THE UNIVERSITY OF MANITOBA

STUDIES ON GASTRIN RELEASE, HEPATIC AND PANCREATIC EXOCRINE SECRETIONS IN SHEEP

BY

Jerry Martin Bergen

A THESIS

Submitted to the Faculty of Graduate Studies in

Partial Fulfilment of the Requirements for

the Degree of Master of Science

Department of Animal Science

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Abstract

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Gastrin, a hormone released from the antral mucosa of monogastric animals in response to mechanical and chemical stimuli was studied in sheep under different feeding schedules and after intravenous injections of secretin or cholecystokinin. The radioimmunoassay used to determine serum gastrin concentrations indicated that gastrin was present in sheep and that changes in feeding schedules may alter the serum gastrin concentrations. Sheep under fasting conditions had the lowest concentrations of gastrin, followed by sheep on continuous feed while sheep fed once daily had the highest serum gastrin concentrations. Immunoreactive serum gastrin concentrations increased in all sheep following feeding, and this increase was inhibited by an intravenous injection of secretin but not by cholecystokinin-pancreozymin (CCK-PZ).

The examination of factors controlling both hepatic and pancreatic exocrine secretions indicate that feeding schedules, secretin and CCK-PZ play important regulatory functions. Sheep fed continuously generally had elevated concentrations of electrolytes in their bile compared to fasting sheep. Phosphate was the only electrolyte in the bile of sheep fed continuously, which decreased as level of feed intake increased. The volume of bile secreted increased over that observed for fasting sheep when the level of feed intake increased to 1200 g. feed/24 hrs.

Pancreatic secretions were stimulated to increase with increased levels of feed intake. The electrolytes potassium and calcium increased in sheep fed continuously as compared to fasting sheep while sodium

concentrations did not change and phosphate decreased.

Feeding sheep once daily was used to study the effect of feeding as a stimulus for bile and pancreatic juice secretions. Following feeding an increase in both secretory volumes was observed. The electrolyte concentrations in bile decreased following feeding while no change occurred in pancreatic juice electrolyte composition.

Once a day feeding was also a potent stimulus to increase the enzyme concentrations in pancreatic exocrine secretions.

Secretin injections increased bile and pancreatic juice flow rates, had no effect on sodium or calcium concentrations in either secretion, increased potassium concentrations in both secretions, increased phosphate concentration in bile but decreased it in pancreatic secretion, and did not stimulate pancreatic enzyme secretion.

Injections of CCK-PZ stimulated an increased flow rate of both bile and pancreatic juice. CCK-PZ did not change the concentrations of electrolytes in bile or pancreatic juice with the exception of an increased phosphate concentration in bile. CCK-PZ appeared to have as its main stimulatory action, an increase in pancreatic juice enzyme concentration.

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LIST OF ABBREVIATIONS

Ach - acetylcholine

BTEE - butyl tyrosine ethyl ester

C - Celsius

Ca^{++} - calcium ion

CCK-PZ - cholecystokinin-pancreozymin

Cl^{-} - chloride ion

cm. - centimeter

C.U. - clinical units

dl. - deciliter

DNA - deoxyribonucleic acid

FFA - free fatty acid

G - gastrin

g - gram

GHRIH - growth hormone release - inhibiting hormone

GIP - gastrin inhibitory polypeptide

H^{+} - hydrogen ion

HCO^{-}_{3} - bicarbonate

H_2PO_4 - phosphate

hr. - hour

I - iodine

I.D. - inside diameter

I.V. - intravenous

K^{+} - potassium ion

kg. - kilogram

l - liter

mEq - milliequivalent

mg. - milligram

min - minute

ml - milliliter

mM - millimole

Na^+ - sodium ion

NaOH - sodium hydroxide

ng. - nanogram

nm - nanometer

O.D. - outside diameter

pg. - picogram

PZ - pancreozymin

RIA - radioimmunoassay

RNA - ribonuclei acid

S.E. - standard error

TAME - tyrosyl-arginine-methyl ester

U - unit (S)

ug - microgram

umole - micromole

U.V. - ultraviolet

VIP - vasoactive intestinal peptide

$\overline{X}$ - mean

INTRODUCTION

The regulation of hepatic and pancreatic exocrine secretions is under both hormonal and neural control, in both monogastrics and ruminant animals. A large number of factors appear to be important in the regulation of the two major intestinal secretions.

The present studies were designed to examine the importance of a number of factors which may regulate hepatic and pancreatic exocrine secretions in the sheep. Most of the information available for control of these digestive secretions has come from the study of monogastric animals. Whether the mechanisms for control are similar between the monogastric and the ruminant digestive system is not well understood. An objective of the present experiments was to determine whether certain regulatory systems known for control of monogastric secretions, were also present in sheep.

Gastrin a hormone which is released from the antral mucosa of monogastrics appears to regulate a large number of gastrointestinal functions of which the major ones are gastric secretion and pancreatic enzyme secretion. The release of gastrin is stimulated by mechanical and chemical stimuli following a meal. In the ruminant animal, ingested food enters the reticulo-rumen following feeding and may therefore not stimulate gastrin release assuming that the gastrin releasing cell is present in the abomasum which may be considered analogous to the stomach of monogastrics. The present studies examined the effects of different feeding schedules on levels of immunoreactive serum gastric. Interactions of secretin and cholecystokinin-pancreozymin (CCK-PZ) on serum gastrin

levels were also examined.

A surgical method was used to catheterize the common bile duct and pancreatic duct of sheep, which enabled the separate collection of each secretion. The bile and pancreatic juice samples so collected were analysed for electrolyte composition. Pancreatic juice samples were also analysed for composition of certain proteolytic enzymes. The sheep studied were subjected to different feeding schedules and injections of secretin and CCK-PZ in order to determine the importance of these conditions on the production and composition of bile and pancreatic juice.

LITERATURE REVIEW

Gastrin History, Structure and Radioimmunoassay

Edkins (1905) suggested that postprandial release of acid by the oxyntic glands of the stomach mucosa was under partial chemical control. This chemical control was largely attributed to the secretagogue gastrin which could be demonstrated in extracts of antral stomach mucosa. Gastrin, suggested to be the major humoral control factor in gastric acid secretion was not isolated until 1961 by Gregory and Tracy from hog antral mucosa. Anderson et. al. (1964) synthesized gastrin to provide adequate amounts of the hormone for development of radioimmunoassay (RIA) techniques. RIA findings have subsequently shown that gastrin may exist as G17, ie: a gastrin composed of 17 amino acids, big gastrin (G 34) composed of 34 amino acids (Gregory and Tracy, 1975), big big gastrin (Yallow and Wu, 1973), intermediate gastrin (Rehfeld et. al. 1974), and little little gastrin made up of 13 amino acids (Gregory and Tracy, 1974). Biological activity of big big gastrin, intermediate gastrin and little little gastrin is speculative at present. The major forms, G17 and G34, are present in much higher concentrations than the aformentioned other types and probably contribute most to the physiological response of "gastrin." Gregory and Tracy (1974) suggested that little little gastrin is a breakdown product of big gastrin, (fig. 1).

The heterogeneity of "gastrin" raised difficulties in utilizing RIA techniques, namely in the production of antibodies to a specific gastrin. Since both G17 and G34 were shown to have the greatest biological activity, quantitating G17 alone or G34 may be useful in explaining physiological responses to gastrin. Berson and Yallow (1971)

have indicated that both G17 and G34 are released in response to gastrin secretagogues but tissue heterogeneity exists in which G17 is more abundant in the antrum of the stomach while G34 is progressively more abundant from duodenum to jejunum. Gregory (1974) also showed that fasting levels of gastrin in man, dog and pig were predominantly G34 whereas stimulation for gastrin release resulted in G17 becoming the more concentrated serum gastrin.

The minimal segment of gastrin with measureable biological activity was shown by Lin (1972) to be the last four amino acids or tetrapeptide at the carboxyl end of the polypeptide. The tetrapeptide was shown to possess the same range of biological actions as G17 but not to be as potent a stimulator. Pentagastrin a derivative of tetrapeptide "gastrin" also possesses the same range of actions as G17 and is used widely in research and clinical work as a cheaper replacement for purified G17 (Peterson and Meyer, 1975).

In addition to the variable sizes of the polypeptide "gastrin", Gregory and Tracy (1975) demonstrated that gastrins may be either sulfated or non sulfated at tyrosine in position 12 on the gastrin molecule. Biological potency does not appear to be altered due to either form. Gastrin, G17 structure also varies between mammalian species as indicated in figure 2 (Bentley,et. al. 1966 and Johnson, 1974). Johnson (1974) indicated that differences in structure occurred primarily at amino acid positions 5 and 10, with little loss in potency when tested for biological activity across species.

Figure 1 FROM Gregory and Tracy (1974).

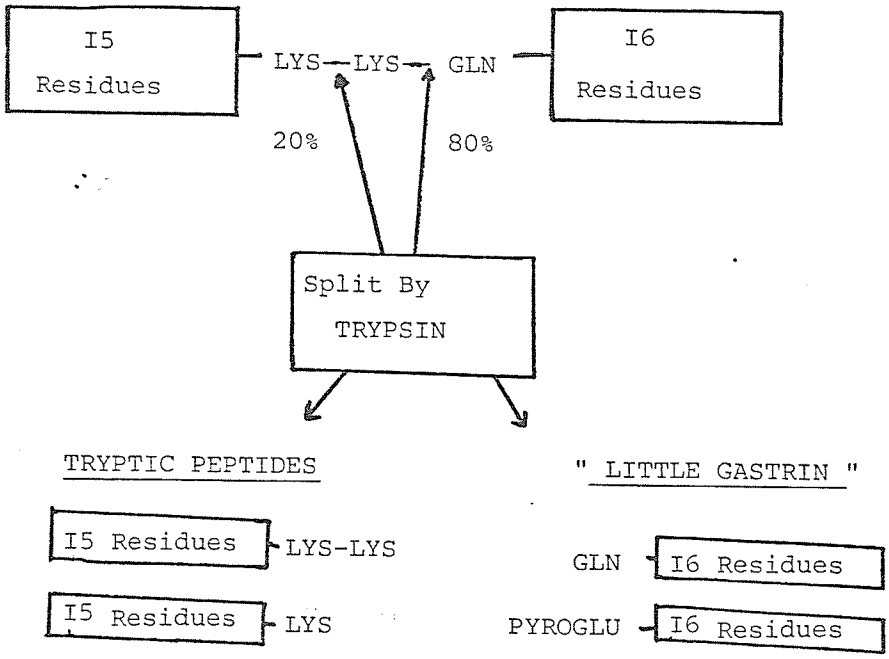


Figure 2 FROM Bentley et. al. (1966, and Johnson (1974).

STRUCTURE OF HUMAN G17

1	2	3	4	5	6	7	8	9	10	11	12
PYR -	GLY -	PRO -	TRP -	LEU -	GLU -	GLU -	GLU -	GLU -	GLU -	ALA -	TYR -

13	14	15	16	17	
GLY -	TRP -	MET -	ASP -	PHEN -	NH_2

HSO_3 (on TYR)

C terminal active tetrapeptide is underlined.

Gastrin I - denotes gastrin with no sulfate group on position 12.

Gastrin II - denoted gastrin with a sulfate group on position 12.

	5	6	7	8	9	10
MAN	- LEU -	GLU -	GLU -	GLU -	GLU -	GLU
HOG	MET					
CAT						ALA
DOG	MET			ALA		
COW AND SHEEP	VAL					ALA

SPECIES VARIATION IN THE STRUCTURE OF GASTRIN

Degradation and Localization

Walsh and Grossman (1975) indicated the $t_{\frac{1}{2}}$ (half life) of G17 to be about 5 minutes and big gastrin (G34) to be about 42 minutes. G34 was shown to remain at levels about four times G17 serum concentrations because of a slower clearance rate for G34, but G17 still appeared to be the major stimulus for gastric acid secretion according to Walsh and Grossman (1975). Bloom (1977) suggested that both G17 and G34 are probably released by one cell type with no evidence of differential secretion. Bloom (1977) demonstrated that nephrectomized patients had elevated serum gastrin levels which suggested the importance of the kidney in the destruction and or filtration of gastrin. However, whether different rates of breakdown for G17 and G34 exist has not been shown.

Gastrin has been shown, by immunoflourescence studies, to be stored, released and probably synthesized by the G cell of the mucosa (McGuigan and Greider, 1971). Most gastrin has been located in the antrum and some so called "G" cells have been observed in the duodenum and upper jejunum of human gastrointestinal tissue. Berson and Yalow (1971) demonstrated that G17 was the predominant form in antral mucosa while G34 was predominant in small intestinal mucosa.

Actions of Gastrin

The actions of gastrin have been demonstrated to be wide and varied. Bloom (1977) suggested that relatively few actions fall within the physiological range of the hormone. The major action demonstrated at physiological levels of gastrin infusion is the secretion and production of gastric acid (Edkins, 1905; Emas & Grossman, 1967). Much of the work regarding actions of gastrin was performed with monogastrics

as the animal model. The author has been unable to find any reference to actions of gastrin in ruminants and therefore has had to rely on evidence from work on monogastrics, mainly human, dog and rat. Walsh and Grossman (1975) suggest a multitude of actions for gastrin, many of which may be pharmacological. The following are put forth as possible actions for gastrin in gastrointestinal functions.

1) Gastrin stimulates gastric acid secretion (Edkins, 1905 and Emas and Grossman, 1967).
2) Gastrin increases smooth muscle contractility in the stomach, upper small intestine and gallbladder (Isenberg and Grossman, 1969; Vagne and Grossman, 1968; Bennet and Misiewicz, 1967).
3) Gastrin strongly stimulates pancreatic enzyme secretion (Stening and Grossman, 1969a).
4) Gastrin weakly stimulates production of alkaline pancreatic secretion (Stening and Grossman, 1969a).
5) Gastrin stimulates water and bicarbonate secretion by the liver (Jones and Grossman, 1970).
6) Gastrin stimulates mucous secretion from Brunner's glands (Stening and Grossman, 1969b).
7) Gastrin reduces the net absorption of Na^+, K^+ and water from jejunum and ileum (Gingell et. al., 1968).
8) Gastrin stimulates protein synthesis in duodenal and gastric mucosa (Johnson et. al., 1969a).
9) Pentagastrin was shown by (Johnson et. al., 1969b) to act as a trophic hormone on the gastric and duodenal mucosa, and also as a trophic agent on pancreatic acinar cells (Mayston and

Barrowman, 1971).

Johnson (1977) also showed that G17I, G17II and G34II infused into rats increased the DNA content and synthesis in gastric mucosa. Enochs and Johnson (1977) indicate that secretin may act to control the trophic action of gastrin, since secretin inhibited the trophic response of pentagastrin induced mucosal hypertrophy.

Humans exhibit a circadian gastric acid secretory pattern with the maximal acid output in the evening and minimal during the morning (Moore and Wolfe, 1973). However serum gastrin levels which may have been expected to increase causing increased gastric acid production did not show a circadian rhythm in humans and remained in the range of 30 to 45 pg./ml. of serum (Moore and Wolfe,1973).

Shaw and Heath (1972) demonstrated that intravenous infusion of pentagastrin resulted in no change in HCO^-_3 concentration or flow of either bile or pancreatic juice in rats, contrary to findings in the dog (Stening and Grossman, 1969a; Jones and Grossman, 1970).

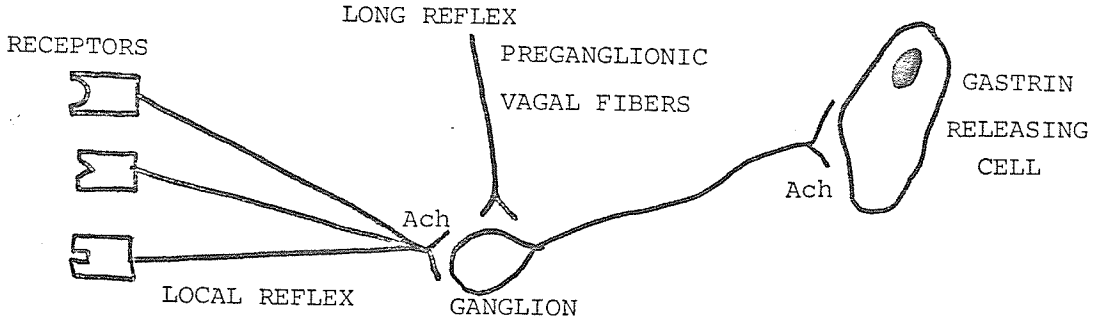
Gastrin levels in serum as measured by radioimmunoassays appear to vary depending on the immunospecificity of the antiserum. Fasting has been shown to decrease gastric acid output but this finding does not agree well with the relatively high levels of immunoreactive serum gastrin observed in fasting humans, dogs and pigs (Brandsborg et. al. 1975). Yallow and Wu (1973) have shown that big gastrin (G34) remains relatively constant before and after feeding while smaller forms of gastrin were shown to increase greatly

after feeding. This may explain Brandsborg et. al. (1975) findings if their antiserum had a high immunospecificity for big gastrin.

Release of Gastrin

The release of gastrin from the antral G cell is regulated by neuroendocrine reflexes. All neural control occurs via cholinergic nerves via short local reflexes which are activated by stimuli affecting receptors in the gastric mucosa or long reflexes involving vagal nerve activation (Grossman, 1967). Figure 3 illustrates a possible neural control linking stimuli to the gastrin releasing cell.

Figure 3 Adapted from Grossman, 1967



Grossman (1967) has suggested that distension of the antrum increases gastrin release via the local reflex. Blair et. al. (1975) observed the protein fraction of an ingested meal to be a major stimulus for gastrin release. They also observed that peptides and amino acids were the most potent stimulators of gastrin release within the protein component of the meal. Alcohols, as ethanol and propanol, also act on local receptors in the stomach mucosa, leading to increased gastrin release (Grossman, 1967). The receptors in the stomach mucosa are also sensitive to inhibition by local anesthetics.

Vagal activity and the long reflex control of gastrin release was demonstrated by Smith et. al. (1975). They demonstrated that electrical stimulation of the vagus nerve greatly enhances gastrin release in dogs. Insulin induced hypoglycemia, which elevates vagal activity, also increases serum gastrin levels (Walsh and Grossman, 1975). Walsh and Grossman (1975) also demonstrated that vagotomy will eliminate the increased serum gastrin levels seen in canine insulin hypoglycemia. Contrary to the finding for the canine, Faroog and Walsh (1975) observed that vagotomy, in humans with insulin hypoglycemia, actually resulted in higher levels of serum gastrin and that administration of atropine to these subjects also enhanced gastrin secretion.

Walsh & Grossman (1975) observed that acid actively inhibits gastrin release when pH of the antral mucosa is lower than 3.5. This negative feedback control which prevents excessive gastric acid production and release, operates even after local anaesthesia suggesting that H^+ ions may act directly on the G cell and not via a neural mechanism.

Secretin History, Structure and Radioimmunoassay

Secretin was discovered by Bayliss and Starling (1902) when they demonstrated that, after nervous connections to the duodenum were severed and acid was infused intraduodenally, the pancreas increased production and secretion of HCO_3^- and water. This response was also shown to occur when duodenal extracts were injected intravenously. Discovery of a blood born chemical mediator acting on a distant organ, as secretin appeared to do, originated the discipline of endocrinology. Difficulties with available biochemical techniques at the time delayed obtaining a pure sample of secretin. Fifty nine years after its discovery, Jorpes and Mutt (1961) isolated a pure preparation of secretin which they sequenced (Mutt and Jorpes, 1966). Secretin was shown to be a polypeptide composed of 27 amino acids, which was successfully synthesized by Bodansky, Ondetti and Levine (1966). Unlike gastrin and cholecystokinin-pancreozymin, the entire secretin molecule was found to be required for full biological activity and substitution for any one of the amino acids probably destroys the tertiary structure of the molecule which is required for its action (Bodansky et. al., 1969).

The structural sequence of secretin as determined by Jorpes and Mutt (1961) is seen in figure 4.

Figure 4 Amino Acid Sequence of Porcine Secretin

1	2	3	4	5	6	7	8	9	10	11	12
HIS -	SER -	ASP -	GLY -	THR -	PHE -	THR -	SER -	GLU -	LEU -	SER -	ARG -
13	14	15	16	17	18	19	20	21	22	23	24
LEU -	ARG -	ASP -	SER -	ALA -	ARG -	LEU -	GLN -	ARG -	LEU -	LEU -	GLN -
25	26	27									
GLY -	LEU -	VAL -	NH_2								

Presently only porcine secretin has been sequenced, and since amino acid substitutions appear to destroy secretin's biological activity it appears that species differences do not exist or receptors may be different across species.

Understanding of literature on the amounts of secretin used for infusion studies is confused by the fact of different units being used to express the activity of the hormone. Boots' secretin, a relatively impure preparation utilizes Crick - Harper - Raper units (Crick et. al., 1949), while the GIH laboratory in Stockholm uses clinical units. Prior to 1968 one clinical unit was equivalent to two Crick-Harper-Raper units. However Stening et al (1968) demonstrated that the potency of the clinical unit had increased to equal 8 or 9 Crick-Harper-Raper units. Further work using a synthetic secretin has shown that 1 ug secretin is equal to 3.4 clinical units (Vagne et. al., 1968). The use of the Crick-Harper-Raper unit is seldom reported in the current literature.

