

Assay 2

Reticular	40	0.30	9.94
	20	0.15	4.97
Penta.	40	0.95	31.91
	20	0.45	14.91

Assay 3

Reticular	40	0.40	13.25
	20	0.21	6.96
Penta.	40	1.20	39.76
	20	0.59	19.55

OMASAL EXTRACTS

	Dose (μg)	pH Change	μ mol H^+.30 min^{-1}
EXTRACT 1:			
Assay 1			
Omasal	40	0.11	3.64
	20	0.05	1.66
Penta.	40	0.28	9.28
	20	0.15	4.97
Assay 2			
Omasal	40	0.16	5.30
	20	0.09	2.98
Penta.	40	0.53	17.56
	20	0.26	8.61
Assay 3			
Omasal	40	0.13	4.31
	20	0.06	1.99
Penta.	40	0.39	12.92
	20	0.20	6.63
EXTRACT 2:			
Assay 1			
Omasal	40	0.10	3.31
	20	0.05	1.66
Penta.	40	0.31	10.27
	20	0.15	4.97

Assay 2			
Omasal	40	0.13	4.31
	20	0.07	2.32
Penta.	40	0.33	10.93
	20	0.17	5.63
Assay 3			
Omasal	40	0.19	6.29
	20	0.09	2.98
Penta.	40	0.58	19.22
	20	0.28	9.28

EXTRACT 3:

Assay 1			
Omasal	40	0.15	4.96
	20	0.07	2.31
Penta.	40	0.41	13.24
	20	0.20	6.62
Assay 2			
Omasal	40	0.09	2.97
	20	0.04	1.32
Penta.	40	0.23	7.61
	20	0.11	3.64
Assay 3			
Omasal	40	0.30	9.93
	20	0.12	3.97
Penta.	40	0.67	22.31
	20	0.33	10.98

EXTRACT 4:

Assay 1

Omasal	40	0.15	4.96
	20	0.08	2.64
Penta.	40	0.44	14.56
	20	0.21	6.95

Assay 2

Omasal	40	0.18	5.96
	20	0.09	2.98
Penta.	40	0.52	17.21
	20	0.25	8.27

Assay 3

Omasal	40	0.20	6.62
	20	0.11	3.64
Penta.	40	0.52	17.21
	20	0.25	8.27

EXTRACT 5:

Assay 1

Omasal	40	0.23	7.61
	20	0.11	3.64
Penta.	40	0.44	14.56
	20	0.21	6.95

Assay 2

Omasal	40	0.26	8.60
	20	0.13	4.30
Penta.	40	0.52	17.21
	20	0.25	8.27

Assay 3

Omasal	40	0.22	7.28
	20	0.10	3.31
Penta.	40	0.52	17.21
	20	0.25	8.27

EXTRACT 6:

Assay 1

Omasal	40	0.18	5.95
	20	0.09	2.97
Penta.	40	0.56	18.53
	20	0.29	9.59

Assay 2

Omasal	40	0.15	4.96
	20	0.09	2.97
Penta.	40	0.42	13.90
	20	0.20	6.62

Assay 3

Omasal	40	0.13	4.30
	20	0.07	2.31
Penta.	40	0.37	12.24
	20	0.19	6.28

ABOMASAL BODY EXTRACTS

	Dose (µg)	pH Change	µ mol H^+.30 min^{-1}
EXTRACT 1:			
Assay 1			
Ab. Body	40	0.19	6.33
	20	0.09	3.00
Penta.	40	0.47	15.65
	20	0.24	7.99
Assay 2			
Ab. Body	40	0.20	6.66
	20	0.10	3.32
Penta.	40	0.49	16.32
	20	0.24	7.99
Assay 3			
Ab. Body	40	0.20	6.66
	20	0.10	3.32
Penta.	40	0.56	18.65
	20	0.28	9.32
EXTRACT 2:			
Assay 1			
Ab. Body	40	0.19	6.33
	20	0.10	3.32
Penta.	40	0.52	17.32
	20	0.26	8.66

Assay 2

Ab. Body	40	0.22	7.37
	20	0.11	3.66
Penta.	40	0.57	18.98
	20	0.28	9.32

Assay 3

Ab. Body	40	0.17	5.66
	20	0.09	3.00
Penta.	40	0.50	16.65
	20	0.25	8.33

EXTRACT 3:

Assay 1

Ab. Body	40	0.29	9.66
	20	0.15	5.00
Penta.	40	0.70	23.31
	20	0.35	11.66

Assay 2

Ab. Body	40	0.24	7.99
	20	0.12	4.00
Penta.	40	0.55	18.32
	20	0.28	9.32

Assay 3

Ab. Body	40	0.24	7.99
	20	0.12	4.00
Penta.	40	0.59	19.65
	20	0.30	9.99

ANTRAL EXTRACTS

	Dose (μg)	pH Change	μ mol H^+.30 min^{-1}
EXTRACT 1:			
Assay 1			
Antral	40	0.44	14.65
	20	0.22	7.33
Penta.	40	0.47	15.65
	20	0.23	7.66
Assay 2			
Antral	40	0.47	15.65
	20	0.23	7.65
Penta.	40	0.49	16.32
	20	0.24	7.99
Assay 3			
Antral	40	0.56	18.65
	20	0.28	9.32
Penta.	40	0.56	18.65
	20	0.28	9.32
EXTRACT 2:			
Assay 1			
Antral	40	0.51	16.98
	20	0.25	8.33
Penta.	40	0.52	17.32
	20	0.26	8.66

Assay 2			
Antral	40	0.51	16.98
	20	0.25	8.33
Penta.	40	0.57	18.98
	20	0.28	9.32
Assay 3			
Antral	40	0.49	16.32
	20	0.25	8.33
Penta.	40	0.50	16.65
	20	0.25	8.33

EXTRACT 3:

Assay 1			
Antral	40	0.68	22.64
	20	0.33	10.90
Penta.	40	0.70	23.31
	20	0.35	11.66
Assay 2			
Antral	40	0.52	17.32
	20	0.27	8.99
Penta.	40	0.55	18.32
	20	0.28	9.32
Assay 3			
Antral	40	0.57	18.98
	20	0.29	9.66
Penta.	40	0.59	19.65
	20	0.30	9.99

DUODENAL EXTRACTS

		Dose (µg)	pH Change	µ mol H^+.30 min^{-1}
EXTRACT 1:				
Assay 1				
Rat 1	Duodenal (0-20 cm)	40	0.27	8.99
		20	0.14	4.66
	Duodenal (20-40 cm)	40	0.28	9.32
		20	0.14	4.66
	Penta.	40	0.54	17.98
		20	0.25	8.33
Rat 2	Duodenal (40-60 cm)	40	0.21	6.99
		20	0.11	3.66
	Duodenal (60-80 cm)	40	0.15	5.00
		20	0.08	2.66
	Penta.	40	0.55	18.32
		20	0.26	8.66
Assay 2				
Rat 1	Duodenal (0-20 cm)	40	0.33	10.99
		20	0.18	5.99
	Duodenal (20-40 cm)	40	0.32	10.66
		20	0.16	5.33
	Penta.	40	0.86	28.64
		20	0.43	14.32

Rat 2	Duodenal (40-60 cm)	40	0.18	5.99
		20	0.09	3.00
	Duodenal (60-80 cm)	40	0.15	5.00
		20	0.08	2.66
	Penta.	40	0.67	22.71
		20	0.33	10.99

Assay 3

Rat 1	Duodenal (0-20 cm)	40	0.27	8.99
		20	0.14	4.66
	Duodenal (20-40 cm)	40	0.27	8.99
		20	0.13	4.39
	Penta.	40	0.68	22.64
		20	0.35	11.66
Rat 2	Duodenal (40-60 cm)	40	0.23	7.66
		20	0.11	3.66
	Duodenal (60-80 cm)	40	0.14	4.66
		20	0.06	2.00
	Penta.	40	0.87	28.01
		20	0.42	15.99

EXTRACT 2:

Assay 1

Rat 1	Duodenal (0-20 cm)	40	0.26	8.66
		20	0.12	4.00
	Duodenal (20-40 cm)	40	0.09	3.00
		20	0.04	1.33
	Penta.	40	0.54	17.98
		20	0.29	9.66