Localization

Bloom and Bryant (1973) localized radioimmunoassayable secretin in the mucosal cells of the duodenum and upper jejunum. Polak et al. (1971b) indicated that secretin was produced by the S cell whose distribution in rat, dog and baboon was also in the mucosa of the duodenum and upper jejunum. Immunofluorescent localization of secretin producing cells (S cell) was also located primarily in the upper small intestine of humans (Polak et. al., 1971a).

Actions of Secretin

The actions of secretin, as for gastrin, are widely varied, involving

much of the gastrointestinal tract and associated organs. Secretin was shown to decrease gastric acid secretion in dogs when given intravenously (Konturek et. al., 1977). Immunoreactive serum secretin levels increased when a meal was infused into the duodenum and was pH dependent with maximal secretin release at a pH of 2. As secretin levels increased with decreasing pH, gastrin levels decreased suggesting an inhibition of gastrin release by secretin. Secretin infusions mimicked gastrin inhibition by intraduodenal acidification but not to the same extent, which suggested that gastric acid release inhibition was attributed to both secretin and a neural feedback mechanism in the dog (Konturek, 1977).

Konturek (1970) also showed secretin given intravenously inhibited basal acid output but only mildly inhibited pentagastrin induced gastric secretion in man. Ward and Bloom (1974) indicated that intraduodenal acidification, which increased serum secretin, strongly inhibited gastric acid secretion whereas intravenous infusions of secretin only weakly inhibited acid secretion in man. Johnson (I974) has suggested that the degree of gastric acid inhibition by secretin may be related to the capacity of the liver, pancreas and Brunners' glands to neutralize the acid chyme entering the duodenum. In the dog the capacity for neutralization is much less than gastric capacity to secrete acid and therefore secretin acts as a strong inhibitor of gastric acid output. In man neutralization capacity is much higher and secretin inhibition on gastric secretion is relatively weak compared to the dog. In the sheep the neutralizing capcity of the liver, pancreas and Brunners' glands falls greatly below the capacity for abomasal acid production, which would tend

to favor secretin as a strong inhibitor of gastric acid output (Caple and Heath, 1974).

Stening & Grossman (1969) demonstrated that secretin strongly stimulated pepsin secretion from the chief cells in the cat and dog, and Brooks et. al. (1969) showed the same type of action in man. No evidence for this action has been demonstrated in the ruminant animal.

Secretin also reduces gastric motility in man which effectively reduces the amount of acid chyme entering the duodenum (Dinoso et al., 1966). Motor activity of the upper small intestine in man was reduced when secretin was given I.V. as demonstrated by Gutierrez et. al. (1974). Recently Brown et. al. (1973) discovered a hormonal peptide in porcine intestinal mucosa which they named motilin. Ruppin et. al. (1975) and Rosch et. al. (1976) demonstrated motilin to increase esophageal sphincter pressure and to delay gastric emptying. Mitznegg et. al. (1977) indicated that infusion of graded doses of secretin resulted in decreased serum levels of motilin suggesting a mechanism for control of gastric emptying in man. No evidence is presently available for a secretin - motilin regulation of abomasal emptying in ruminants.

Bruce and Huber (1973) showed that intravenous infusion of secretin at 6.88 U/kg/hr. in sheep inhibited amplitude and the frequency of rumen contractions similar to but not as greatly as did intraduodenal acidification with lactic acid.

Perhaps the major action of secretin in gastrointestinal functions is the strongly stimulatory effect on pancreatic and hepatic production of watery bicarbonate secretions. Konturek (1970) gave intravenous infusions of secretin to humans at 2 U/kg/hr. and observed an increase in pancreatic

flow from 5 ml/15 minutes to 60 ml/15 minutes, with a simultaneous increase in pancreatic juice bicarbonate output from 0.25 m equiv./15 min. to 6.5 m eqiv/15 min. Domschke et. al. (1976) used an endoscopic cannulation of the main pancreatic duct in man to collect secretin stimulated secretion. They showed an increased pancreatic juice flow with an increased HCO_3^- output, decreased Cl^- concentration, and no change in sodium or protein concentrations.

Konturek (1971) used dogs to demonstrate that either secretin infusions intravenously or intraduodenal acidification increased bile flow and HCO_3^- concentration in bile. Secretin was also more potent as a stimulator of bile HCO_3^- concentration and flow, demonstrating a 25% higher maximal output than maximal output attained by intraduodenal acidification.

Gardiner and Small (1976) used a flow meter with electronic stream splitter to sample only 5% of bile and pancreatic juice from two separate catheters in monkeys. By avoiding interruption of enterohepatic and enteropancreatic circulations Gardiner & Small (1976) showed that intravenous infusions of secretin increased bile flow, pancreatic flow, pancreatic juice HCO_3^- concentration and produced no significant increase of pancreatic enzyme production.

Contrary findings are reported for the rat by Shaw and Heath (1972) who indicate that exogenous secretin did not alter bile flow or composition compared to controls, while pancreatic juice volume and HCO_3^- concentration increased. These findings suggest the rat pancreas is actively involved in duodenal neutralization to a much greater extent than the liver.

Horn and Huber (1975) examined the effects of intraduodenal acidification with a lactic acid solution of pH 2.0, and intravenous secretin

at 6.88 U/kg/hr. on mixed pancreatic biliary secretions obtained from wethers with chronically catheterized bile ducts. Results obtained indicated an increase of pH due to increased HCO_3^- production concomitant with increased production of mixed secretions. Their results however do not indicate the relative proportions that bile or pancreatic juice contributed to this increase in flow.

Magee (1961) examined the relationship between volume of pancreatic juice secreted and pH of solutions infused intraduodenally or intravenous secretin infusions of 5 to 22 C.V. given as a single injection in sheep. Magee found decreasing flows as intraduodenal pH was increased from 2 to 8. Likewise increasing the dose of secretin increased pancreatic juice flow rate. These data suggest the possibility of pH regulating secretin release which in turn regulates volume of pancreatic juice produced. Bicarbonate concentration was demonstrated to increase as volume and chloride concentration dropped (Magee, 1961).

The effect of secretin on bile formation in sheep was examined by Heath (1970) by infusion of secretin into the portal vein at 0.92 C.U. / min. for 20 minutes. No signs of distress, no change in portal venous pressure, arterial pressure and no pulse rate or respiratory rate changes occurred following secretin infusions. These parameters are mentioned as indicators of possible increases in blood flow or hepatic blood pressures which may increase production of bile. Heath's (1970) results demonstrate an increased flow rate from control sheep (0.19 ml./ min. and 0.27 ml./min.) values to secretin stimulated secretion (0.57 ml/ min. and 0.77 ml/min. respectively). There was no change in bile flow for the first 3 min. of secretin infusion followed by a rapid increase

to peak values at 7 minutes. The increase in bile flow due to secretin should be considered with results by Heath et. al. (1970) which demonstrated that taurocholate infusions intravenously could stimulate bile flow rates to the same degrees as 5 C.U./kg./hr. secretin. Consideration must be given to levels of serum bile salts when considering effects of secretin on bile formation and disruption of the enterhepatic circulation of these salts. Grossman et. al. (1949) reported that, in humans, low levels of bile salts were efficiently transferred to the bile from serum, but efficiency decreased with increasing levels of bile salts in the enterohepatic circulation until a maximum rate of transfer was reached. Heath, et. al. (1970) demonstrated that infusion of secretin increased bile salt uptake and secretion by the liver above the maximum values found for taurocholate alone, suggesting that secretin acts directly on hepatocytes. Wheeler &Mancusi-Ungaro(1966)showed that the stimulus for increased bile flow by secretin is not by the same mechanism for increasing bile salt choleresis alone, but rather that secretin may increase bile flow in the absence of bile salts.

The additive effect of secretin and taurocholate or bile salts on bile flow and composition in sheep was further studied by Caple and Heath (1974), Caple and Heath (1972) and Harrison and Hill (1962). Harrison and Hill (1962) demonstrated an average secretory rate of bile during a 4 - 24 hr period after non return of bile to the duodenum to be 0.41 ml/kg/hr. They further suggested that the ratio of bile to pancreatic juice secreted is 2 or 3 to 1. Measurements on mixed secretions with return of bile to the duodenum yielded a value of 0.90 ml./kg./hr. which would therefore place bile

flow values between 0.60 ml./kg./hr. and 0.68 ml./kg./hr. Caple and Heath (1974) using bile duct cannulated sheep obtained basal flow of bile of 0.31 ± .02 ml./min. when bile was not returned to the duodenum over a 12-48 hr. collection period. Sodium taurocholate infused intraduodenally at 55 umol./min. increased basal flow to 0.66 ± .04 ml./min. further emphasizing the importance of enterohepatic circulation on bile production.

The importance of bile salts has been demonstrated on bile flow but little emphasis has been placed on pancreatic juice. Caple and Heath (1972) studied pancreatic juice electrolyte composition and volume and were able to demonstrate no effect of taurocholate infusions on these parameters. (Fig. 5). They did however observe that when secretin was infused into the portal vein, concentration and flow of pancreatic juice did increase, but simultaneous infusions of taurocholate had no effect on this response. Therefore it appears that the major action of bile salts is on bile salt concentration in bile production and its flow rate.

Electrolyte composition of bile in response to taurocholate infusion and secretin has also been examined. Caple and Heath (1972) showed increased concentration of bile salts and bicarbonate, decreased chloride concentration with no change in sodium or potassium, with taurocholate infusions. Secretin markedly stimulated HCO_3^- concentration increase and output, decreased Cl^- concentration but increased Cl^- output, with little change in Na^+ or K^+ concentration in both bile and pancreatic juice of sheep (Fig. 6). Table. I from Caple and Heath (1974) illustrated composition of basal secretions of bile and pancreatic juice.

Figure 5 Effect of secretin on the response of the liver and pancreas to sodium taurocholate.

Each histogram represents the mean of 4 sheep.

Open blocks represent taurocholate infusion into the portal vein.

Closed blocks represent taurocholate infusion with 0.88 units/min of secretin into the portal vein (Caple and Heath, 1972).

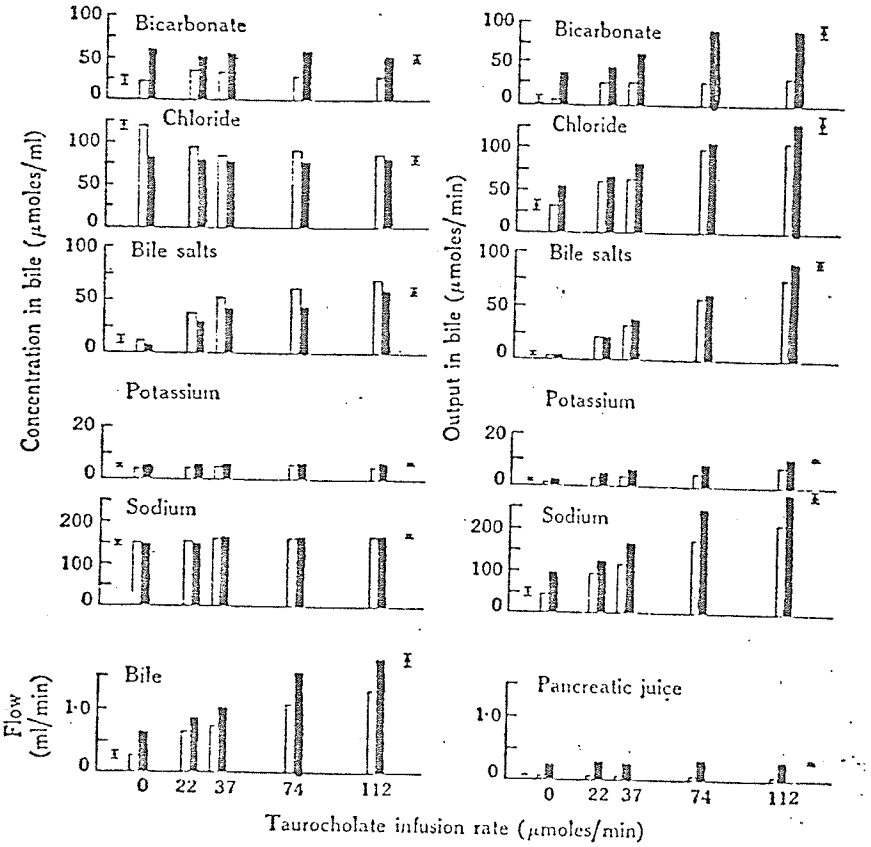


TABLE I FROM CAPLE and HEATH (1974)

	Conc. (umol./ml)		Output (umol/min.)		Conc. (umol/ml)
	Bile	Pancreatic Juice	Bile	Pancreatic Juice	Plasma
Bile Salt	10.9 ± 1.7		3.1 ± 1.1		
Bicarbonate	23. ± 1.7	28 ± 6.0	6.5 ± 1.9	1.8 ± 0.7	26. ± 2.4
Chloride	118 ± 9.1	123 ± 6.4	33 ± 7.1	7.2 ± 1.6	114 ± 4.2
Sodium	150 ± 2.4	147 ± 4.3	40 ± 9.7	8.8 ± 2.0	146 ± 3.6
Potassium	4.4 ± 0.9	4.6 ± 0.4	1.1 ± 0.2	0.3 ± 0.1	4.8 ± 0.6

Basal Secretion of Bile and Pancreatic Juice

Bile and pancreatic juice were collected from four sheep that had been deprived of bile and pancreatic juice and of food for 40 hr, but received daily infusions of an electrolyte solution. Bile flow was 0.27 ± 0.66 ml/min; pancreatic juice flow was 0.06 ± 0.01 ml/min.

Figure 6 Effect of the rate of administration of secretin into the portal vein on the flow and composition of bile and pancreatic juice collected simultaneously in each of four sheep that had been deprived of bile salts. (Caple and Heath, 1972).

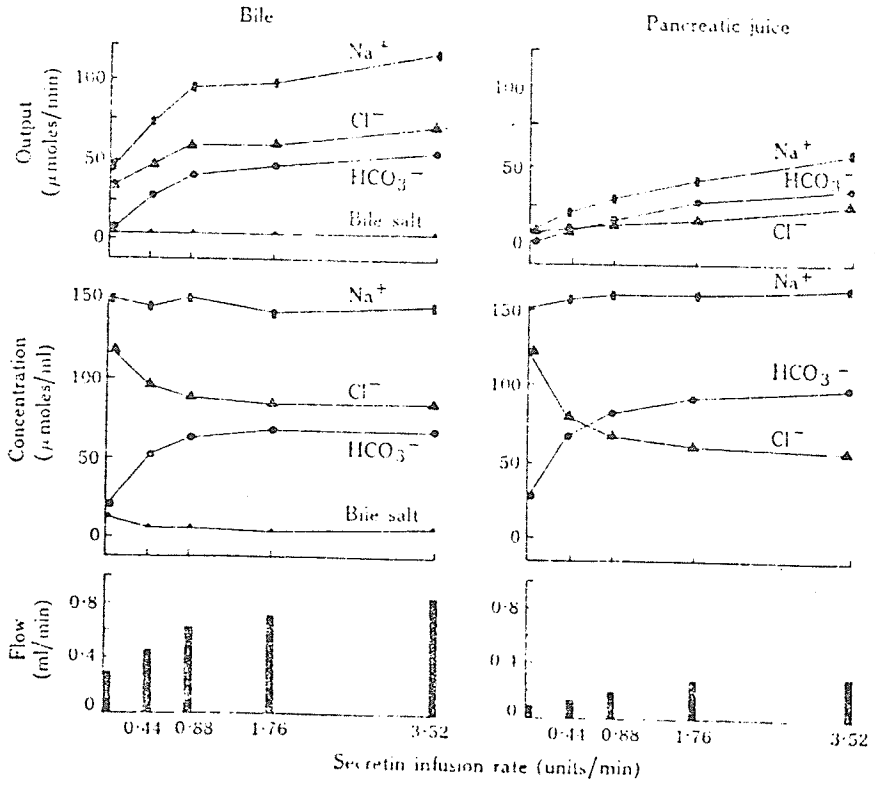


Table I indicates that bicarbonate concentration is higher in pancreatic juice than in bile for sheep (Caple and Heath, 1974). However total bicarbonate output is much higher in the bile than pancreatic juice both at basal and secretin stimulated secretory levels. It therefor appears that secretin may play a major role in stimulating HCO_3^- secretion by the liver and pancreas for neutralization of acid chyme entering the duodenum. However this effect is more pronounced on HCO_3^- secretion in bile suggesting that the liver plays the more major role in acid chyme neutralization in the sheep. This contrasts with the situation in the dog where pancreatic juice bicarbonate output is the greater (Caple and Heath, 1974; Table 2).

If secretin is released upon duodenal acidification it might be expected that duodenal acidification would also produce results similar to secretin infusions. This was demonstrated to be the case in sheep by Caple and Heath (1972) after 1 mmole of HCl in 40 ml of saline was infused intraduodenally. The responses seen in figure 7 also support the contention that the liver plays the major role in total output of bicarbonate.

Some discussion as to the effects of varying the route of exogenous secretin administration was also presented by Caple and Heath (1972). Harper (1967) demonstrated in canines that infusions of secretin into the jugular vein stimulated pancreatic secretion to a greater degree than did portal infusions. Chey, et. al. (1970) did similar studies on dogs and concluded that the liver may be a major site for secretin degradation. Testing this hypothesis for the sheep Caple and Heath (1972) observed no differences on pancreatic and bile flow or bicarbonate output, when

TABLE 2 Comparison of Response of Sheep and Dog. Liver and Pancreas to Infusion of Secretin at 4 U/kg/min. (Caple and Heath, 1974).

	Sheep	Dog
Bile flow (ml/kg/10 min)	0.22	0.19
Bile bicarbonate concentration (umol/ml)	67.0	55.0
Bile bicarbonate output (umol/kg/10 min)	14.7	10.5
Pancreatic juice flow (ml/kg/10 min)	0.07	0.4
Pancreatic juice bicarbonate concentration (umol/ml)	93.2	135.0
Pancreatic juice bicarbonate output (umol/kg/10 min)	6.5	54.0

secretin was infused either into the portal or jugular vein, suggesting that species differences may account for possible different deactivation sites (table 3).

Figure 7 Effect of intraduodenal acidification on bile and pancreatic flow, chloride and bicarbonate composition in sheep (Caple and Heath, 1972).

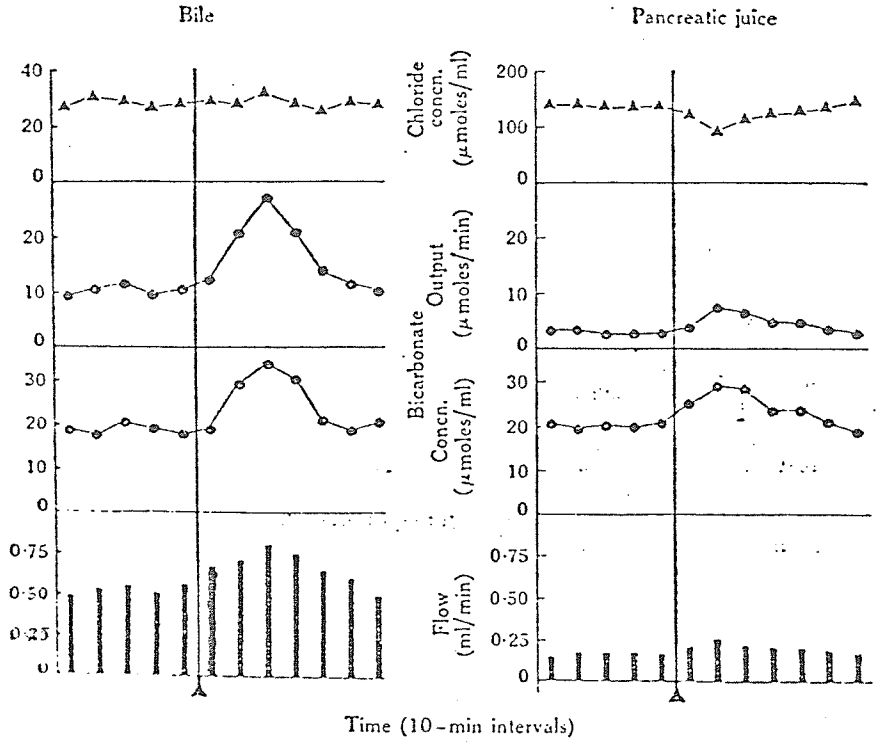


TABLE 3 From Caple and Heath, 1972

Effects of Altering the Route Administration on the Responses of Infusions of Secretin

Three sheep each received a continuous infusion of sodium taurocholate to the portal vein 22 umoles/min, and infusions of secretin at 0.88 units/min were given alternately into the portal vein and the jugular vein

	Flow (ml/min)		Bicarbonate output (u moles/min)	
	Bile	Pancreatic juice	Bile	Pancreatic juice
No secretin	0.49 ± 0.01	0.05 ± 0.01	10 ± 0.9	1.7 ± 0.3
Secretin to portal vein	1.01 ± 0.05	0.21 ± 0.05	57 ± 10.4	20 ± 7.9
Secretin to jugular vein	1.03 ± 0.04	0.18 ± 0.02	50 ± 3.9	14 ± 2.9

The importance of secretin in stimulating enzyme production and secretion by the pancreas appears to be insignificant in all species studied when compared to the stimulating capacity of CCK (cholecystokinin). However a small increase in protein output by the dog pancreas was observed after prolonged infusion with secretin (Stening, et. al., 1969a). Initial infusions of secretin generally increase the protein output by the "wash out phenomenon" which has been described as a flushing out of enzymes already present in the pancreatic ducts (Wormsley, 1968). To further study this matter Singh and Webster(1975) measured the rate of L-^{14}C- phenylalanine incorporation into pancreatic slices in vitro from Spraque-Dawley rats. It was found that secretin infusions, did not change the rate at which labelled protein from ribosomes was incorporated into zymogen granules and did not affect the secretory rate of labelled proteins on the zymogen content of pancreatic acinar cells. These results suggest that the "wash out phenomenon" probably accounts for most of the enzyme or protein output increases observed after secretin administration. Contamination of some secretin preparations with cholesystokinin, (CCK) which has a potent stimulating effect on pancreatic enzyme secretion, may also be a factor. The increased amylase output by the sheep pancreas observed by Magee (1961) following secretin infusions was probably largely due to contamination of the preparation with CCK-PZ.

The evidence however suggests that secretin administration causes some increase in protein output by the dog pancreas (Laval et al, 1977; Meyer et al, 1971). It is also possible that the infused secretin may potentiate the action of the CCK in the circulation, however in vitro

experiments on perfused dog pancreas do not support this (Laval et. al. 1977).

For the ovine species, Horn and Huber (1975) were unable to demonstrate a significant increase ($p \leq .05$) in pancreatic protein output after exogenous secretin infusion. Clain et. al. (1974) also showed that secretin infusion alone did not enhance protein or enzyme secretion by the human pancreas.

Pancreatic blood flow has been demonstrated to increase after exogenous secretin infusions in the cat (Holton and Jones, 1960) and dog (Goodhead et. al., 1970). Goodhead et. al. (1970) demonstrated increased blood flow to the pancreas following infusions of secretin, or pancreozymin, or pentagastrin, and that secretin also increased the volume of viscous pancreatic juice and increased cardiac output. The action of secretin to increase pancreatic blood flow does not necessarily mean that increased pancreatic juice volume, after secretin infusion, is dependent on the increased rate of pancreatic perfusion.

The ability of secretin to produce gall bladder contraction has been attributed to its potentiating action on CCK induced contraction. Stening and Grossman (1969a) observed an augmentation of gall bladder contraction in dogs following secretin and CCK infusion, compared with CCK alone. Secretin alone however appears to have no effect.

Other work by Stening and Grossman (1969b) showed that secretin stimulated bicarbonate secretion by Brunner's glands in dogs. Harrison and Hill (1962) described Brunner's glands in sheep which are largely concentrated between the pyloris and the sphincter of Oddi. They observed an average hourly secretion of 13.3 ml in sheep on once a day feeding

and that this value increased to 26.0 ml./hr. when the flow rate of acid chyme entering the duodenum was increased by feeding the same total quantity of food in three meals daily. This suggests that secretin is probably involved although no evidence for a direct action by secretin on Brunner's glands is available at present.

Release of Secretin

Due to difficulties in producing a reliable radioimmunoassay (RIA) for secretin, much of its release pattern is poorly understood. Boden et. al. (1974) found that intraduodenal acidification with HCl increased immunoreactive serum secretin concentrations. Meyer et. al. (1970) has previously suggested that an intraduodenal pH of 4.5 or below was required to reach threshold for secretin release. Further work by Meyer and Grossman (1970) showed that intraduodenal pH values less than 3.0 did not significantly increase secretin release if titratable acid levels were held constant. They suggested that below pH 3.0 the amount of titratable acid entering the duodenum per unit time would determine the amount of secretin release and this was directly related to the length of intestine acidified. The work of Meyer et. al. (1970) was based on bioassay methods utilizing pancreatic flow volume and bicarbonate output as indices of serum secretin. Bioassays are not able to assess the possible simultaneous release of vasoactive intestinal peptide (VIP), which was demonstrated by Said and Mutt (1972) to also increase pancreatic bicarbonate output. Therefore an RIA method for secretin with no crossreactivity with VIP would be the most reliable for quantitative measurements of serum secretin. Horn and Huber (1975) attempted to illustrate that secretin release by intra-

duodenal acidification with lactic acid was responsible for increased mixed biliary-pancreatic secretions, in sheep. The results only indicate that the acidification response can be mimicked by exogenous secretin administration. At present quantitative determinations of serum secretin levels following intestinal acidification have not been reported for ruminant animals.

Recent studies utilizing RIA methods have shown increases in plasma secretin after intraduodenal acidification in man (Bloom and Ward, 1975) and in dogs (Boden et. al., 1974). Rhodes and Prestwich (1966) had previously indicated that only a small portion of the proximal duodenum became significantly acidified in man following a meal, suggesting little secretin release. Bloom, Byrant and Cochrane (1975) presented evidence that showed no significant increase in serum secretin in humans following a normal meal. The neutralization of acid chyme entering the duodenum may be achieved largely via neural reflexes in humans since bicarbonate output by the liver and pancreas is significantly increased following a meal.

The importance of secretin release in the ruminant is somewhat questionable. Although intestinal acidification occurs to a much greater degree than in monogastrics (Caple and Heath 1974, Harrison and Hill 1962) there is little neutralization of acid chyme entering the duodenum, between the Pylorus and the jejunum. However the stimuli for large quantities of secretin to be released are probably present, considering the pH of 3 to 4 and quantities of titratable acid present in the duodenum (Harrison and Hill, 1962).

Other possible stimuli for secretin release have also been examined. Osnes et. al. (1978) demonstrated an increase in serum immunoreactive secretin after intraduodenal infusion of 6 grams of bovine bile in humans.

The action of secretin to stimulate insulin release was considered to prepare for an increase in plasma glucose. Glucose as an intraduodenal stimulator for secretin release has been recently examined by Boden et. al. (1975). Their results indicated that immunoreactive serum secretin does not rise following intraduodenal instillation of amino acids, fatty acids or sugars in dogs.

Factors other than intraduodenal acidification, which may increase serum secretin, have not been reported for the sheep. At best the increased serum secretin is presently only implied following intraduodenal acidification since no direct measurements of serum secretin are available for the ruminant animal.

Cholecystokinin-Pancreozymin History and Structure

The early studies of cholecystokinin-pancreozymin were somewhat confusing due to the two major different actions of the hormone. Ivy and Oldberg (1928) demonstrated a humoral mechanism producing gall bladder contraction in dogs following intraduodenal infusion of fat; which led to the name cholecystokinin for the hormone involved. Harper and Raper (1943) observed stimulation of a pancreatic secretion rich in enzymes due to a hormone released from duodenal mucosa which they named pancreozymin. It was not until 1968 that these two hormones were shown by Mutt and Jorpes (1968) to be the same and hence the name cholecystokinin-pancreozymin (CCK-PZ). Jorpes (1968) using hog mucosa isolated and sequenced CCK-PZ which was found to be a polypeptide composed of 33 animo acids (fig. 8).

Figure 8 Amino Acid Sequence of CCK-PZ

H-LYS-ALA-PRO-SER-GLY-ARG-VAL-SER-MET-ILE-LYS-ASN-LEU-GLN-

SER-LEU-ASP-PRO-SER-HIS-ARG-ILE-SER-TYR-THR-GLY-TRP-MET-

ASP-PHE-NH_2

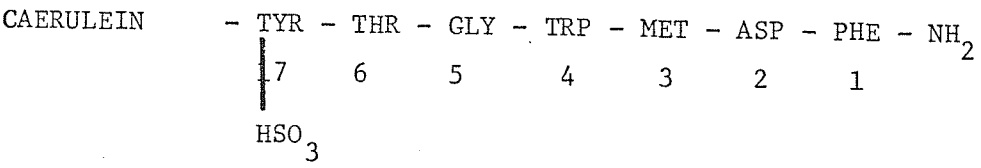
A larger form of CCK-PZ termed CCK variant composed of 39 amino acids was also discovered, however its biological function and importance are not known (Mutt and Jorpes, 1968).

The full biological activity of CCK-PZ required only the COOH

terminal 8 amino acids (Debas and Grossman, 1973), of which the 5C-terminal amino acids are identical to gastrin. As was expected CCK-PZ was found to be a weak stimulator of gastric secretion, in comparison to gastrin as a weak stimulator of gall bladder contraction. CCK exists naturally as the sulfated form where tyrosine at position 7 is sulfated. The sulfated form is highly potent compared to the desulfated form unlike gastrin where both sulfated and unsulfated forms are equipotent (Johnson et. al., 1970; Kaminski, et. al., 1977).

Much of the work examining the actions of CCK-PZ has been done utilizing caerulein, a decapeptide amide isolated from the skin of the Australian frog, Hyla caerulea (Anastasi, et. al., 1967) (Fig. 9).

Figure 9 From Anastasi et. al., 1967



Caerulein (fig. 9) has the same 5-C terminal residues as CCK-PZ and the sulfated tyrosine at position 7, which is also required for maximum biological activity (Johnson et. al., 1970).

Localization

CCK-PZ was demonstrated by electron microscopy to be produced and released by the "I" cell of human and canine upper intestine (Polak et. al., 1975; Buffa , et. al., 1976). Bloom (1974) found the highest concentrations of CCK in the jejunum of man, whereas Buffa , et. al. (1976) observed the duodenum of dogs to have equal abundance of "I" cells as the proximal jejunum.

Radioimmunoassay

As for secretin, development of sensitive radioimmunoassays for the determination of serum CCK levels has been extremely difficult. Bloom (1974) attributes these difficulties to the following:

1) very low CCK concentrations in plasma;
2) large quantities of purified uncontaminated CCK required for production of high affinity antibodies;
3) CCK is a poor antigen;
4) difficulty labelling the sulfated tyrosine with ^{125}I.

Presently RIA methods for CCK do exist but widespread use of them has not occurred (Young et. al., 1969; Go, et. al., 1971; Harvey, et. al., 1974; Reeder, et. al., 1973). Bioassays for CCK-PZ are also in use (Johnson and McDermott, 1973).

Interpretation of studies utilizing infusions of CCK-PZ is made somewhat confusing because of differences in units used to express the amount of CCK-PZ. Two types of units are used to express CCK-PZ activity. The Ivy dog unit, is, according to Jorpes (1968), equal to 4 Crick-Harper-Raper units. Boot's CCK-PZ a relatively crude preparation contains substantial quantities of secretin while the purified CCK-PZ from the GIH laboratory in Stockholm also contained 50 clinical units of secretin to 300 Ivy dog units of CCK-PZ (Ertan, et. al., 1971).

Actions of CCK-PZ

The actions of CCK-PZ, as for gastrin and secretin, appear wide and varied. Most actions mentioned may be pharmacological, however their importance should not be ignored.

Stening and Grossman (1969a) showed that CCK-PZ weakly stimulated gastric acid and pepsin secretion in cats but inhibited gastric acid secretion in dogs. Odori & Magee (1970) also demonstrated that CCK-PZ inhibited gastric acid production which had been stimulated by gastrin, probably because of competition for the gastrin receptor.

The effect of CCK-PZ on gastric and small intestinal motility is not clear. Gutierrez' et. al. (1974) used an intraluminal pressure recording method in humans to demonstrate that CCK stimulated upper intestinal motility, and that this action could be suppressed by secretin. Dollinger, et. al. (1975) noted the contrary where CCK-PZ inhibited motility of the human jejunum. In humans CCK-PZ usually inhibits motility of the stomach but enhances motility in the quiet stomach (Cameron, et. al., 1967). Bruce and Huber (1973) demonstrated the inhibitory action of secretin on rumen contractions in sheep and also suggested that CCK-PZ may have a similar action although no direct evidence was obtained. Debras, et. al. (1975) have also suggested that inhibition of gastric emptying by CCK-PZ is a physiological action of the hormone.

As mentioned previously, CCK-PZ was first implicated by Ivy and Oldberg (1928) in causing gall bladder contraction, and hence the name cholecystokinin (CCK). The contractile response to CCK-PZ, caerulein, or the C-terminal octapeptide of CCK has been observed in most animals studied including man (Ivy, 1934; Vaysse, et. al., 1974). In the ruminant however the gall bladder was considered to be non contractile (Magee,et.al.,1965). Harrison and Hill (1962) demonstrated that the rate of bile entry into the duodenum increased when frequency of feeding was increased in sheep.

Caple and Heath (1971) noted an increased frequency in gall bladder contractions following feeding and that, in sheep, the contraction response could be produced by administration of CCK-PZ. Evidence for the presence of CCK-PZ in ovine intestinal mucosa is lacking, however muscosal extracts from sheep do stimulate contraction of the canine gall bladder (Kloster, et. al., 1929). Caple and Heath (1971) concluded that chyme entering the duodenum of sheep may stimulate the release of CCK-PZ which has a physiological role in ovine gall bladder emptying.

Next to gall bladder contraction the other major physiological role of CCK-PZ is the strong stimulation of pancreatic enzymes output, hence the name pancreozymin (Harper and Raper, 1943). Robberecht, et. al. (1975) and Vayssee, et. al. (1974) noted an increase in enzyme secretion following CCK given I.V. to humans. Similar evidence using protein as an indication of total enzyme output has been shown for the dog, (Meyer, et. al., 1971; Laval, et. al., 1977), man (Debas and Grossman, 1973), monkey (Gardiner and Small, 1976), rat (Shaw and Heath, 1972) and sheep (Horn and Huber, 1975). Horn and Huber (1975) infused CCK-PZ into the jugular vein of sheep at a rate of 6.88 units/kg./hr. for four 7 minute periods. They observed a significant increase in pancreatic protein output during the initial first two periods following infusions of CCK followed by a drop in protein output, but post infusion levels remained above pre infusion protein levels.

CCK-PZ increased parallel enzyme output in studies with humans (Goebell, et. al., 1973), and also in rats (Ihse, et. al., 1976). However Vandermeers-Piret, et. al. (1974), demonstrated a parallel increase of chymotrypsin and trypsin to CCK injection in humans, followed by

delayed increases in lipase and amylase. Differential enzyme secretion in response to CCK-PZ has been suggested, as has the concept of more than one intracellular pool of enzymes which may respond differently to the same stimulus (Robberecht, et. al., 1977).

Singh, et. al. (1975) examined the effect of CCK-PZ on isolated rat pancreas slices, noting an increased release of zymogen granules but no increased zymogen granule production. According to Albano et. al. (1976) enzyme release in response to CCK-PZ was associated with stimulation of intracellular increases of cGMP, acting as a second messenger. Goodhead, et. al. (1970) have shown an increase in pancreatic blood flow and perfusion rate following intravenous CCK-PZ in dogs, which suggests another factor possibly contributing to CCK-PZ's stimulating action on pancreatic secretory protein production.

Information regarding the action of CCK-PZ on secretion of water and electrolytes into pancreatic juice or bile is sparse (Caple and Heath, 1972). CCK-PZ appears to weakly stimulate water and bicarbonate secretion into both bile and pancreatic juice of dogs (Stening and Grossman, 1969; Meyer et. al., 1971) and of monkeys (Gardiner and Small, 1976), and of rabbits (Esteller, et. al., 1977). A number of studies also indicate that CCK's stimulatory action on protein secretion by the pancreas is paralleled by an increase in pancreatic calcium output in humans (Goebell, et. al., 1973) and dogs (Goebell et. al., 1972). Goebell, et. al. (1972, 1973) also noted the absence of a stimulatory action by CCK-PZ on other pancreatic electrolytes. Petersen and Veda (1976) also demonstrated a lack of stimulatory action by CCK-PZ on pancreatic output by rat

pancreas perfused with a calcium deficient perfusate. Secretin was also without effect when a bicarbonate deficient perfusate was used. There is no comparable information on the requirement for calcium in the sheep pancreas stimulated with CCK or whether calcium secretion parallels protein secretion.

The major role of CCK-PZ in stimulating fluid production by the pancreas, is in potentiating actions of secretin. Evidence for such potentiation as shown for man (Wormsley, 1969), cats (Brown, et. al., 1967), and dogs (Henriksen and Worning, 1967; Meyer, et. al., 1971; Douglas and Duthie , 1971). Evidence for CCK-PZ's potentiation on hepatic production of bile stimulated by secretin is lacking in large part due to CCK-PZ's strong action on gall bladder contraction.

Stening and Grossman (1969) demonstrated a stimulation of Brunner's glands by CCK-PZ in the dog. Information regarding this action in other species awaits further study.

Release of CCK-PZ

As mentioned previously, CCK-PZ is produced and secreted by the "I" cell of the upper and mid small intestine. Stimuli for the release of CCK-PZ are amino acids, fatty acids and hydrogen ions. Work by Go, et. al. (1970) demonstrated that phenylalanine, methionine and valine released CCK-PZ in man, while in the dog phenylalanine, and tryptophan were more potent releases of CCK-PZ than were valine and leucine. Johnson (1974) notes that protein itself does not cause release but required degradation to amino acids. Meyer et. al. (1970) also demonstrated L-phenylalanine as an active stimulant for CCK release while the D isomer was ineffective.

The amount of CCK-PZ released appears to be a function of the area of mucosa exposed to amino acids rather than to concentration (Johnson, 1974), much like secretin's release in response to acid.

Fatty acids of chain length longer than C_8 appear to cause the release of CCK-PZ in dogs. In sheep Magee (1961) demonstrated that fatty acid infusions intraduodenally increased pancreatic amylase output, however this increase may have been due to secretin release which also appeared to increase amylase output though not to the extent of fatty acid infusions. Magee (1961) does not mention the strong possibility of CCK-PZ stimulation on amylase output. Johnson et. al. (1974) infused coconut oil and safflower oil intraduodenally into sheep and observed a decrease in total bile-pancreatic volume, total protein and lipase activity, which suggests no release of CCK-PZ or perhaps even inhibition. The difficulty in these interpretations lies in the absence of good quantitative data for CCK-PZ release using RIA methods rather than bioassay techniques.

Acid may also be of importance for CCK-PZ release, however, Moreland and Johnson (1971) suggested that high rates of hydrogen ion entry into the duodenum are required. Hong et. al. (1967) showed that, in dogs and pigs, acid stimulated pancreatic protein secretion which was suggestive of CCK-PZ release. Caple and Heath (1974) also suggested that the passage of chyme into the duodenum of sheep may stimulate release of CCK-PZ, however the possible stimulating factor(s) in the chyme were not speculated on. Horn and Huber (1975) however suggested that duodenal acidification does release secretin and also CCK-PZ although to a lesser degree. Unfortunately the evidence available is based entirely on bioassays which depend on specific actions such as CCK-PZ being the only hormone to stim-

ulate pancreatic protein output, but as previously reported, potentiation interactions also occur.

Johnson (1974) noted that release of CCK-PZ may also occur indirectly via a neuro hormonal reflex, which has the stimulatory agents acting on specialized receptor sites which may have neural connections with the CCK-PZ releasing cell. However vagal stimulation does not release CCK-PZ but vagotomy, atropine and local anaesthetics reduce CCK-PZ release suggesting a modulating or permissive role by cholinergic input (Johnson, 1974).

Other Hormones

Pancreatic glucagon was found in the canine mucosa (Sasaki, et. al., 1975) and shown by Bloom (1975) to inhibit gastric acid secretion, gastric motility and pancreatic enzyme output, but to stimulate bile flow and secretion of Brunner's glands.

Enteroglucagon was discovered in the mucosa of the ileum and colon of man (Bloom, et. al., 1975) and was found to be released in response to glucose and long chain fatty acids. Enteroglucagon appears to function mainly in reducing intestinal motility following rapid exposure of the intestine to glucose or fatty acids. Functionally it may play a physiological role to ensure adequate digestion and absorption of digesta entering the small intestine.

Motilin a 22 amino acid polypeptide (Brown, et. al., 1973) was isolated and purified from porcine duodenum Brown, et. al., 1971). Bloom, et. al. (1975) have shown motilin to be concentrated in the duodenal and jejunal mucosa. The major actions of motilin appear to be strong gastric contractions (Brown, et. al., 1976), and contractile stimulation of duodenum and jejunum (Strunz, et. al., 1975). Release of motilin is probably due to alkalinization of the duodenum, at least in the dog (Brown, et. al., 1976).