Rat 2	Duodenal (40-60 cm)	40	0.05	1.67
		20	0.02	0.67
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.03	1.00
	Penta.	40	0.65	21.65
		20	0.30	9.99
	Assay 2			
Rat 1	Duodenal (0-20 cm)	40	0.26	8.66
		20	0.12	4.00
	Duodenal (20-40 cm)	40	0.10	3.33
		20	0.05	1.67
	Penta.	40	0.59	19.65
		20	0.29	9.66
Rat 2	Duodenal (40-60 cm)	40	0.19	6.33
		20	0.09	3.00
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.03	1.00
	Penta.	40	0.61	20.36
		20	0.32	10.66
	Assay 3			
Rat 1	Duodenal (0-20 cm)	40	0.28	9.32
		20	0.13	4.33
	Duodenal (20-40 cm)	40	0.09	3.00
		20	0.05	1.67
	Penta.	40	0.63	20.98
		20	0.33	10.99

Rat 2	Duodenal (40-60 cm)	40	0.05	1.67
		20	0.02	0.67
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.03	1.00
	Penta.	40	0.56	18.65
		20	0.32	10.66

EXTRACT 3:

Assay 1

Rat 1	Duodenal (0-20 cm)	40	0.17	5.66
		20	0.09	3.00
	Duodenal (20-40 cm)	40	0.15	5.00
		20	0.07	2.33
	Penta.	40	0.40	13.32
		20	0.22	7.33
Rat 2	Duodenal (40-60 cm)	40	0.14	4.66
		20	0.06	2.00
	Duodenal (60-80 cm)	40	0.04	1.33
		20	0.02	0.67
	Penta.	40	0.45	14.99
		20	0.23	7.66

Assay 2

Rat 1	Duodenal (0-20 cm)	40	0.16	5.33
		20	0.08	2.66
	Duodenal (20-40 cm)	40	0.14	4.66
		20	0.07	2.33
	Penta.	40	0.49	16.32
		20	0.22	7.33

Rat 2	Duodenal (40-60 cm)	40	0.13	4.33
		20	0.06	2.00
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.02	0.67
	Penta.	40	0.41	13.65
		20	0.21	6.99

Assay 3

Rat 1	Duodenal (0-20 cm)	40	0.15	5.00
		20	0.07	2.33
	Duodenal (20-40 cm)	40	0.14	4.66
		20	0.07	2.33
	Penta.	40	0.45	14.99
		20	0.24	7.99
Rat 2	Duodenal (40-60 cm)	40	0.13	4.33
		20	0.07	2.33
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.02	0.67
	Penta.	40	0.48	15.98
		20	0.23	7.66

EXTRACT 4:

Assay 1

Rat 1	Duodenal (0-20 cm)	40	0.27	8.99
		20	0.14	4.66
	Duodenal (20-40 cm)	40	0.22	7.33
		20	0.11	3.66
	Penta.	40	0.63	20.98
		20	0.31	10.32

Rat 2	Duodenal (40-60 cm)	40	0.13	4.33
		20	0.07	2.33
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.03	1.00
	Penta.	40	0.69	22.98
		20	0.35	11.66

Assay 2

Rat 1	Duodenal (0-20 cm)	40	0.18	5.99
		20	0.09	3.00
	Duodenal (20-40 cm)	40	0.17	5.66
		20	0.07	2.33
	Penta.	40	0.68	22.64
		20	0.34	11.32
Rat 2	Duodenal (40-60 cm)	40	0.12	4.00
		20	0.05	1.67
	Duodenal (60-80 cm)	40	0.06	2.00
		20	0.03	1.00
	Penta.	40	0.74	24.64
		20	0.35	11.66

Assay 3

Rat 1	Duodenal (0-20 cm)	40	0.21	6.99
		20	0.09	3.00
	Duodenal (20-40 cm)	40	0.25	8.33
		20	0.12	4.00
	Penta.	40	0.71	23.64
		20	0.36	11.99

Rat 2	Duodenal (40-60 cm)	40	0.13	4.33
		20	0.06	2.00
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.03	1.00
	Penta.	40	0.65	21.65
		20	0.33	10.99

EXTRACT 5:

Assay 1

Rat 1	Duodenal (0-20 cm)	40	0.50	16.65
		20	0.24	7.99
	Duodenal (20-40 cm)	40	0.40	13.32
		20	0.25	8.33
	Penta.	40	0.74	24.64
		20	0.37	12.32
Rat 2	Duodenal (40-60 cm)	40	0.15	5.00
		20	0.07	2.33
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.03	1.00
	Penta.	40	0.54	17.98
		20	0.29	9.66

Assay 2

Rat 1	Duodenal (0-20 cm)	40	0.21	6.99
		20	0.43	14.32
	Duodenal (20-40 cm)	40	0.12	4.00
		20	0.05	1.67
	Penta.	40	0.56	18.65
		20	0.28	9.32

Rat 2	Duodenal (40-60 cm)	40	0.27	8.99
		20	0.14	4.66
	Duodenal (60-80 cm)	40	0.19	6.33
		20	0.09	3.00
	Penta.	40	1.18	39.29
		20	0.54	17.98

Assay 3

Rat 1	Duodenal (0-20 cm)	40	0.33	10.99
		20	0.18	5.99
	Duodenal (20-40 cm)	40	0.22	7.33
		20	0.11	3.66
	Penta.	40	1.16	38.63
		20	0.55	18.32
Rat 2	Duodenal (40-60 cm)	40	0.26	8.66
		20	0.16	5.33
	Duodenal (60-80 cm)	40	0.09	3.00
		20	0.05	1.67
	Penta.	40	0.77	25.64
		20	0.39	12.99

EXTRACT 6:

Assay 1

Rat 1	Duodenal (0-20 cm)	40	0.24	7.99
		20	0.12	4.00
	Duodenal (20-40 cm)	40	0.16	5.33
		20	0.08	2.66
	Penta.	40	0.59	19.65
		20	0.31	10.32

Rat 2	Duodenal (40-60 cm)	40	0.19	6.33
		20	0.09	3.00
	Duodenal (60-80 cm)	40	0.09	3.00
		20	0.05	1.67
	Penta.	40	1.08	35.96
		20	0.51	16.98
	Assay 2			
Rat 1	Duodenal (0-20 cm)	40	0.32	10.66
		20	0.16	5.33
	Duodenal (20-40 cm)	40	0.15	5.10
		20	0.08	2.66
	Penta.	40	0.74	24.64
		20	0.35	11.66
Rat 2	Duodenal (40-60 cm)	40	0.11	3.66
		20	0.05	1.67
	Duodenal (60-80 cm)	40	0.05	1.67
		20	0.02	6.67
	Penta.	40	0.60	19.98
		20	0.32	10.66
	Assay 3			
Rat 1	Duodenal (0-20 cm)	40	0.14	13.65
		20	0.18	5.99
	Duodenal (20-40 cm)	40	0.23	7.66
		20	0.11	3.66
	Penta.	40	1.06	35.30
		20	0.51	16.98

Rat 2	Duodenal (40-60 cm)	40	0.10	3.33
		20	0.05	1.67
	Duodenal (60-80 cm)	40	0.09	3.00
		20	0.05	1.67
	Penta.	40	0.73	24.31
		20	0.36	11.99

CAECAL EXTRACTS

	Dose (µg)	pH Change	µ mol H^+.30 min^{-1}
EXTRACT 1:			
Assay 1			
Caecal	40	0.24	7.99
	20	0.19	6.28
Penta.	40	0.67	22.31
	20	0.33	10.98
Assay 2			
Caecal	40	0.13	4.30
	20	0.07	2.32
Penta.	40	0.36	11.92
	20	0.16	5.30
Assay 3			
Caecal	40	0.23	7.61
	20	0.12	3.97
Penta.	40	0.62	20.52
	20	0.34	11.25
EXTRACT 2:			
Assay 1			
Caecal	40	0.52	17.32
	20	0.25	8.33
Penta.	40	1.22	40.63
	20	0.61	20.31

Assay 2			
Caecal	40	0.39	12.99
	20	0.18	5.99
Penta.	40	0.86	28.64
	20	0.42	13.99
Assay 3			
Caecal	40	0.43	14.32
	20	0.23	7.66
Penta.	40	0.99	32.97
	20	0.51	16.98

EXTRACT 3:

Assay 1			
Caecal	40	0.53	17.65
	20	0.27	8.99
Penta.	40	1.22	40.63
	20	0.61	20.31
Assay 2			
Caecal	40	0.37	12.32
	20	0.18	5.99
Penta.	40	0.86	28.64
	20	0.42	13.99
Assay 3			
Caecal	40	0.40	13.32
	20	0.24	7.99
Penta.	40	0.99	32.97
	20	0.51	16.98

EXTRACT 4:

Assay 1

Caecal	40	0.65	21.65
	20	0.32	10.66
Penta.	40	1.01	33.63
	20	0.52	17.32

Assay 2

Caecal	40	0.62	20.65
	20	0.33	10.99
Penta.	40	1.20	39.90
	20	0.61	20.31

Assay 3

Caecal	40	0.34	11.32
	20	0.17	5.66
Penta.	40	0.69	22.98
	20	0.35	11.66

EXTRACT 5:

Assay 1

Caecal	40	0.47	15.65
	20	0.21	6.99
Penta.	40	1.01	33.63
	20	0.52	17.32

Assay 2

Caecal	40	0.42	13.99
	20	0.19	6.33
Penta.	40	1.20	39.90
	20	0.61	20.31

Assay 3

Caecal	40	0.30	9.99
	20	0.16	5.33
Penta.	40	0.69	22.98
	20	0.35	11.66

EXTRACT 6:

Assay 1

Caecal	40	0.39	12.99
	20	0.20	6.66
Penta.	40	0.80	26.64
	20	0.41	13.65

Assay 2

Caecal	40	0.40	13.65
	20	0.19	6.33
Penta.	40	0.93	30.97
	20	0.46	15.32

Assay 3

Caecal	40	0.50	16.65
	20	0.26	8.66
Penta.	40	1.21	40.29
	20	0.60	19.98

APPENDIX VII: pH change and acid secreted by the anaesthetized rats stomach during bioassay of antral, abomasal body, rumenal, reticular and omasal muscosal extracts. The secretory activity of equal doses (4.0×10^{-5}g) of extract and pentagastrin were compared.

	Bioassay	pH Change	μ mol H^+.30min^{-1}
Antral	1	0.65	21.64
	2	0.88	29.30
	3	0.70	23.30
Ab. Body	1	0.27	8.91
	2	0.37	12.32
	3	0.28	9.32
Rumenal	1	0.24	7.99
	2	0.34	10.65
	3	0.28	9.32
Reticular	1	0.25	5.32
	2	0.34	11.32
	3	0.24	7.99
Omasal	1	0.26	8.65
	2	0.31	10.32
	3	0.26	8.65
Pentagastrin	1	0.67	22.32
	2	0.89	29.64
	3	0.69	22.97

APPENDIX VIII: pH change and acid secreted by the anaesthetized rats stomach during bioassay of extracts obtained from the Dolphin forestomach, mainstomach, pyloric stomach, duodenal ampulla and intestine. The secretory activity of equal doses (2.0×10^{-5}g) of extract and of pentagastrin were compared.

	Bioassay	pH Change	μ mol.H^+.30min^{-1}
Forestomach	1	0.12	3.8
	2	0.09	2.9
Mainstomach	1	0.07	2.3
	2	0.08	2.6
Pyloric stomach	1	0.15	4.9
	2	0.11	3.6
Duodenal Ampulla	1	0.05	1.7
Intestine	1	0.05	1.8
	2	0.054	1.6
Pentagastrin	1	0.18	6.0
	2	0.18	6.0

APPENDIX IX: Gel buffer used for S.D.S. electrophoresis.

	$g.l^{-1}$
Sodium di-Hydrogen Phosphate (Analar, B.D.H. Chemicals Ltd., England)	7.8
di-Sodium Hydrogen Phosphate (Analar, B.D.H. Chemicals Ltd., England)	38.6
S.D.S.	2.0g

APPENDIX X: Sodium phosphate buffer used for S.D.S. electrophoresis.

	$g.l^{-1}$
Sodium di-hydrogen Phosphate (Analar, B.D.H. Chemicals Ltd., England)	7.8
di-Sodium Hydrogen Phosphate (Analar, B.D.H. Chemicals Ltd., England)	38.6

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