Gastric inhibitory polypeptide (GIP) which was isolated and sequenced (Brown, et. al., 1971), was shown to be in highest concentrations in the jejunum (Bloom, Bryant and Polak, 1975). The major known action of GIP demonstrated was as a powerful inhibitor of gastric acid secretion (Pederson & Brown, 1972). GIP was released by fats and sugars (Cleator and Courlay, 1975), and was therefore thought to be "enterogastrone"

since it was a powerful inhibitor of gastric acid secretion. However the enterogastrone activity has been demonstrated for secretin and CCK-PZ.

Vasoactive intestinal peptide (VIP) appears to be distributed throughout the gastrointestinal mucosa (Said and Mutt, 1970). Bloom, et. al. (1975) demonstrated VIP concentrations to greatly exceed those of secretin. VIP inhibits gastric acid secretion (Barbezat and Grossman, 1971) and was shown to stimulate pancreatic fluid and bicarbonate secretion (Said and Mutt, 1972). Release of VIP was shown to occur after a meal, however it was not clear which factors stimulate its release (Bloom, et. al., 1975).

Growth hormone release-inhibitory hormone (GHRIH) or somatostatin was found in rat to slow gastric acid and pepsin secretion(Gomez-Pan, et. al., 1975), to slow gastric emptying (Bloom et. al., 1975b), and to inhibit CCK-PZ stimulated gall bladder contraction and enzyme output by the pancreas (Bloom, Joffe, Polak, 1975).

The pancreatic hormones, avian pancreatic polypeptide and bovine pancreatic polypeptide (BPP) appear to have such varied effects as inhibition of gall bladder contraction, reduced gastrointestinal motility and decreased pancreatic enzyme secretion (Lin,et. al.,1973).

The complexity of hormonal regulation of liver, pancreatic and gastrointestinal functions must be considered in light of these "newly" discovered hormones.

Neural Control of Liver and Pancreatic Secretion

Neural control of hepatic and pancreatic secretion is an area of controversy and contradictory findings. The cephalic phase is thought to be largely mediated via the vagus nerve. Stimulation of the vagus nerve was shown by Hickson (1970), to stimulate water, bicarbonate and enzyme secretion by the porcine pancreas, and by Taylor (1962) for ovine pancreas and liver. Atropine blocked the enzyme secretion, but had no effect on water and bicarbonate production. Preshaw et. al. (1966) demonstrated that sham feeding in dogs resulted in increased pancreatic flow rate and protein output. Sarles, et. al. (1968) showed similar results for humans exposed to the sight and smell of an appetizing meal.

Gerolami and Sarles (1977) noted that vagal stimulation generally decreased bile flow, caused gall bladder relaxation and either increased or decreased sphincter of Oddi pressure in the dog. They also found that vagal influence was predominantly on increased muscle activity of the hepatic ductal system. Gerolami and Sarles (1977) also reviewed sympathetic actions on bile production and ductal motility. The difficulty of interpretation lies in variable responses to stimulation between species and within species, however the most probable sympathetic action is to decrease ductal and sphincter muscle tone. These actions may however be secondary to changes in blood flow to the ductal system.

The gastric phase of neural control of pancreatic exocrine function was found to involve an increase in pancreatic enzyme output in dogs (Passaro, et. al., 1963) and man (White, et. al., 1962). However

it should be noted that vagal stimulation also increases gastrin release which may contribute to the pancreatic enzyme output increases observed.

Gastric distension which may increase vagal activity possibly acts on the biliary system by a reflex which alters ductal muscle activity.

The intestinal phase of control centers largely about vagal influence on secretin or CCK-PZ actions. Vagotomy may increase (Moreland, et. al., 1971) or decrease (Magee, et. al., 1965) pancreatic secretion in response to secretin. Cholinergic control has also been implicated in the release of secretin and CCK-PZ. Increased cholinergic activity may increase release of these hormones, therefore the intestinal phase of neural control is largely secondary via the hormonal reflexes (Thomas, 1964).

The neural control of hepatic or pancreatic secretion in ruminants is poorly understood, however the possible importance of neural reflexes should not be minimized.

Pancreatic Enzymes

Enzymes produced by the pancreas of the cow have been examined by Keller (1968), who noted the presence of trypsin, chymotrypsin A, B and C, elastase, carboxypeptidase A and B, amylase, RNAase, DNAase, and lipase. Armstrong and Hutton (1974) noted that all proteases are secreted as inactive zymogens which are activated by trypsin. Trypsin secreted as the inactive zymogen, trypsinogen, is activated by enterokinase of the duodenal mucosa (Hermon-Taylor, et. al., 1977).

The activity and composition of pancreatic juice enzymes such as

phospholipase (Leat and Harrison, 1974), amylase (Magee, 1961), and lipase (Johnson, et al., 1974) have been studied in sheep. Information on total protein output by the sheep pancreas has also been studied (Horn and Huber, 1975) however information is lacking concerning concentration of the individual enzymes following stimulation of pancreas with secretin, CCK-PZ or gastrin. Difficulties in standardization of enzyme activities have been encountered due to the various units of activity expressed depending on the particular substrate utilized for a particular assay. This poses problems of comparison, of enzyme activity units, between the various studies.

At present, information is lacking on the enzyme composition of pancreatic juice in ruminants following feeding or hormonal stimulation. Individual enzymes which have been studied, have not been studied simultaneously in the same samples following stimulation of the exocrine pancreas.

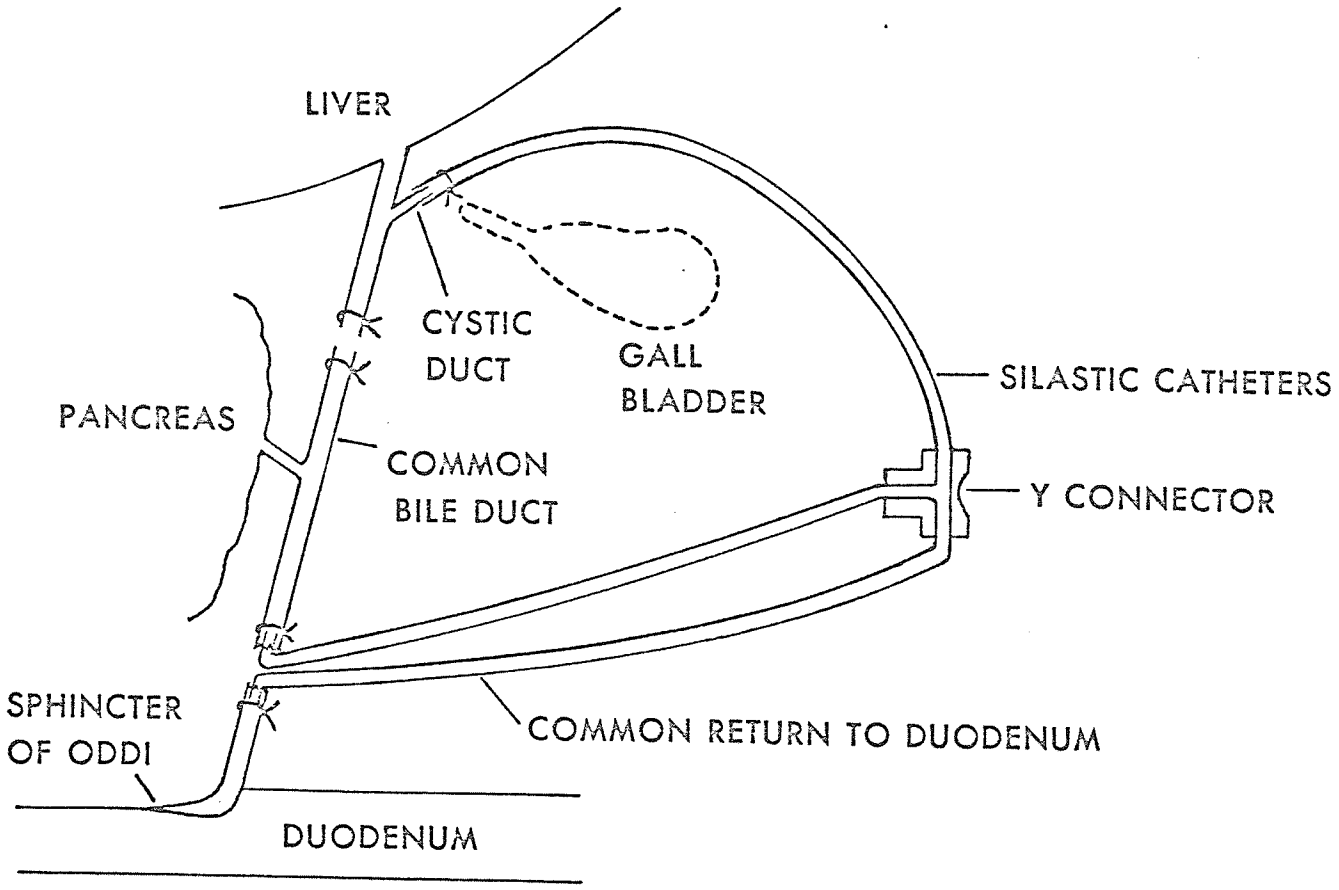
MATERIAL AND METHODS

CATHETERIZATION TECHNIQUE

The general surgical procedure as outlined below was performed on ram lambs weighing from 30 to 38 kg. Post surgical mortality rate was high (approx. 67%), which resulted in only three animals used for experiments involving bile and pancreatic juice collection. The critical period was usually the first two weeks post surgery, during which time obstruction of bile or pancreatic flow were the major problems. The surgical protocol is outlined as follows.

1) Wool shaved off right lateral side and area washed and disinfected with Bridine.
2) Approx. 4 inch incision made over last rib; rib removed for better access to the gall bladder.
3) Gall bladder was opened; contents aspirated; cystic duct catheterized; cholecystectomy performed.
4) Common bile duct distal to pancreatic duct junction was catheterized with two catheters, one for sampling, the other to return fluid through the sphincter of Oddi into the duodenum. (fig. C).
5) Ligated common bile duct proximal to pancreatic bile duct junction which diverted all bile flow through the cystic duct catheter.
6) All 3 catheters were exteriorized through stab incisions on right lateral side just caudal to the primary incision.
7) The 3 catheters were joined by means of a Y shaped polypropylene connector.

Figure C Catheterization Technique



This method allowed for separate collection of hepatic bile and pancreatic juice and between experiments with return of the secretions via one catheter into the common bile duct proximal to the sphincter of Oddi. A minimum three weeks recovery period was used before sampling experiments were done. Anesthesia for surgery was induced with sodium pentobarbital (Nembutal) I.V. and maintained with Halothane administered by endotracheal tube and a closed circuit anesthesia machine. The catheter material was silastic tubing (3 mm O.D. x 1.5 mm. I.D.) approximately 15 cm. long with collars 2 mm. from one end. Ligatures around the duct behind the collar of the catheter prevented slippage of the catheters out of the ducts.

EXPERIMENTS Ia, Ib, Ic, Id, Ie

Animals

Six ewe lambs (Finnish Landrace) weighing between 35 and 48 kg were used for experiments Ia, Ib, Ic, and 4 of these were used in experiments Id and Ie. One week between experiments was alloted. The animals were housed in separate 3½' x 7' plexiglass pens with expanded metal flooring, water buckets and feed boxes. All animals were fed alfalfa pellets (16% protein) according to each experimental protocol.

FEEDING SCHEDULE

Experiment Ia

Sheep were given 1200 g. per 24 hours of alfalfa pellets using a continuous belt feeder. Water was given ad. libitum.

Experiment Ib, Id, Ie

Sheep were given 1200 g. of alfalfa pellets once a day at 1:45 p.m.

and ad libitum water; for one week prior to blood sampling.

Experiment Ic

Sheep were given 1200 g. of alfalfa pellets at 11:45 A.M. after 48 hr. feed deprivation. All feed was consumed within ½ hour following feeding. Water was given throughout the experiment ad libitum.

BLOOD SAMPLING

Blood samples were taken from either jugular vein via 10 cc. non heparinized vacutainer tubes with a number 21G needle. Each sample was allowed to clot at room temperature for 15 minutes, centrifuged at 2000 rpm. for 10 minutes at 4°C, serum pipetted into clean 3 ml. test tubes, capped and frozen at -20°C within one hour of sampling.

BLOOD SAMPLING SCHEDULE

Experiment Ia

A blood sample was taken from each animal at 10:00 A.M., 11 A.M., 12:00, 1 P.M., 2 P.M. and 3 P.M.

Experiment Ib

Blood samples were taken at 12:00 (noon), 1, 2, 2:30, 3, 3:30, 4, 5, 6, and 7 P.M. Feed was given at 1:45 P.M.

Experiment Ic

Blood samples were taken at 10 A.M., 11 A.M., 12:00 (noon), 12:30, 1, 1:30, 2, 3, 4 and 5 P.M. Feed was given at 11:45 A.M.

Experiments Id and Ie

Blood samples were taken at 1:30, 2, 2:30, 2:45, 3 and 3:30 P.M. Feed was given at 1:45 P.M. and either secretin or CCK-PZ was injected at 2:15 P.M.

CCK-PZ and SECRETIN INJECTIONS FOR EXPERIMENTS Id and Ie

Secretin was injected as 75 C.U. in 5 ml. of physiological saline solution into the jugular vein at 2:15 P.M. for experiment Id. The secretin (75 C.U./vial from GIH laboratory, Sweden) was dissolved in the saline solution 10 minutes prior to injection to minimize degradation time. CCK-PZ (75 C.U./vial from GIH laboratory, Sweden) was used for experiment Ie in the same way as secretin in experiment Id.

GASTRIN ANALYSIS

Immunoreactive serum gastrin (G17 and G34 both sulfated and non sulfated) levels were determined for each serum sample by the RIA method utilizing the Schwarz/Mann gastrin radioimmunoassay kit ^{125}I (Schwarz/Mann, Orangeburg, N.Y. 10962). Details of the method are included with each kit. All assays were completed within 10 days of sampling and all carried out in polypropylene test tubes (Fisher Scientific Co,). The radioactivity was counted in an automatic γ counter system (Searle Analytic Inc., Model 1185).

STATISTICAL ANALYSIS

Analysis of variance was performed on the values in experiments Ia, Ib and Ic to compare period and treatment differences.

EXPERIMENTS 2, 3, 4, 5, 6

Animals

Three ram lambs weighing 30 to 33 kg. were used for experiment 2 and two of these sheep were used in experiments 3,4,5 and 6. Animals were prepared by the catheterization technique previously described at least 3 weeks before experiments were begun.

EXPERIMENTAL DESIGN

Experiments 2a, 2b, 2c

The feeding schedule was 600, 1300 and 1700 g. of alfalfa pellets given by continuous feeder each 24 hr. for experiments 2a, 2b and 2c respectively. Bile and pancreatic juice samples were collected in nine, five minute periods for a total of 45 consecutive minutes. Each 45 minutes sampling period was chosen randomly to fall between 10 A.M. and 6 P.M. Three 45 minute samplings over 3 separate days were taken for each sheep on each level of feed. Bile and pancreatic juice were collected separately into 10 ml. glass vials which were frozen at $-20^{\circ}C$ for analysis at a later date. During the collection period neither bile or pancreatic juice was returned to the duodenum.

ANALYSIS OF BILE AND PANCREATIC JUICE

VOLUME

Volumes were recorded for each five minute sampling period for both bile and pancreatic juice and expressed as ml./min.

SODIUM AND POTASSIUM

Sodium and potassium analysis were performed using a flame photometer (Techicon Corp.) in conjunction with an Auto Analyzer II (Technicon Corp.). All results are expressed as mEq. per liter for both bile and pancreatic juice.

CALCIUM AND PHOSPHATE

Calcium and phosphate concentrations (mg%) were determined using an AutoAnalyzer II (Technician Corp.) for both bile and pancreatic juice.

CHLORIDE

Chloride determinations were performed using a diazo colormatic method on a Chloride Analyzer (I. L. 279) for bile samples only. Due to low volumes of pancreatic juice collected per sample chloride analysis was not possible.

STATISTICAL ANALYSIS

Means, standard errors and analysis of variance were calculated for each parameter recorded on an IBM 370/158 computer using a statistical package for social sciences (SPSS). Tests of significance with differences $p < 0.05$ were accepted as significant.

EXPERIMENTAL DESIGN EXPERIMENT 3

Two sheep were starved for 3 days over which period 9 random 5 minute samples of bile and pancreatic juice were taken from each animal. (ie: 3 random samples per sheep each day).

Bile samples were analyzed for sodium, potassium calcium, phosphate and volume as in experiment 2. Pancreatic juice samples were analyzed for electrolytes as in bile. All samples of bile and pancreatic juice were stored at $-20^{\circ}C$ until analysis. Enzyme analysis of pancreatic juice were performed within 10 days of sampling and storage at $-20^{\circ}C$. Pancreatic juice samples were frozen within 1 hr. of sampling.

TRYPSIN DETERMINATION

The method used for trypsin determination was based on the rate of substrate degradation by the enzyme. The substrate which was used tyrosyl-arginine-methyl ester (TAME) (Sigma Chemical Co.) absorbs U.V. light at 247 mm maximally. As substrate is degraded the absorbance of

light by the spectrophotometric cell increases at a rate dependant on the enzyme concentration in the sample.

CHYMOTRYPSIN DETERMINATION

The method for chymotrypsin was a spectrophotometric one as for trypsin. Chymotrypsin analysis used butyl tyrosine ethyl ester (BTEE) (Sigma Chemical Co.) as substrate and a U.V. wavelength of 256 mm.

AMYLASE METHOD

Amylase was measured by the visual colorimetric method developed by Somogyi(1960). The method is based on the length of time required for an unknown amount of amylase to degrade a fixed quantity of starch to oligasaccharides, maltose and glucose. The rate of reaction is directly proportional to the concentration of the enzyme. Full details are provided with the amylase kit (Sigma Chemical Co., Tech. Bulletin No. 700).

LIPASE METHOD

Lipase concentration were determined using a lipase kit (Sigma Chemical Co., Tech. Bulletin No. 800). The method is based on an incubation of the sample with an olive oil substrate which is degraded by lipase to fatty acids and diglycerides. The amount of fatty acids were determined by titration with NaOH to indicate the lipase activity in the sample.

STATISTICAL ANALYSIS

Means and standard errors were determined for each electrolyte and enzyme analysis of the two sheep over the three day feed deprivation period.

EXPERIMENTAL DESIGN EXPERIMENT 4

Two sheep were fed 1200 g. of alfalfa pellets once daily at 11:45 A.M. for one week prior to sampling of bile and pancreatic juice. Collections were made at 10 A.M., 11 A.M., 12, 12:30 P.M., 1 P.M., 1:30 P.M., 2, 3, 4, 5 P.M. for 2 days. Each sample was taken for 5 minutes with return of bile and pancreatic juice to the duodenum between samples. Electrolyte and enzyme analysis were performed as in experiment 3. Statistical analysis was similar to experiment 3.

EXPERIMENTAL DESIGN EXPERIMENTS 5 AND 6.

Pancreatic and bile secretion samples were taken for 6 consecutive 5 minute periods in two sheep for each of two days during which time the animals were fasted. The samples for experiment 5 were taken following 2.5 C.U./kg of secretin (GIH laboratory, Sweden) injection in 5 ml. of sterile physiological saline into the jugular vien. Samples for experiment 6 were taken following an injection of 2.5 C.U./kg of CCK-PZ (GIH laboratory, Sweden). Analysis of electrolytes, enzymes and statistical analysis were performed as for experiment 3. The hormone solutions injected were prepared 10 minutes prior to injection at time 0.

STATISTICAL ANALYSIS

One way analysis of variance was performed on the data from experiments 2,3,4,5 and 6 to test for period differences within each experiment. Between experiment differences were made using analysis of variance and a multiple range test. The comparison between experiments was not the best method of comparison since unequal number of animals and observations were obtained. However the comparisons (figures 33,34 and 35) were taken to indicate treatment effects on the parameters measured.

RESULTS

EXPERIMENT Ia

The results (Table 4) indicate a range of means 29 ± 2 to 41 ± 2 pg./ml. of immunoreactive serum gastrin between sheep on continuous feed (1200 g. alfalfa pellets per 24 hr.). The data also indicate no significant differences between time periods samples with a range of 34 ± 3 to 41 ± 3 pg./ml. of gastrin, and a mean value of 35 ± 1 pg./ml.. Although each individual animal demonstrates a cyclic pattern for gastrin release the mean gastrin values plotted against time in figure 10 do not clearly demonstrate this since the cyclic pattern is not coordinated between animals. Generally it appears that the low points for each animal's gastrin cycle occurs every three hours.

EXPERIMENT Ib

The results for sheep on once a day feeding (1200 g. alfalfa pellets) indicate an increase in serum gastrin levels from a mean of 51 ± 6 pg./ml. just prior to feeding to a peak value of 69 ± 6 pg./ml. approximately one hour after feeding (table 5). The results graphed in figure 11 indicate this increase to be significant with a drop in serum gastrin values (to levels observed before feeding) about 3 hours after feeding. Due to large differences in gastrin concentrations between animals, standard errors tended to be quite large, however the increase observed after feeding was consistent for all animals (fig. 12). The results were therefore taken to be of physiological significance.

TABLE 4 Immunoreactive serum gastrin levels (pg./ml.) in six sheep on continuous feed (1200 g./24 hr.).

	ANIMAL							
TIME	1	2	3	4	5	6	$\overline{X}$	S.E.
10:00	30	40	25	44	40	40	37	3
11:00	49	54	26	36	34	41	40	4
12:00	38	31	33	36	49	23	35	4
1:00	38	25	36	45	31	28	34	3
2:00	42	31	22	43	34	33	34	3
3:00	43	34	29	40	51	46	41	3
$\overline{X}$	40	36	29	41	40	35	35	1
S.E.	3	4	2	2	3	4		

FIGURE 10 Means and standard errors for immunoreactive serum gastrin levels of 6 sheep on continuous feed (1200 g./ 24 hr.)

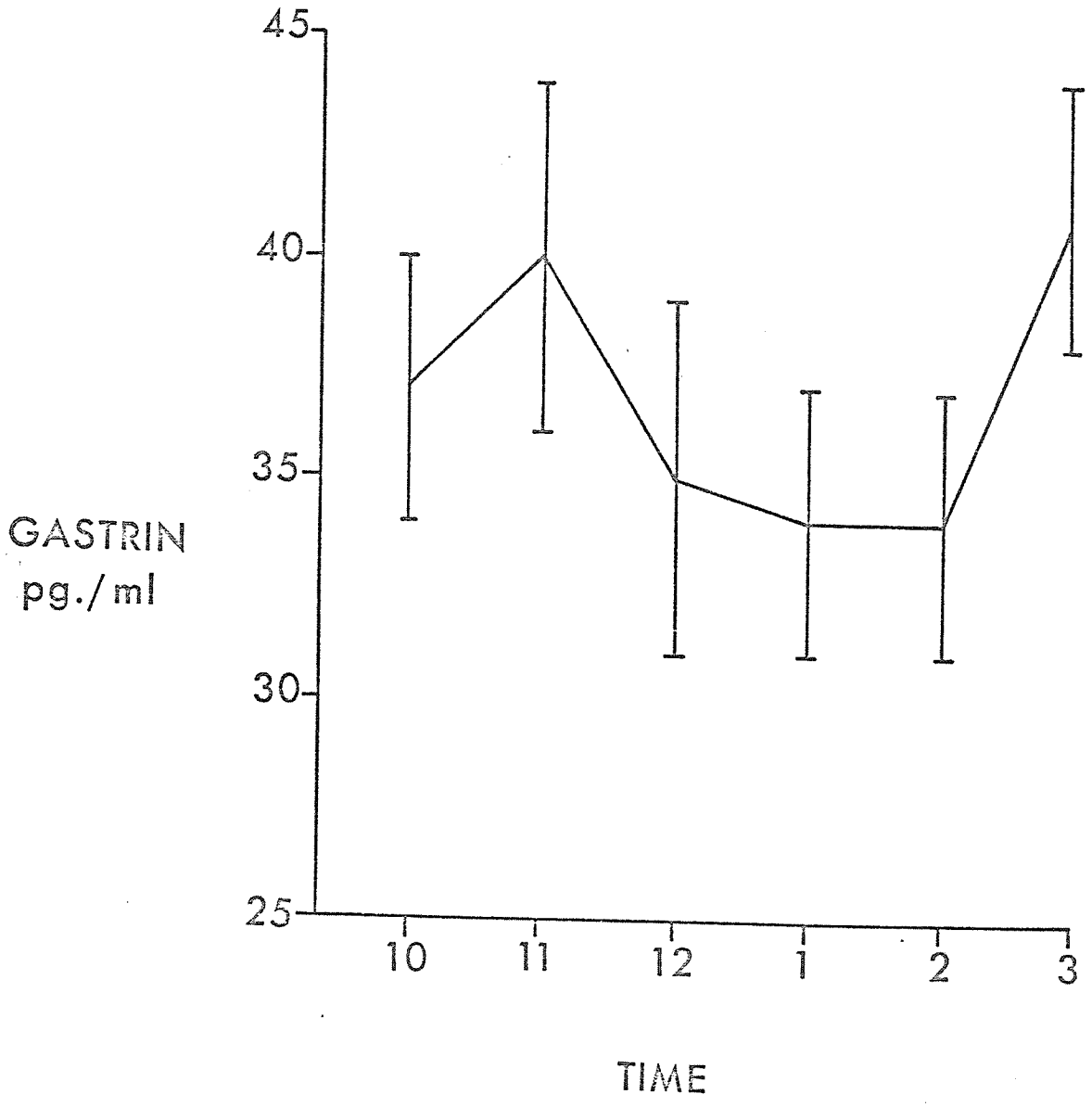


TABLE 5 Immunoreactive serum gastrin levels (pg./ml.) in six sheep fed 1200 g. alfalfa pellets once daily

		ANIMAL							
	TIME	1	2	3	4	5	6	$\bar{X}$	S.E.
	12:00	73	58	43	61	50	58	57	4
	1:00	73	49	43	58	30	55	51	6
Fed	1:45								
	2:00	80	50	43	73	30	64	57	8
	2:30	86	55	40	67	42	64	59	7
	3:00	73	90	55	70	52	76	69	6
	3:30	82	80	50	55	61	76	67	6
	4:00	76	50	43	58	55	80	60	6
	5:00	67	55	34	58	46	61	54	5
	6:00	61	45	32	55	38	52	47	4
	7:00	64	58	55	52	41	49	53	3
	$\bar{X}$	73	59	44	62	45	63	57	2
	S.E.	3	5	3	2	3	3		

FIGURE]] Means and standard errors for immunoreactive serum gastrin levels of 6 sheep fed]200 g. alfalfa pellets once daily.

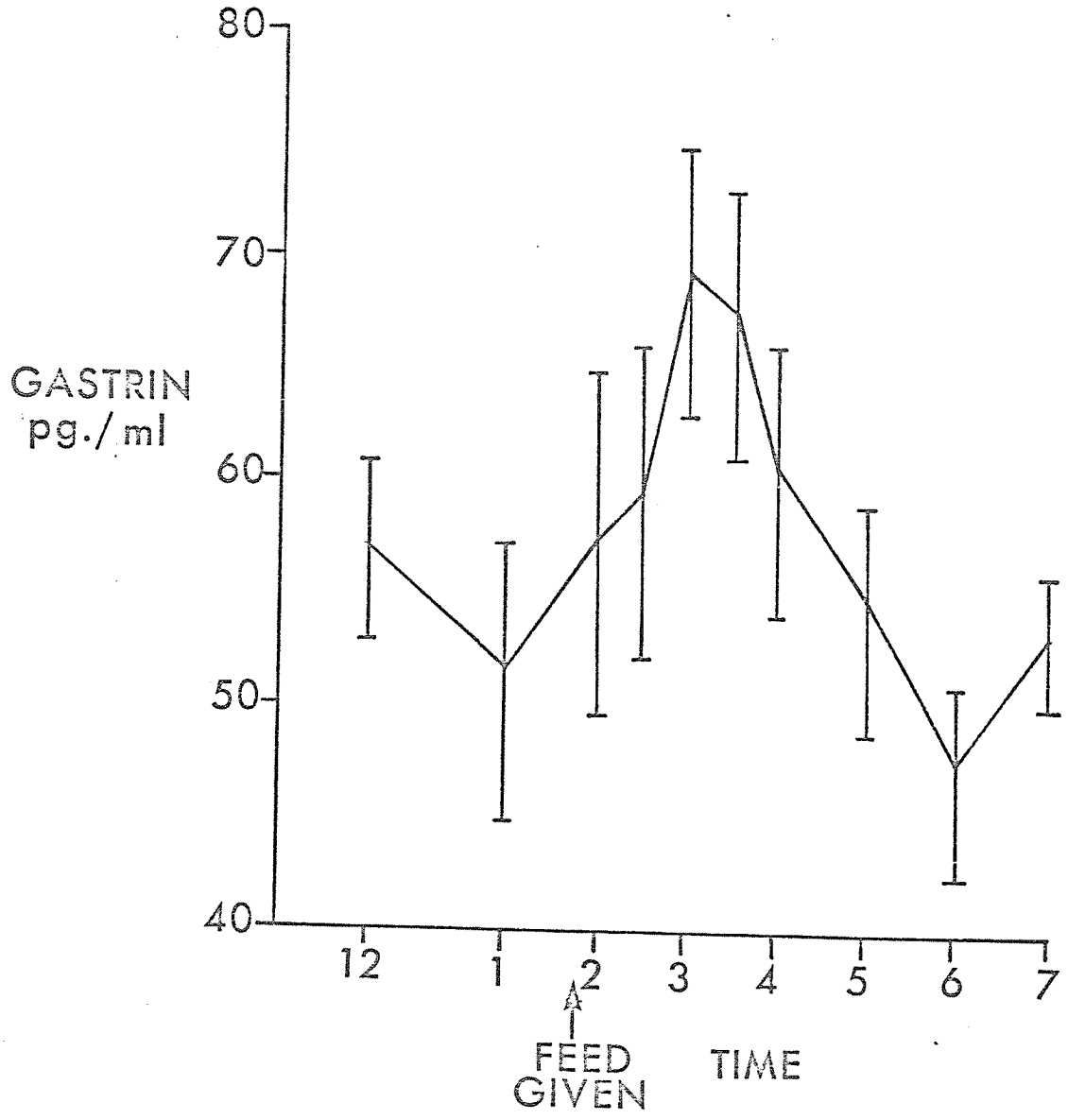
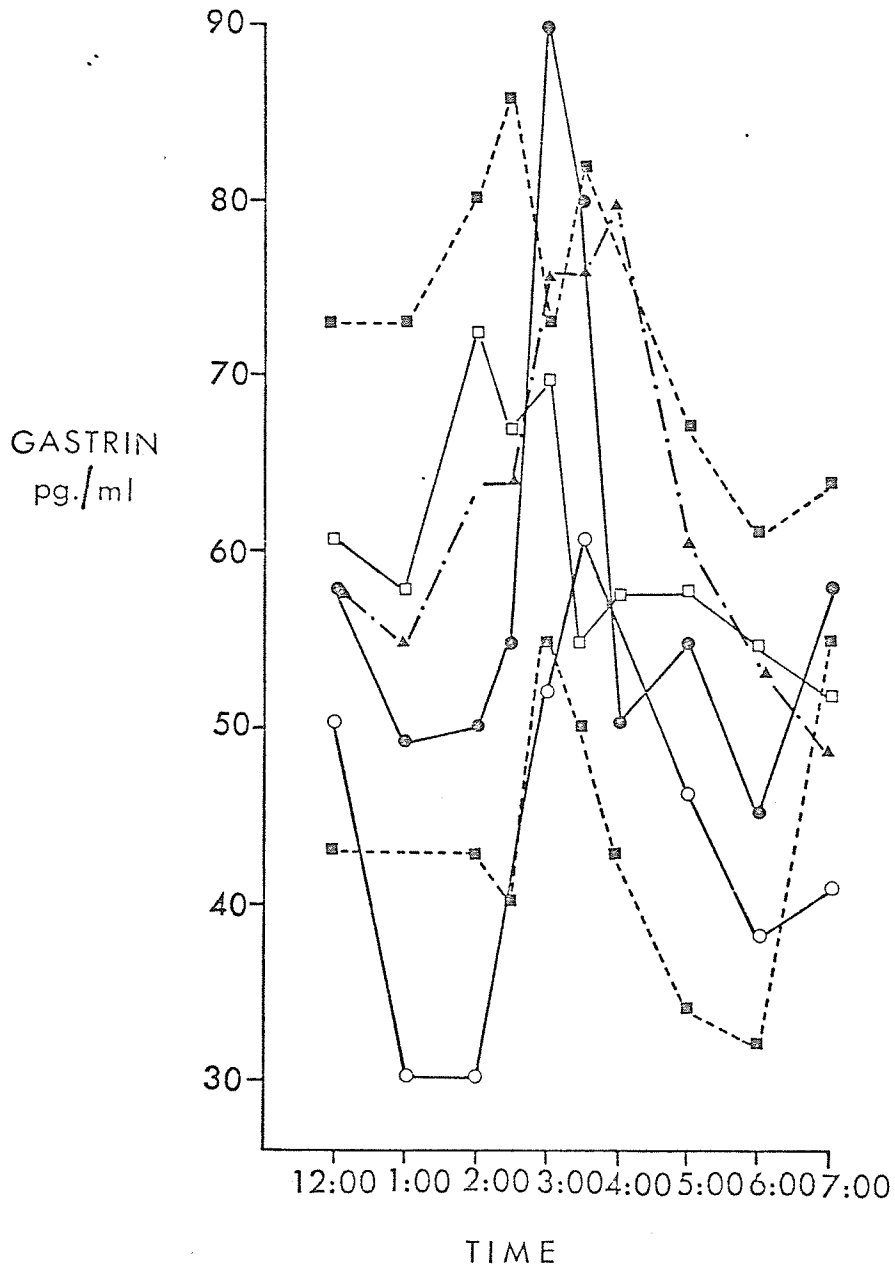


FIGURE 12 Immunoreactive serum gastrin levels of 6 sheep before and after feeding 1200 g. alfalfa pellets.

Sheep were fed at 1:45 P.M.



EXPERIMENT Ic

The results for 6 sheep fed 1200 g. alfalfa pellets following a 48 hr. starvation period indicate a significant increase in serum gastrin levels from a mean of 19.5 pg./ml. prior to feeding to a high mean value of 29 pg./ml., 45 minutes after feeding (table 6 and fig. 13). Unlike the previous experiment with once a day feeding in which serum gastrin levels returned to prefeeding values within 3 hours after feeding, these sheep showed elevated serum gastrin levels (25 pg./ml.) five hours after feeding compared to prefeeding values of 19.5 pg./ml. serum which were taken to be the basal level for gastrin concentration with the assumption that abomasal contents were minimal following 48 hours without feed.

SUMMARY OF RESULTS FOR EXPERIMENTS Ia, Ib and Ic

Results for the three experiments are illustrated in figure 14. Continuous feeding experiment Ia indicates a mean overall serum gastrin concentration of 35 ± 1 pg./ml. The dramatic drop in serum gastrin to values around 20 pg./ml. after 48 hr. food deprivation is seen for experiment Ic while once daily feeding resulted in gastrin levels between 50 and 60 pg./ml.. These results as illustrated in figure 14 are in agreement with the hypothesis that gastrin is released from the abomasum of sheep in proportion to stimulation by quantity of digesta provided the same feed is given in all cases (as was the case).

TABLE 6 Immunoreactive serum gastrin levels (pg./ml.) in 6 sheep before and after feeding following 48 hr. starvation

		ANIMAL							
	TIME	1	2	3	4	5	6	$\bar{X}$	S.E.
	10:00	24	27	17	23	23	5	20	3
	11:00	11	25	15	23	23	19	19	2
FED	11:45								
	12:00	23	17	18	24	36	27	24	3
	12:30	27	34	28	31	27	25	29	1
	1:00	31	26	26	27	29	27	28	1
	1:30	28	28	19	27	27	29	26	1
	2:00	15	27	19	29	23	30	24	2
	3:00	23	33	20	30	23	31	27	2
	4:00	24	24	27	21	21	31	25	2
	5:00	23	29	17	25	29	28	25	2
	$\bar{X}$	23	27	21	26	26	25	25	1
	S.E.	2	2	1	1	1	2		

FIGURE 13 Means and standard errors for immunoreactive serum gastrin levels of 6 sheep fed 1200 g. alfalfa pellets following a 48 hr. starvation period.

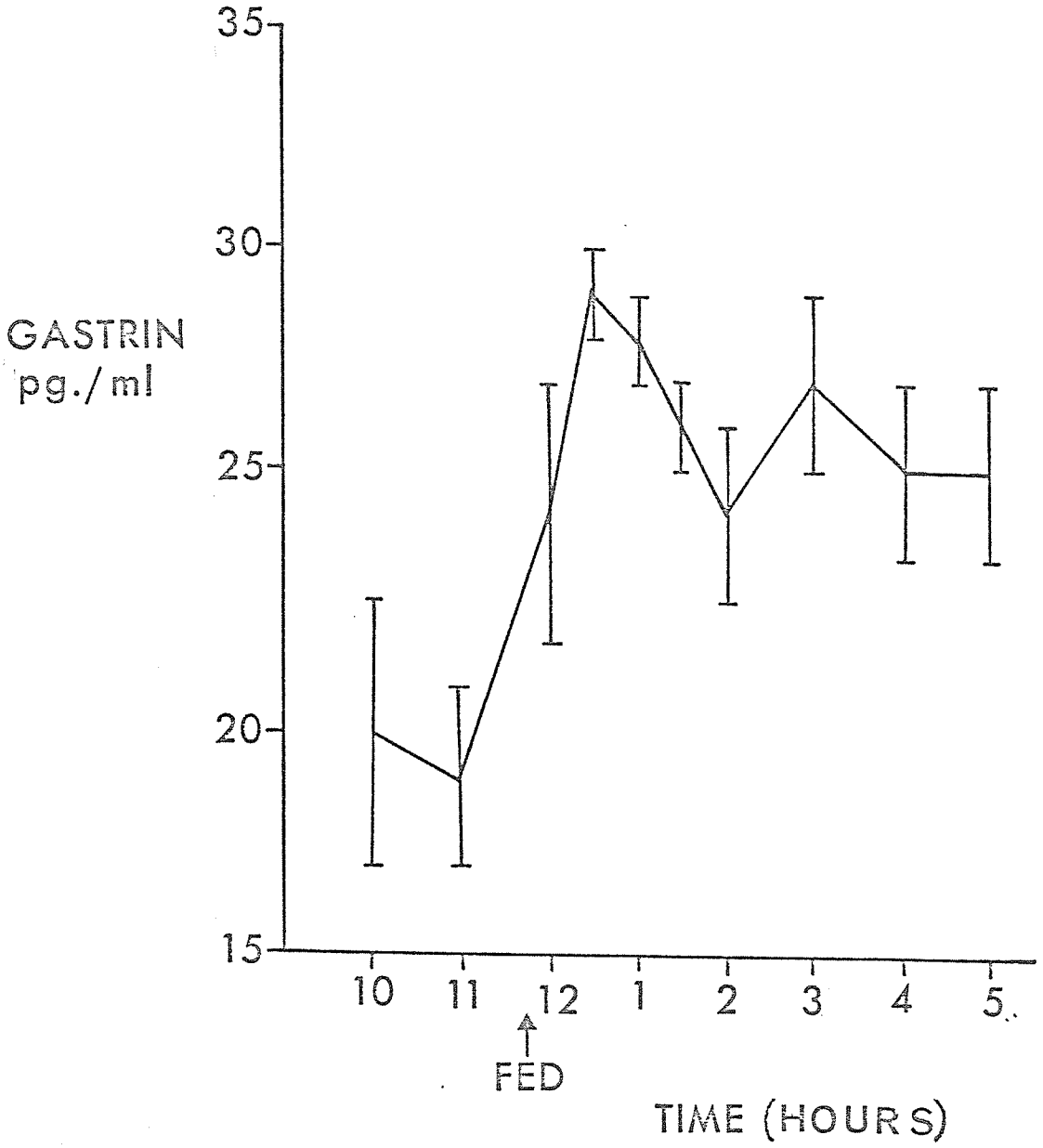


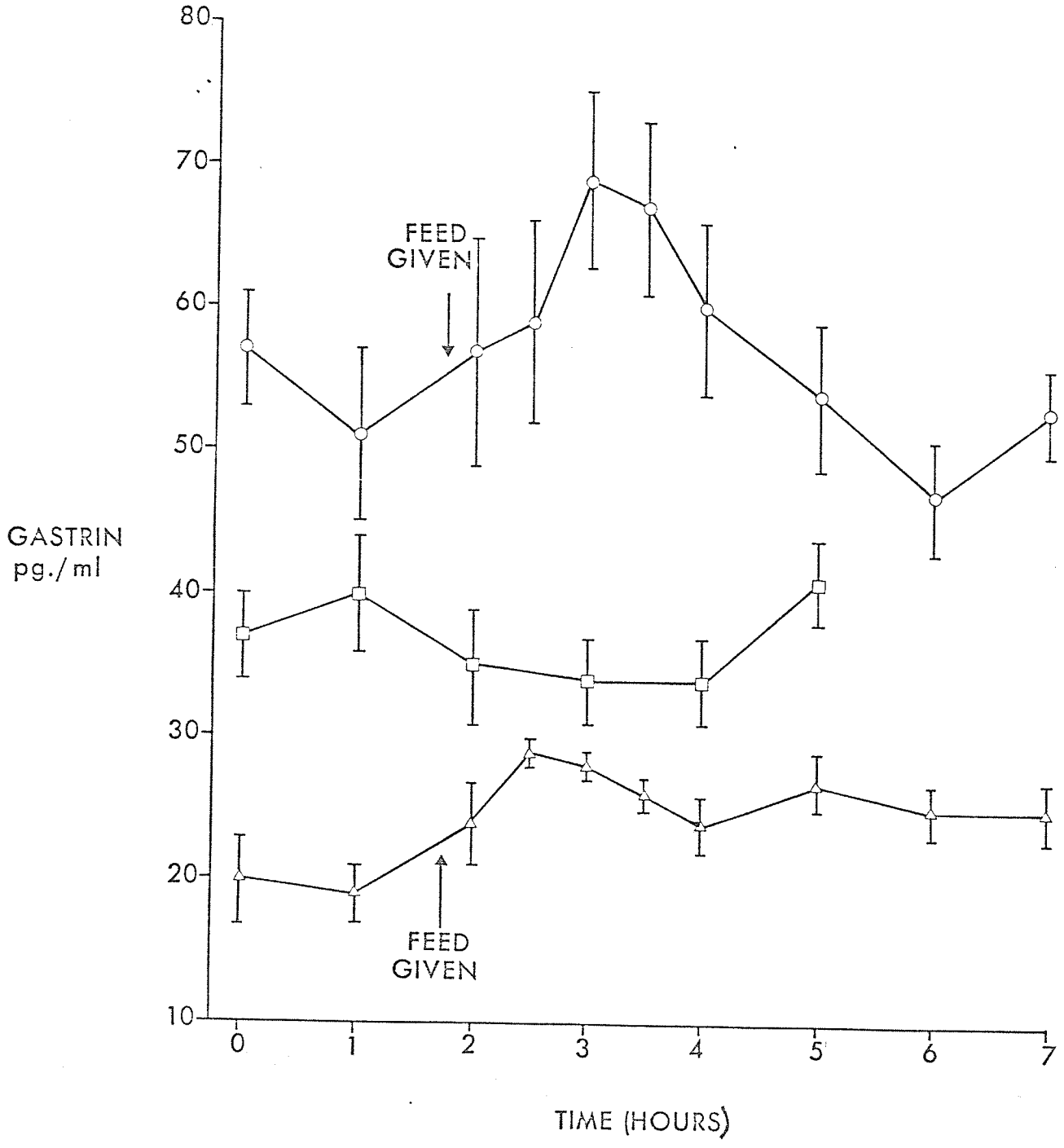
Figure 14 Means and standard errors for serum gastrin levels in sheep on three experiments Ia, Ib and Ic.

□ Experiment Ia continuous feeding

o Experiment Ib fed once daily

△ Experiment Ic fed after 48 hr. food deprivation

FIGURE 14


80
70
60
50
40
30
20
10
FEED GIVEN
FEED GIVEN
GASTRIN pg./ml
0
1
2
3
4
5
6
7
TIME (HOURS)

EXPERIMENT Id

Experiment Ib demonstrated a rise in serum gastrin to peak values within two hours following feeding. To test the effect of CCK-PZ on this rise of serum gastrin, 75 C.U. CCK-PZ was injected ½ hour after feeding. The mean values for gastrin concentrations were inhibited from increasing for ½ hour following CCK-PZ injection (table 7 and figure 15). The expected rise in serum gastrin following feeding was observed and it was resumed 3/4 hour after CCK-PZ injection. Whether this inhibition was a true effect is debatable considering that values of serum gastrin actually increased for animals 3 and 4 following CCK-PZ injection (table 7). The depressant effect of CCK-PZ on serum gastrin levels also assumes that gastrin levels would have increased over the 2 hr. period following feeding.

EXPERIMENT 1e

The rationale for experiment 1e as for 1d assumes that serum gastrin values increase significantly following feeding as demonstrated in experiment 1b. These results indicate that secretin (75 C.U.) injected ½ hour following feeding inhibited a rise in serum gastrin, when considering the means (table 8 and figure 16). However animals 1 and 2 showed no change in serum gastrin following secretin injection. This variability tended to increase the standard errors making interpretation difficult. Unlike the previous experiment (1d), mean gastrin levels did not increase significantly 3/4 hour after secretin injection as they did following CCK-PZ injection. This may indicate a prolonged suppression by secretin on gastrin release.

TABLE 7 Immunoreactive serum gastrin levels (pg./ml.) in 4 sheep on once a day feeding before and after 75 C.U. of CCK-PZ injection.

	ANIMAL					
TIME	1	2	3	4	$\bar{X}$	S.E.
1:30	41	45	23	36	36	5
fed 1:45						
2:00	53	49	33	45	45	4
inject CCK 2:15						
2:30	41	45	38	55	45	4
2:45	30	48	36	43	39	4
3:00	50	50	55	48	51	2
3:30	48	55	33	45	45	5
$\bar{X}$	44	49	36	45	44	2
S.E.	3	2	4	3		

FIGURE 15 Means and standard errors for immunoreactive serum gastrin levels following feeding of 1200 g. alfalfa pellets and then injected with 75 C.U. of CCK-PZ.

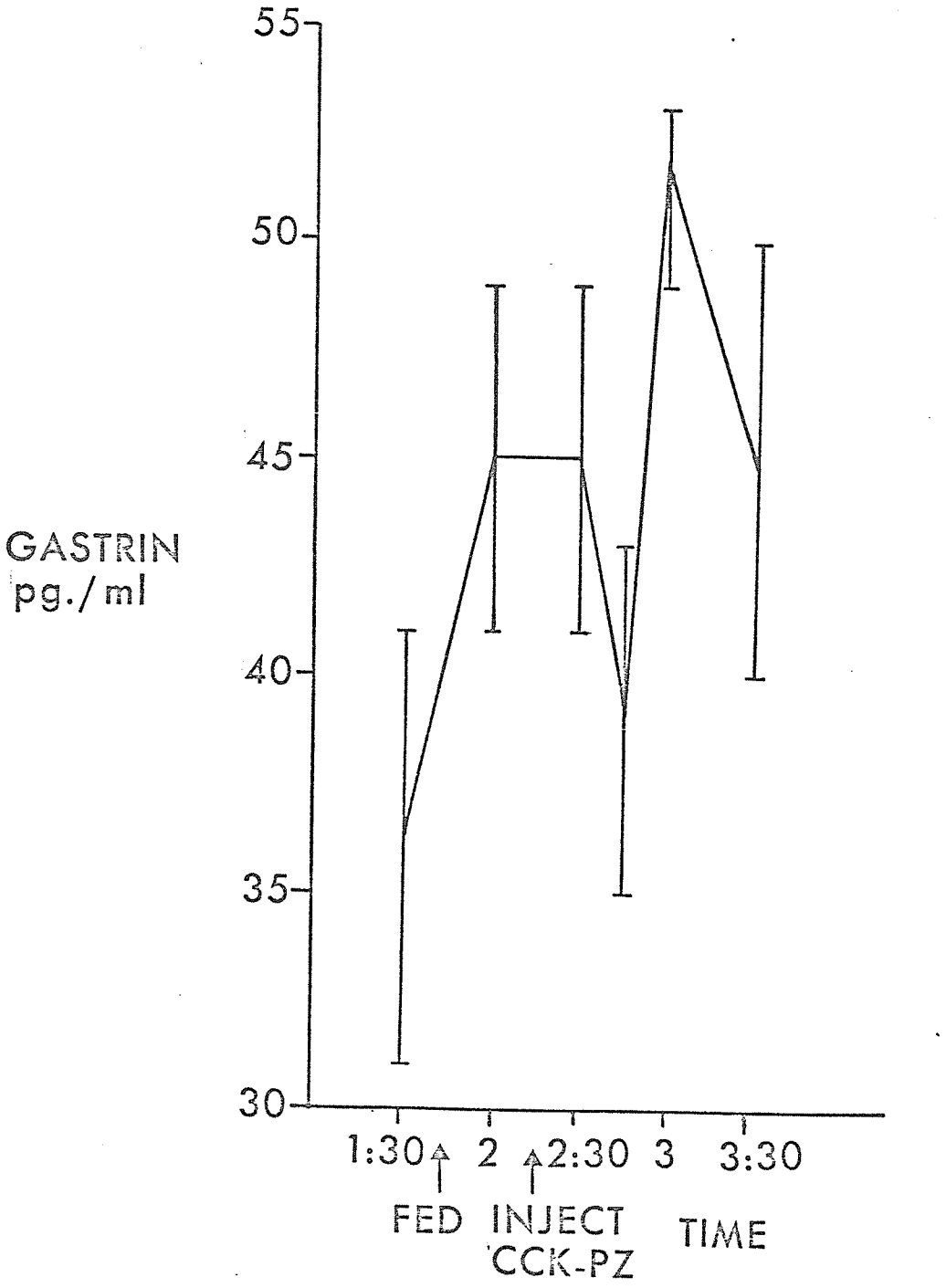
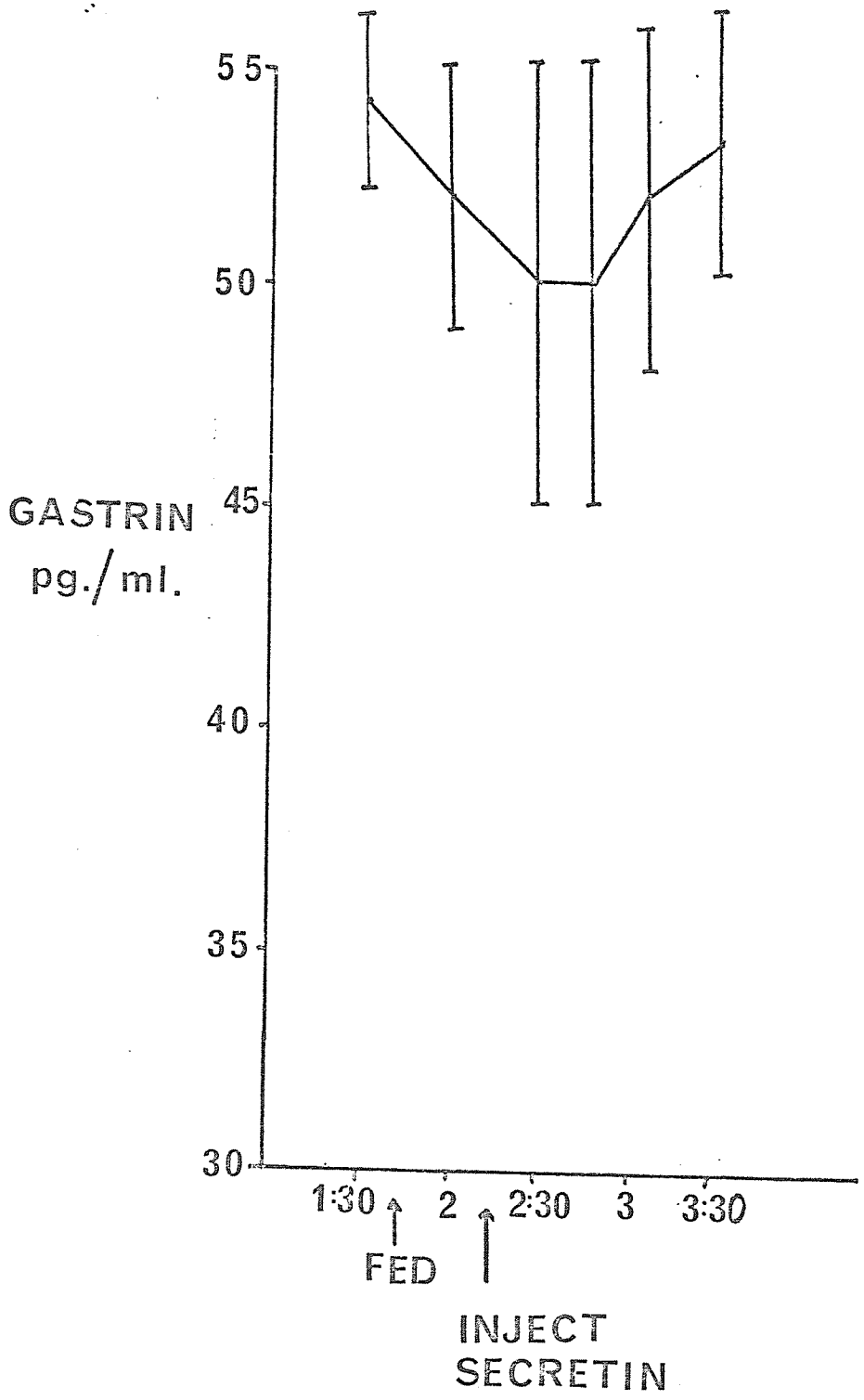


TABLE 8 Immunoreactive serum gastrin levels (pg./ml.) in 4 sheep on once a day feeding before and after intravenous injection of 75 C.U. of secretin.

		ANIMAL					
	TIME	1	2	3	4	$\bar{X}$	S.E.
	1:30	53	50	58	55	54	2
FED	1:45						
	2:00	45	53	53	58	52	3
SECRETIN INJECTION	2:15						
	2:30	53	63	45	38	50	5
	2:45	55	55	53	36	50	5
	3:00	58	61	45	45	52	4
	3:30	56	57	55	43	53	3
	$\bar{X}$	53	57	52	46	52	2
	S.E.	2	2	2	4		

FIGURE 16 Means and standard errors of immunoreactive serum gastrin levels in 4 sheep on once a day feeding before and after intravenous injection of 75 C.U. of secretin.



EXPERIMENTS 2a, 2b and 2c

The results for experiments 2a, 2b and 2c provide information on the changes of bile and pancreatic juice volume and electrolytes in response to three levels of continuous feeding. Means and standard errors for each parameter observed are presented in tables 9 to 14. A summary of treatment effects is provided in table 15 and graphically presented in figures 17 and 18.

VOLUME

A) Bile At 600 g. of feed given per 24 hours a mean bile flow of 0.5 ± .1 ml./min. was obtained, this value increased to 1.6 ± .1 ml./min. for 1300 g. feed/24 hrs. and the increase was statistically significant ($p \leq 0.05$). There was no further increase with 1700 g. feed/24 hrs. which resulted in mean value of 1.6 ± .2 ml./min.

B) Pancreatic fluid A significant increase of pancreatic juice flow was obtained from a mean of .16 ± .01 ml./min. at 600 g./24 hrs. to .22 ± .03 ml./min. at 1300 g./24 hrs. Unlike bile flow a further significant increase in pancreatic flow to .28 ± .03 ml./min. resulted when feed level was increased to 1700 g./24 hrs.

SODIUM

A) Bile The mean sodium concentration of 186 ± 3 mM./l. at 600 g./24 hrs. increased significantly to 196 ± 3 mM./l. at 1300 g./24 hrs. At 1700 g./24 hrs. further significant differences were not apparent, with a mean of 194 ± 3 mM./l.

B) Pancreatic juice A small but significant increase in sodium levels occurred from 151 ± 2 mM/l. to 154 ± 2 mM/l. for 600 and 1300 g./24 hrs. respectively. A further increase in feed to 1700 g./24 hrs.

resulted in a significant drop to 151 ± 2 mM/l. when compared to 1300 g./24 hrs. This mean value for 1700 g./24 hrs. was identical to that for 600 g./24 hrs.

POTASSIUM

A) Bile Values of 6.7 ± 0.1 mM./l. and 6.6 ± 0.1 mM./l. were obtained for bile potassium at 600 g.feed/24 hrs. and 1300 g. feed/24 hrs. respectively. When fed at 1700 g. feed/24 hrs. the potassium concentration increased significantly above the previous two values to 6.9 ± .1 mM./l.

B) Pancreatic juice Significant increases in potassium concentration occurred with each feed level increase from 4.9 ± .1 mM./l. at 600 g./24 hr. to 5.3 ± .2 mM./l. at 1300 g./24 hrs. to 5.5 mM./l. at 1700 g./24 hrs.

CALCIUM

A) Bile A significant inverse relationship between feed level and calcium concentration was observed. At 600 g./24 hrs., 2.6 ± .13 mM./l. calcium was observed which decreased to 2.3 ± .03 mM./l. at 1300 g./24 hrs, and remained at 2.3 ± .05 mM./l. at I700 g./ 24 hrs.

B) Pancreatic juice Mean calcium concentration of 2.7 ± .23 mM./l. at 600 g./24 hrs. decreased significantly to 2.0 ± .18 mM./l. at 1300 g./24 hrs. The mean value of 2.9 ± .03 mM./l. at 1700 g./24 hrs. was significantly higher than the value at 1300 g./24 hrs. but not significantly different from that at 600 g./24 hrs.

PHOSPHATE

A) Bile Bile phosphate concentrations decreased significantly with increased feeding levels from .26 ± .05 mM./l. at 600 g./24 hrs. to .14 ± .02 mM./l. at 1300 g./24 hrs. to .12 ± .01 mM./l. at 1700 g./24 hrs.

B) Pancreatic juice As for bile, significant decreases in phosphate concentration were observed from a mean high of .21 ± .02 mM./l. at 600 g./24 hrs. to .18 ± .02 mM./l. at 1300 g./24 hrs. to a mean low of .12 ± .04 mM./l. at 1700 g./24 hrs.

CHLORIDE

Due to small volumes obtained for pancreatic samples it was possible to determine only bile chloride concentrations. Chloride values decreased significantly from 98 ± 3 mM./l. at 600 g./24 hrs. to 82 ± 2 mM./l. at 1300 g./24 hrs. A further small but significant decrease to 80 ± 2 mM./l. at 1700 g./24 hrs. was obtained.

PERIOD DIFFERENCES

The only significant period difference obtained by sequential sampling of the 5 minute samples occurred for bile potassium concentrations. Tables 9, 11 and 13 indicate a significant decrease in potassium concentrations from period 1 to period 6. Trends of bile calcium and phosphate concentrations increasing with each consecutive sample were observed but these trends were not statistically significant.

TABLE 9 Means and standard errors of bile volumes and electrolyte composition in sheep on 600 g. per day continuous feeding.

Time Period (5 min. each)	Volume ml./min.	Sodium mM/l.	Potassium mM/l.	Calcium mM/l.	Phosphate mM/l.	Cl mM/l.
1	0.7 ± .1	190 ± 3	6.9 ± .1	2.4 ± .23	.25 ± .05	91 ± 3
2	0.6 ± .1	187 ± 2	6.8 ± .1	2.5 ± .12	.25 ± .03	94 ± 3
3	0.5 ± .1	188 ± 3	6.9 ± .1	2.6 ± .23	.28 ± .04	94 ± 3
4	0.5 ± .1	188 ± 2	6.8 ± .2	2.6 ± .23	.27 ± .07	97 ± 3
5	0.4 ± .1	186 ± 2	6.6 ± .1	2.7 ± .23	.26 ± .06	99 ± 3
6	0.5 ± .1	184 ± 3	6.5 ± .1	2.6 ± .12	.29 ± .05	101 ± 3
7	0.5 ± .1	185 ± 3	6.5 ± .1	2.7 ± .12	.29 ± .06	101 ± 3
8	0.5 ± .1	184 ± 2	6.4 ± .1	2.7 ± .12	.22 ± .03	100 ± 2
9	0.5 ± .1	187 ± 3	6.5 ± .1	2.7 ± .12	.24 ± .03	102 ± 3
$\bar{X}$ ± S.E.	0.5 ± .1	186 ± 3	6.7 ± .1	2.6 ± .12	.26 ± .05	98 ± 3

TABLE 10 Means and standard errors for pancreatic juice volume and electrolyte composition in sheep on 600 g. per day continuous feeding.

Time Period (5 min. each)	Volume ml./min.	Sodium mM/l	Potassium mM/l	Calcium mM/l	Phosphate mM/l
1	.18 ± .01	149 ± 1	5.0 ± .1	2.4 ± .23	.23 ± .02
2	.17 ± .01	152 ± 2	5.1 ± .1	2.9 ± .32	.22 ± .02
3	.17 ± .01	149 ± 3	4.9 ± .1	2.6 ± .23	.19 ± .01
4	.17 ± .01	150 ± 1	5.0 ± .1	2.7 ± .12	.20 ± .01
5	.16 ± .02	150 ± 1	4.9 ± .1	2.8 ± .44	.20 ± .02
6	.17 ± .01	149 ± 2	4.9 ± .1	2.7 ± .31	.23 ± .02
7	.16 ± .02	152 ± 2	4.9 ± .2	2.7 ± .12	.22 ± .01
8	.16 ± .02	152 ± 1	4.9 ± .1	2.9 ± .12	.22 ± .01
9	.14 ± .02	152 ± 3	4.8 ± .1	2.5 ± .12	.22 ± .02
$\bar{X}$ ± S.E.	.16 ± .01	151 ± 2	4.9 ± .1	2.7 ± .23	.21 ± .02

TABLE 11 Means and standard errors of bile volumes and electrolyte composition in sheep on 1300 g. per day continuous feeding.

Time Period (5 min. each)	Volume ml./min.	Sodium mM/l	Potassium mM/l	Calcium mM/l	Phosphate mM/l	Chloride mM/l
1	1.7 ± .1	194 ± 4	7.0 ± .1	2.3 ± .04	.13 ± .02	81 ± 2
2	1.6 ± .1	193 ± 3	6.8 ± .2	2.3 ± .04	.14 ± .02	81 ± 2
3	1.7 ± .1	197 ± 4	6.8 ± .2	2.4 ± .03	.17 ± .03	83 ± 2
4	1.5 ± .2	196 ± 3	6.5 ± .1	2.3 ± .08	.17 ± .04	83 ± 2
5	1.7 ± .1	198 ± 3	6.6 ± .2	2.4 ± .03	.13 ± .02	82 ± 2
6	1.6 ± .1	196 ± 3	6.4 ± .1	2.4 ± .03	.12 ± .01	81 ± 1
7	1.6 ± .2	197 ± 3	6.4 ± .1	2.4 ± .03	.14 ± .01	82 ± 1
8	1.5 ± .1	196 ± 3	6.4 ± .1	2.4 ± .03	.14 ± .01	82 ± 2
9	1.6 ± .1	196 ± 3	6.4 ± .1	2.4 ± .03	.13 ± .02	82 ± 2
$\bar{X}$ ± S.E.	1.6 ± .1	196 ± 3	6.6 ± .1	2.3 ± .03	.14 ± .02	82 ± 2

TABLE 12 Means and standard errors for pancreatic juice volume and electrolyte composition in sheep on 1300 g. per day continuous feeding.

Time Period (5 min. each)	Volume ml./min.	Sodium mM/l	Potassium mM/l	Calcium mM/l	Phosphate mM/l
1	.27 ± .05	151 ± 2	5.3 ± .1	1.9 ± .13	.23 ± .02
2	.20 ± .02	152 ± 3	5.6 ± .1	1.8 ± .12	.14 ± .03
3	.24 ± .04	154 ± 2	5.3 ± .2	2.1 ± .23	.22 ± .01
4	.17 ± .04	155 ± 1	5.4 ± .2	1.9 ± .12	.19 ± .02
5	.25 ± .06	155 ± 2	5.3 ± .2	2.1 ± .23	.19 ± .03
6	.21 ± .03	154 ± 1	5.2 ± .1	2.1 ± .13	.13 ± .02
7	.23 ± .03	155 ± 2	5.1 ± .2	2.0 ± .15	.19 ± .02
8	.22 ± .01	155 ± 2	5.3 ± .2	2.1 ± .23	.16 ± .02
9	.20 ± .02	153 ± 2	5.2 ± .1	2.0 ± .13	.18 ± .02
$\bar{X}$ ± S.E.	.22 ± .03	154 ± 2	5.3 ± .2	2.0 ± .20	.18 ± .02

TABLE 13 Means and standard errors for bile volumes and electrolyte composition in sheep on 1700 g. per day continuous feeding.

Time Period (5 min. each)	Volume ml./min.	Sodium mM/l	Potassium mM/l	Calcium mM/l	Phosphate mM/l	Chloride mM/l
1	1.7 ± .2	190 ± 2	7.2 ± .1	2.3 ± .12	.13 ± .01	79 ± 2
2	1.6 ± .2	190 ± 2	7.1 ± .1	2.2 ± .15	.13 ± .01	80 ± 2
3	1.6 ± .1	191 ± 2	6.9 ± .1	2.2 ± .13	.12 ± .02	80 ± 2
4	1.6 ± .2	193 ± 3	6.9 ± .1	2.3 ± .12	.13 ± .01	81 ± 2
5	1.6 ± .2	195 ± 3	6.9 ± .1	2.3 ± .12	.13 ± .01	79 ± 1
6	1.5 ± .1	195 ± 3	6.8 ± .1	2.3 ± .12	.12 ± .02	80 ± 2
7	1.7 ± .2	195 ± 3	6.8 ± .1	2.3 ± .12	.12 ± .02	80 ± 1
8	1.7 ± .1	197 ± 3	6.8 ± .1	2.3 ± .12	.12 ± .01	79 ± 1
9	1.5 ± .1	197 ± 2	6.8 ± .1	2.3 ± .12	.12 ± .01	78 ± 1
$\bar{X}$ ± S.E.	1.6 ± .2	194 ± 3	6.9 ± .1	2.3 ± .12	.12 ± .01	80 ± 2

TABLE 14 Means and standard errors for pancreatic juice volume and electrolyte composition in sheep on 1700 g. per day continuous feeding.

Time Period (5 min. each)	Volume ml./min.	Sodium mM/l	Potassium mM/l	Calcium mM/l	Phosphate mM/l
1	.30 ± .03	147 ± 2	5.6 ± .1	2.8 ± .12	.08 ± .04
2	.27 ± .02	151 ± 2	5.7 ± .1	2.9 ± .13	.11 ± .05
3	.25 ± .03	148 ± 1	5.5 ± .2	2.9 ± .19	.10 ± .04
4	.26 ± .03	151 ± 1	5.6 ± .2	3.1 ± .3	.09 ± .04
5	.27 ± .04	152 ± 1	5.5 ± .2	2.7 ± .20	.11 ± .02
6	.28 ± .03	152 ± 2	5.3 ± .1	3.0 ± .23	.11 ± .05
7	.29 ± .03	153 ± 2	5.3 ± .1	2.9 ± .21	.15 ± .03
8	.28 ± .03	152 ± 2	5.3 ± .1	3.0 ± .22	.17 ± .04
9	.28 ± .03	153 ± 2	5.5 ± .1	2.8 ± .12	.17 ± .03
$\bar{X}$ ± S.E.	.28 ± .03	151 ± 2	5.5 ± .1	2.9 ± .21	.12 ± .04

TABLE 15 Summary of means and standard errors for volume and electrolyte composition of bile and pancreatic juice at three levels of continuous feeding.

Treatment	Volume ml./min.	Sodium mM/l	Potassium mM/l	Calcium mM/l	Phosphate mM/l	Chloride mM/l
Bile						
600 g./24 hr.	0.5 ± .1	186 ± 3	6.7 ± .1	2.6 ± .12	.26 ± .05	98 ± 3
1300 g./24 hr.	1.6 ± .1	196 ± 3	6.6 ± .1	2.3 ± .03	.14 ± .02	82 ± 2
1700 g./24 hr.	1.6 ± .2	194 ± 3	6.9 ± .1	2.3 ± .05	.12 ± .01	80 ± 2
Pancreatic						
600 g./24 hr.	.16 ± .01	151 ± 2	4.9 ± .1	2.7 ± .23	.21 ± .02	
1300 g./24 hr.	.22 ± .03	154 ± 2	5.3 ± .2	2.0 ± .18	.18 ± .02	
1700 g./24 hr.	.28 ± .03	151 ± 2	5.5 ± .1	2.9 ± .18	.12 ± .04	

FIGURE 17 Means and standard errors for bile volume and electrolyte concentrations in sheep on 3 levels of continuous feeding.

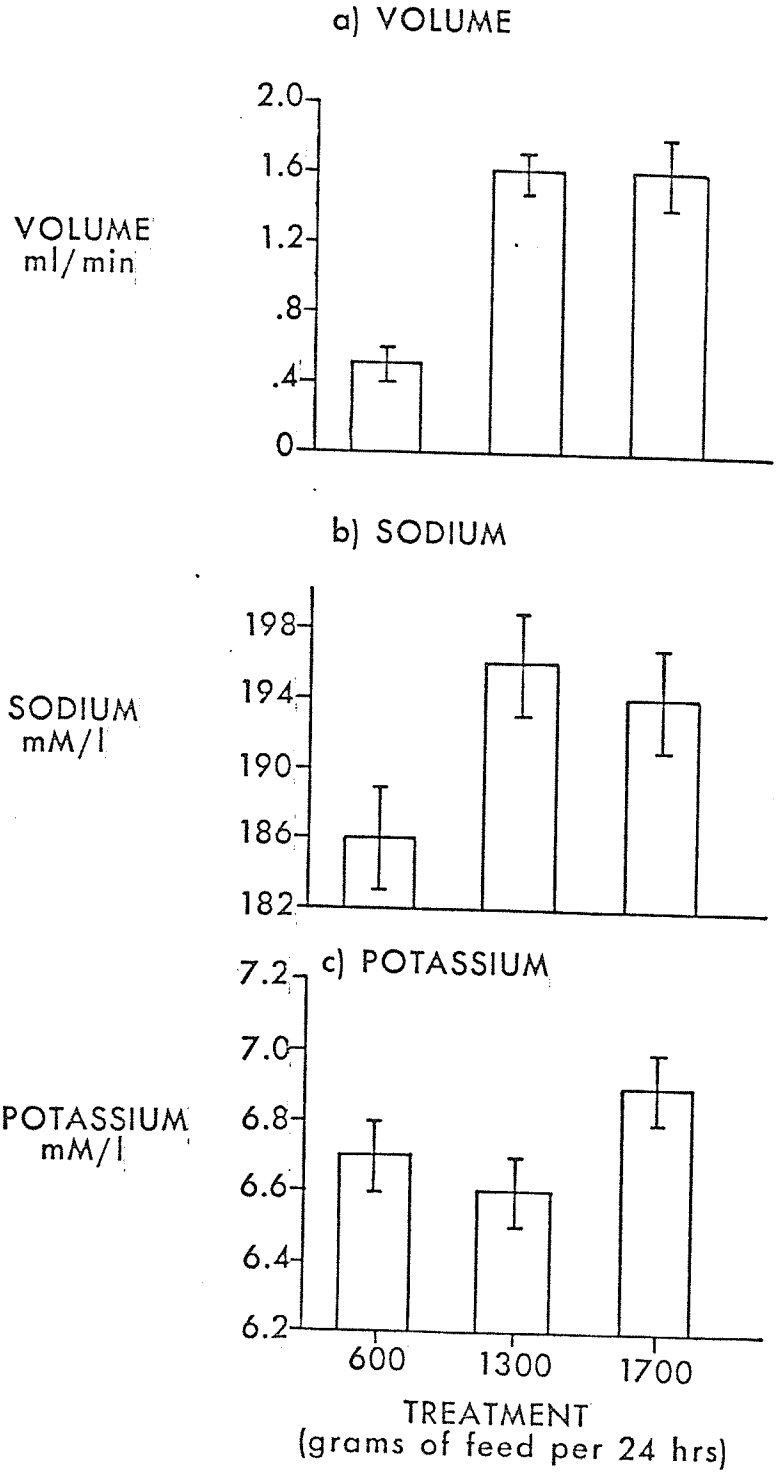


Figure 17

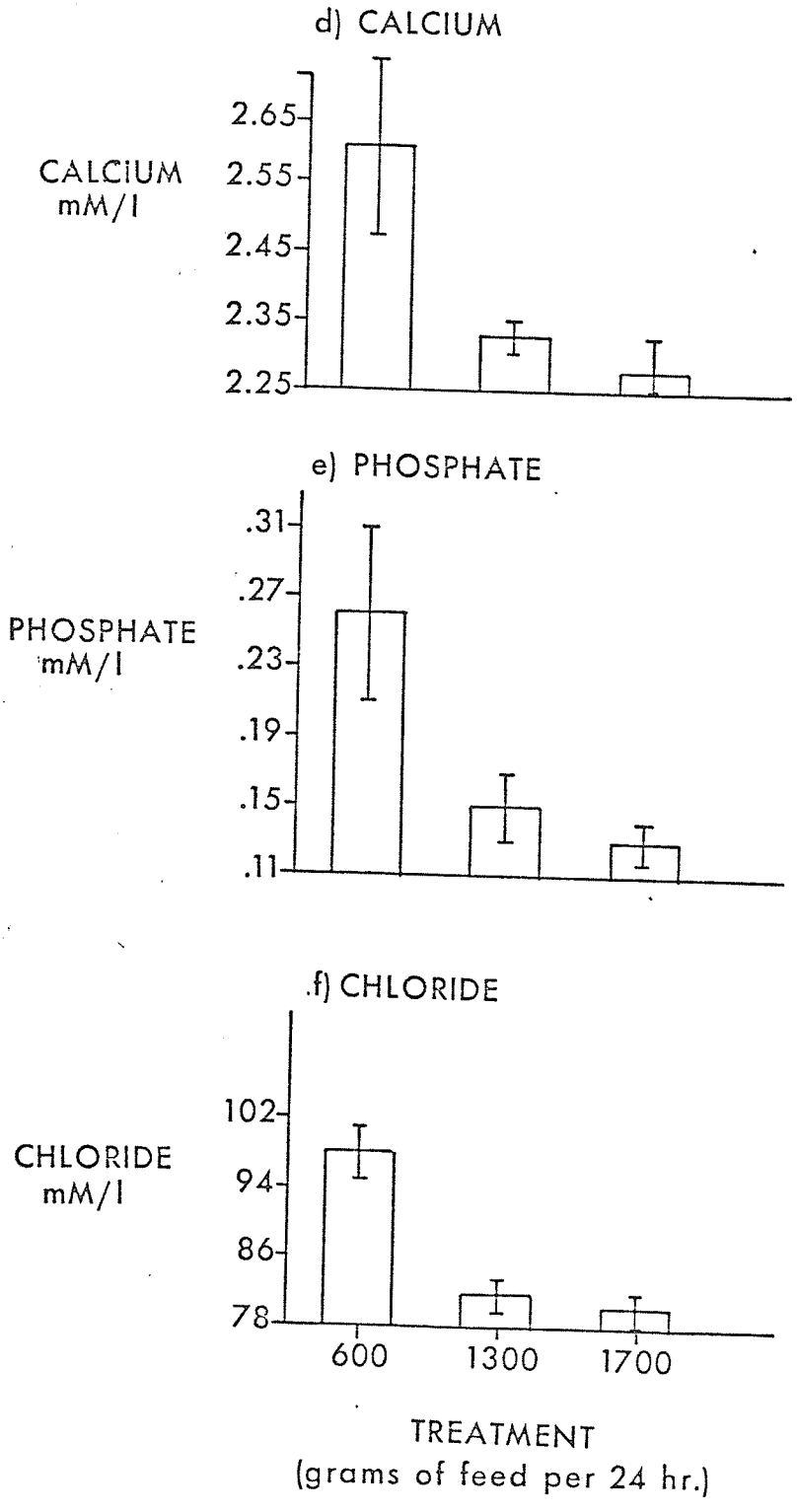

d) CALCIUM
CALCIUM mM/l
2.65
2.55
2.45
2.35
2.25
e) PHOSPHATE
PHOSPHATE mM/l
.31
.27
.23
.19
.15
.11
f) CHLORIDE
CHLORIDE mM/l
102
94
86
78
600
1300
1700
TREATMENT
(grams of feed per 24 hr.)

FIGURE I8 Means and standard errors for pancreatic juice volume and electrolyte concentrations in sheep on 3 levels of continuous feeding.

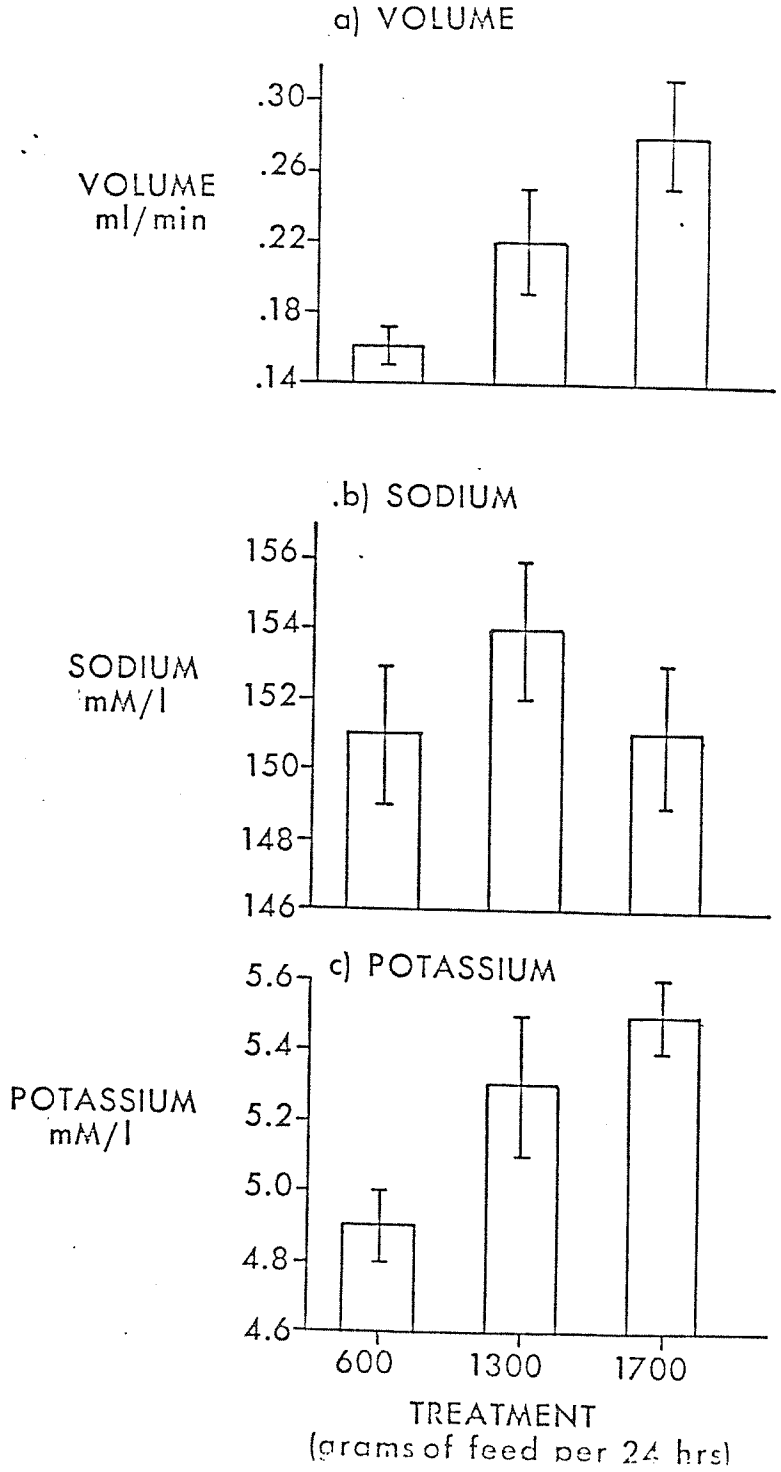
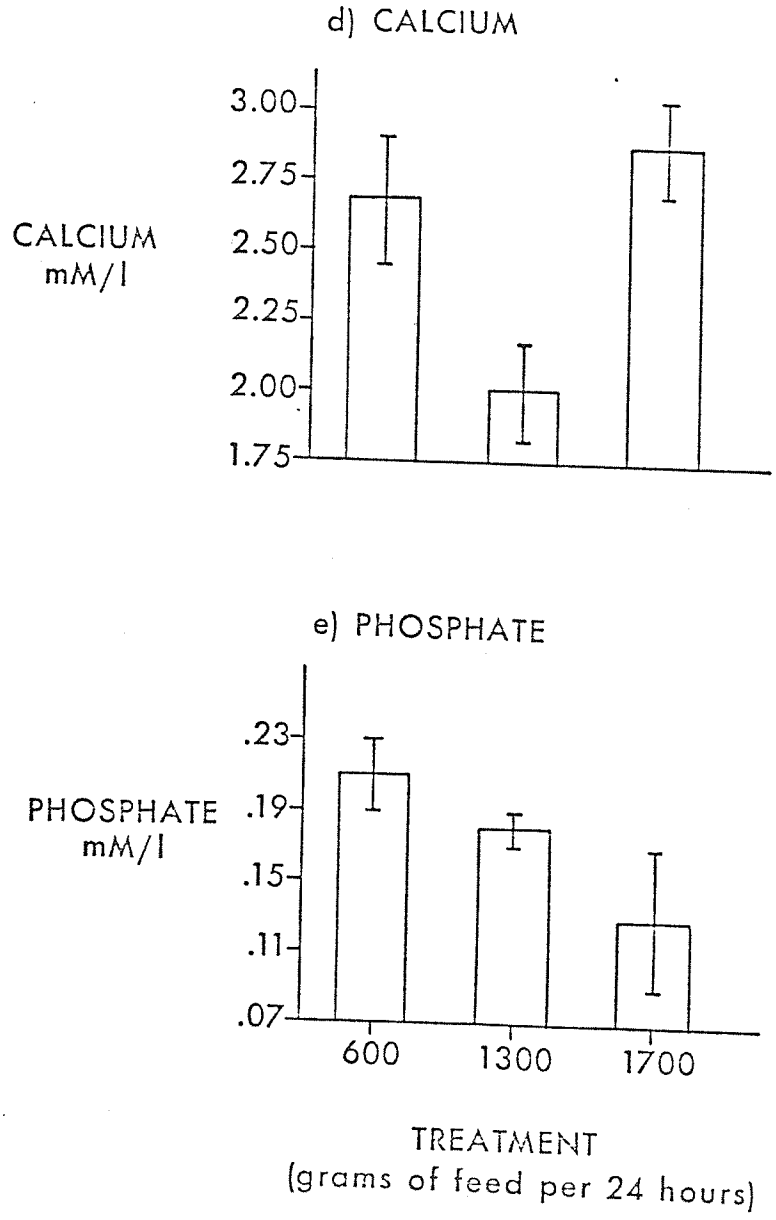


Figure 18


d) CALCIUM
CALCIUM
mM/l
3.00
2.75
2.50
2.25
2.00
1.75
e) PHOSPHATE
PHOSPHATE
mM/l
.23
.19
.15
.11
.07
600
1300
1700
TREATMENT
(grams of feed per 24 hours)

EXPERIMENT 3

Values for bile and pancreatic juice electrolyte composition and volumes were studied under fasting conditions over a period of 3 days. An examination of trypsin, chymotrypsin, lipase and amylase concentrations in pancreatic juice was also made.

BILE (table 16)

Flow

The results (table 16) indicate a mean bile flow of 0.59 ± .02 ml./min. over 3 days of fasting with no significant fall in bile flow over the 3 days. This value was not significantly different from the bile flow rate of 0.5 ± .1 ml./min. seen in sheep on 600 g. feed/24 hrs., but was significantly lower than 1.6 ± .1 ml./min. observed for sheep on 1300 g. feed/24 hrs. (experiment 2b) and significantly lower than 1.6 ± .2 ml/min. observed for sheep on 1700 g. feed/24 hrs. (experiment 2c).

SODIUM

The mean concentration of sodium in bile during fasting was 155 ± 1 mM/l. which was significantly less than values obtained for sheep on 600, 1300 or 1700 g. feed/24 hrs. observed in experiments 2a, 2b and 2c.

POTASSIUM

The mean potassium concentration observed in bile of fasting sheep was 4.5 ± .1 mM/l. which was significantly lower than values obtained for sheep on either 600, 1300 or 1700 g. feed/24 hrs. observed in experiments 2a, 2b and 2c.

CALCIUM

The mean concentration of calcium in bile of fasting sheep was 1.3 ± .1 mM/l. which was significantly lower than values obtained for sheep

TABLE 16 Means and standard errors for bile volume and electrolyte concentration in samples taken randomly over a 3 day period in fasting sheep.

Sample Number	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
1	0.56	155	4.4	1.4	.09
2	0.51	160	4.6	1.1	.08
3	0.64	152	4.3	1.1	.08
4	0.65	152	4.8	1.3	.09
5	0.62	152	4.3	1.3	.10
6	0.58	153	4.4	1.3	.10
7	0.62	156	4.4	1.3	.09
8	0.55	158	4.6	1.3	.09
9	0.58	155	4.7	1.3	.08
$\bar{X}$ ± S.E.	0.59 ± .02	155 ± 1	4.5 ± 1	1.3 ± .1	.09 ± .01

on 600, 1300 or 1700 g. feed/24 hrs. observed in experiments 2a, 2b, 2c.

PHOSPHATE

The mean concentration of phosphate observed in bile of fasting sheep was .09 ± .01 mM/l. which was significantly lower than phosphate concentrations obtained for sheep on 600, 1300 or 1700 g. feed/24 hrs.

PANCREATIC SECRETION (table 17)

Volume

A mean flow of 0.16 ± .01 ml./min. was obtained for fasting sheep which was identical to the mean value obtained for sheep on 600 g. feed/24 hrs. but significantly reduced from .22 ± .03 ml./min. and .28 ± .03 ml./min. observed for sheep on 1300 g. and 1700 g. of feed/24 hrs respectively

SODIUM

A mean concentration for sodium of 149 ± 1 mM./l. was obtained for fasting sheep which was not significantly different from sodium concentrations in pancreatic juice of sheep feed from 600 to 1700 g. feed/24 hrs. These results are quite different for the observations on bile sodium concentrations which increased significantly from the fasting to continuous feeding situation.

POTASSIUM

The mean potassium concentration (table 17) in pancreatic juice of fasted sheep was 4.4 ± .1 mM/l. which was significantly lower than mean concentrations observed in sheep fed continuously at 600, 1300 or 1700 g. feed/24 hrs.

CALCIUM

The mean calcium concentration (table 17) observed for pancreatic juice of fasted sheep was 1.4 ± .1 mM/l. which was significantly lower

TABLE 17 Means and standard errors for pancreatic secretion volume and electrolyte concentrations in samples taken randomly over a 3 day period in fasting sheep.

Sample Number	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
1	0.18	151	4.4	1.4	.25
2	0.16	151	4.4	1.4	.25
3	0.16	148	4.5	1.4	.25
4	0.13	149	4.4	1.3	.25
5	0.21	150	4.4	1.4	.26
6	0.16	150	4.4	1.4	.26
7	0.13	147	4.5	1.4	.26
8	0.18	148	4.4	1.4	.26
9	0.17	149	4.4	1.4	.26
$\bar{X}$ ± S.E.	0.16 ± .01	149 ± 1	4.4 ± .1	1.4 ± .1	.26 ± .01

than calcium concentrations in pancreatic juice of sheep fed continuously at 600, 1300 or 1700 g feed/24 hrs. (experiments 2a, 2b, and 2c).

PHOSPHATE

The mean phosphate concentration observed in pancreatic secretions of fasting sheep was .25 ± .01 mM/l. which was significantly greater than concentrations of phosphate observed for sheep on 600, 1300 or 1700 g feed/24 hrs. (experiments 2a, 2b and 2c).

PANCREATIC ENZYMES (table 18)

The mean concentrations of enzymes in pancreatic juice obtained from fasting sheep was used as an indication of basal enzyme concentrations (table 18). The mean concentration for trypsin was $39 \times 10^3 \pm 2 \times 10^3$ TAME units/ml. No significant differences were observed between sampling periods. The mean chymotrypsin concentration was $38 \times 10^3 \pm 6 \times 10^3$ BTEE units/ml. The mean lipase concentration observed for fasting sheep was 790 ± 25 Sigma Tietz units/ml., while the mean value for amylase was 400 ± 20 Somogyi units/ml. (table 18).

TABLE 18 Means and standard errors for pancreatic enzymes in samples taken randomly over a 3 day period in fasting sheep

Sample Number	Volume ml./min.	Trypsin Tame U/ ml. x 10^3	Chymotrypsin BTEE U/ml. x 10^3	Lipase Sigtietz U/ml.	Amylase Somogyi U/ml.
1	0.18	41	43	825	450
2	0.16	40	41	725	500
3	0.16	35	36	800	425
4	0.13	38	36	750	325
5	0.21	39	37	825	375
6	0.16	42	38	850	300
7	0.13	40	38	800	375
8	0.18	38	38	775	450
9	0.17	39	39	750	425
X ± S.E.	0.16 ± .01	39 ± 2	38 ± 1	790 ± 25	400 ± 20

EXPERIMENT 4

The experiment was designed to determine whether bile and pancreatic juice compositions were significantly altered following feeding of 1200 g. feed. All the feed given was consumed by the sheep within 30 minutes of feeding.

BILE (table 19)

Volume (figure 19)

The rate of bile secretion increased following feeding 1200 g. alfalfa pellets. This increase was not significant over pre fed values due to large standard errors. The increase was considered to be important and probably would be significant if the number of animals examined could have been increased (figure 19). The peak bile flow following feeding was also not significantly different from sheep fed continuously at 1300 g. feed/24 hrs.

Sodium (figure 20)

The concentration of sodium following feeding dropped from 161 ± 2 mM/l. at 2 hours prior to feeding to 145 ± 3 mM/l. at 2 hours after feeding. This significant decrease may have been because of a dilution effect associated with increased bile flow. The value of 145 ± 3 mM/l. for sodium concentration following feeding was also significantly lower than 158 ± 1 mM/l. obtained in fasting sheep.

Potassium (figure 21)

Following feeding, potassium concentration significantly decreased from 7.3 ± .2 mM/l. at 2 hours prior to feeding to 5.3 ± .3 mM/l. at 2 hours after feeding. This decrease in potassium concentration may have

TABLE 19 Means and standard errors for bile volume and electrolyte concentrations before and after feeding 1200 g. of alfalfa pellets. (n=2)

Time	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
10:00 A.M.	1.38 ± .18	161 ± 2	7.3 ± .2	1.9 ± .1	.22 ± .02
11:00 A.M.	0.78 ± .27	159 ± 1	6.9 ± .1	1.7 ± .1	.22 ± .01
Feed 12:00	1.40 ± .08	156 ± 1	6.5 ± .3	1.6 ± .1	.20 ± .01
12:30 P.M.	1.31 ± .06	154 ± 3	6.1 ± .2	1.7 ± .1	.19 ± .01
1:00 P.M.	2.17 ± .47	150 ± 3	6.0 ± .2	1.4 ± .1	.15 ± .01
1:30 P.M.	2.23 ± .78	145 ± 2	5.7 ± .1	1.4 ± .1	.17 ± .01
2:00 P.M.	2.06 ± .25	145 ± 3	5.3 ± .3	1.3 ± .2	.14 ± .02
3:00 P.M.	1.36 ± .28	152 ± 2	6.0 ± .4	1.5 ± .1	.18 ± .01
4:00 P.M.	1.05 ± .34	157 ± 1	6.6 ± .1	1.5 ± .1	.21 ± .01
5:00 P.M.	1.32 ± .21	157 ± 1	6.5 ± .2	1.6 ± .1	.22 ± .02

FIGURE I9 Means and standard errors for bile volume in two sheep before and after feeding I200 g. alfalfa pellets.

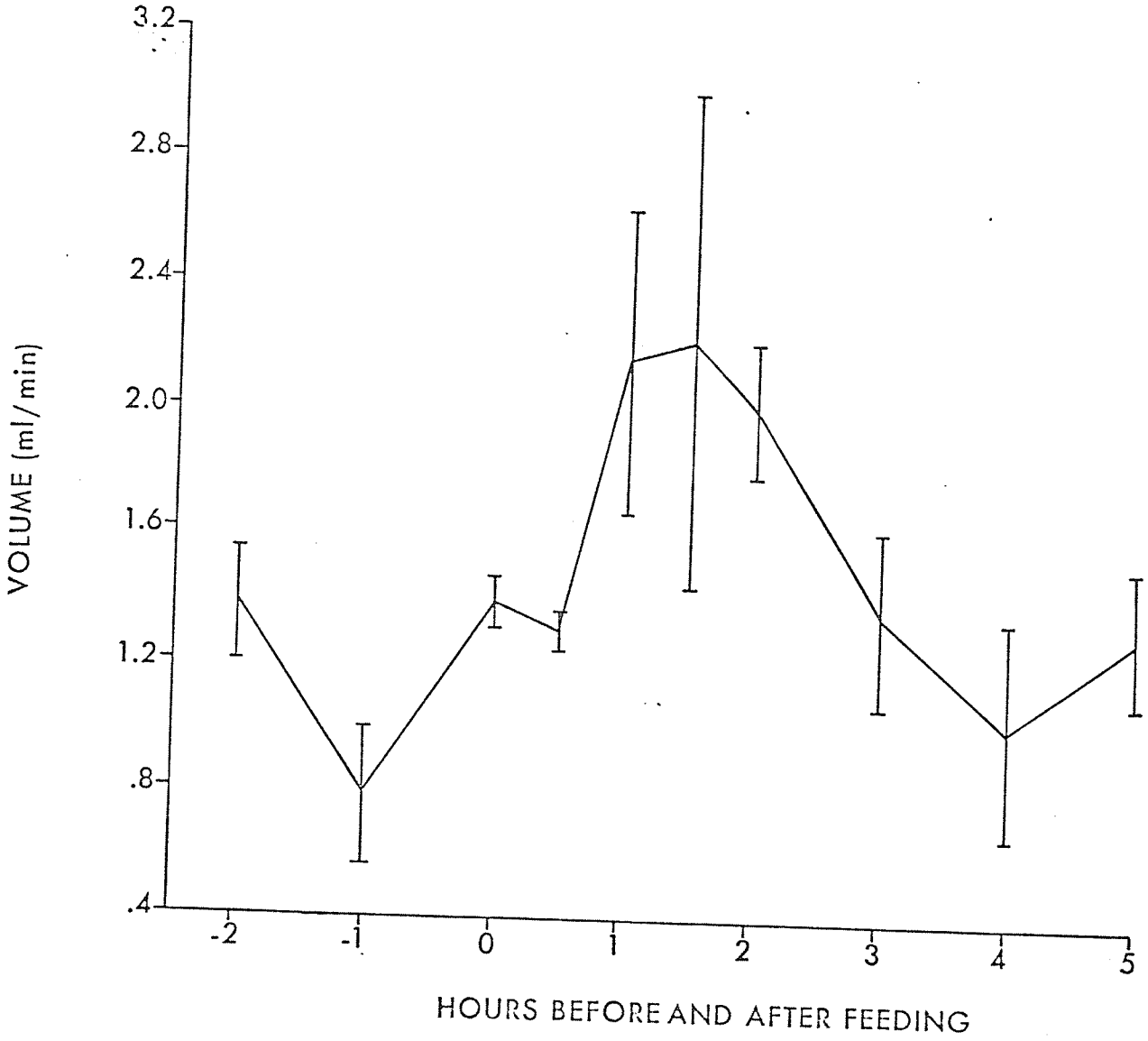


FIGURE 20 Means and standard errors for bile sodium concentration in two sheep before and after feeding I200 g. alfalfa pellets.

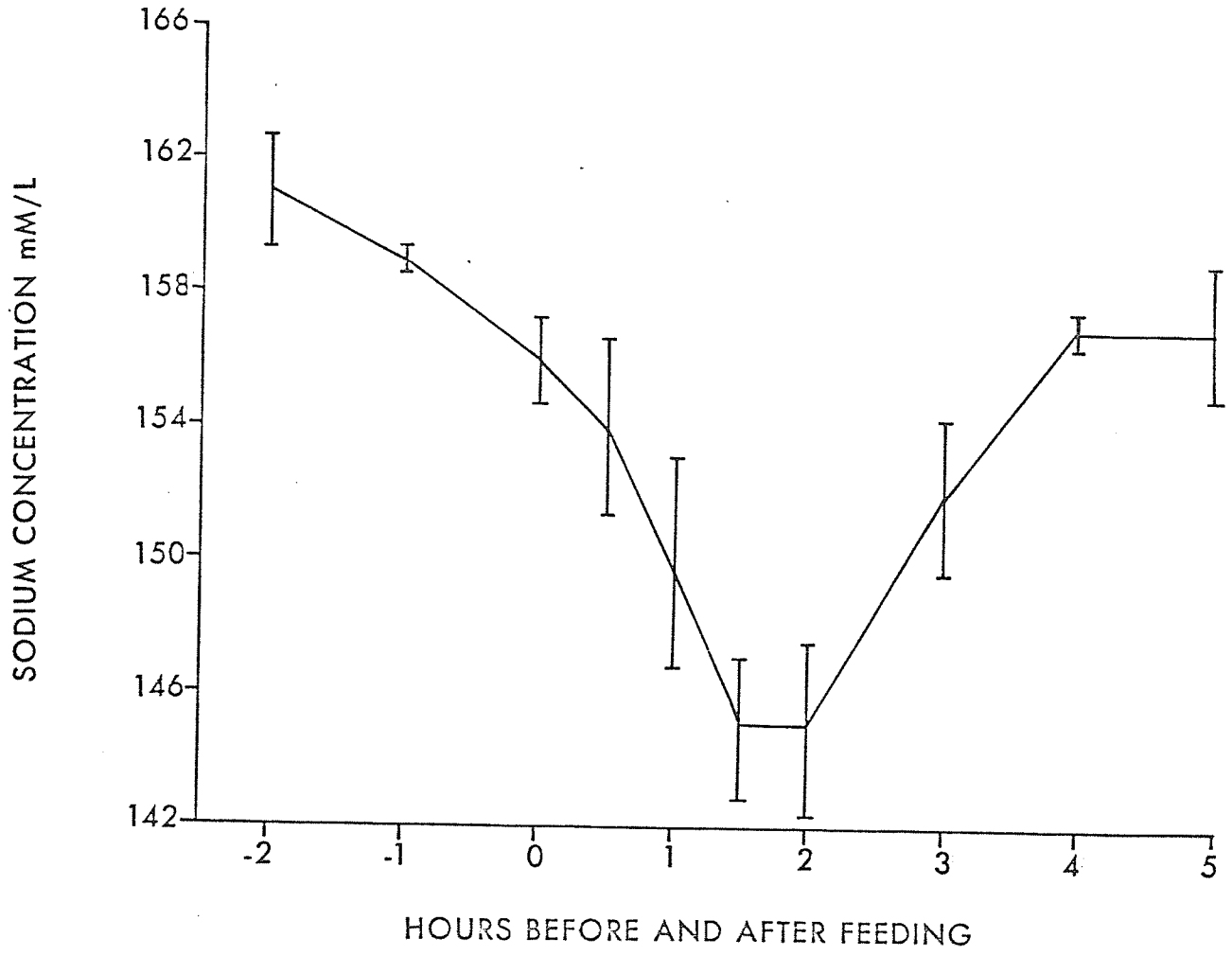
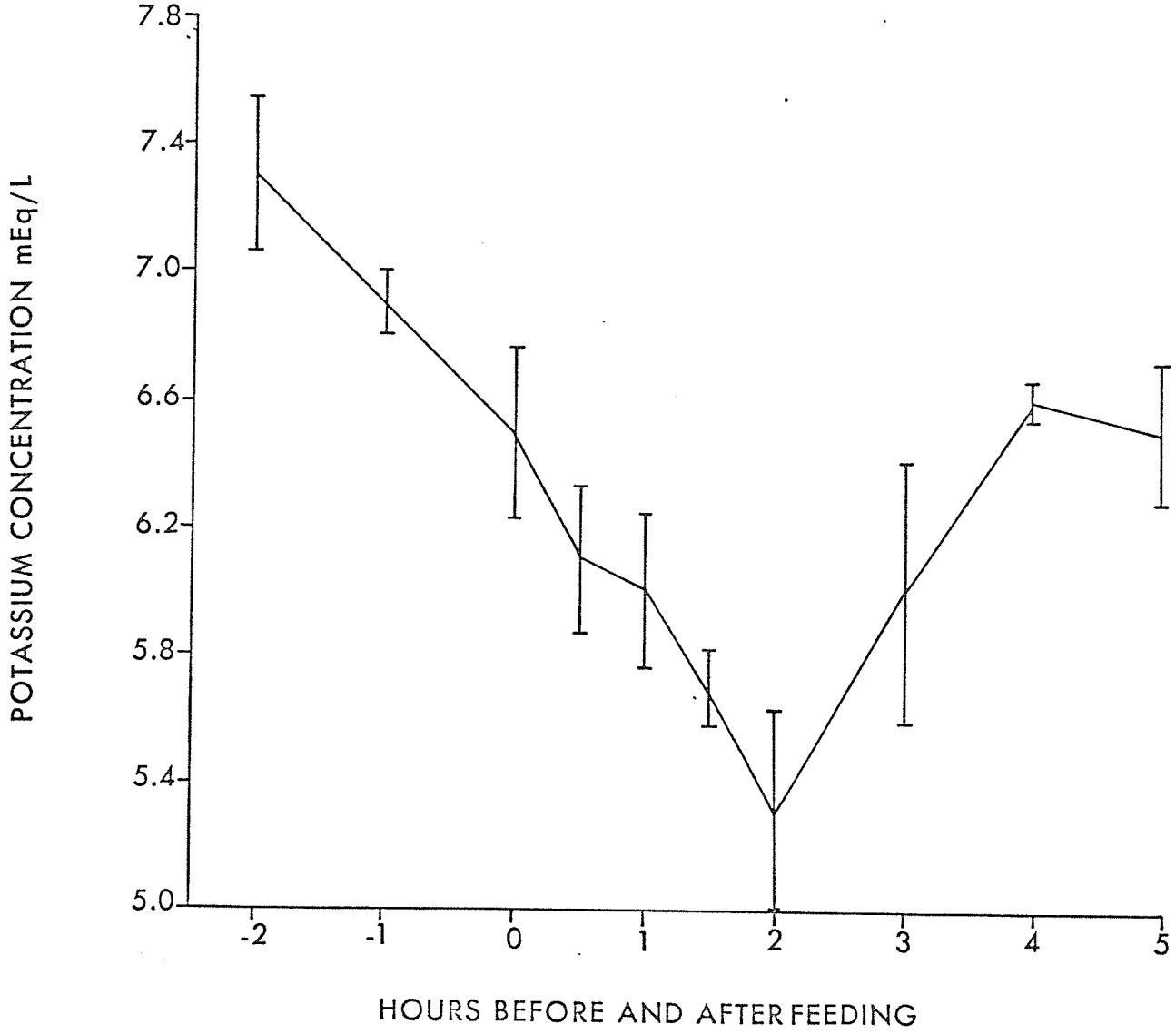


FIGURE 2I Means and standard errors for bile potassium concentration in two sheep before and after feeding I200 g. alfalfa pellets.



been because of a dilution effect of increased bile flow. Unlike sodium the concentration of potassium did not decrease to fasting values of 4.3 ± 0.1 mM/1.

Calcium (figure 22)

Calcium concentrations decreased significantly from 1.9 ± .1 mM/1. at 2 hours prior to feeding to 1.3 ± .2 mM/1. at 2 hours after feeding. As for potassium this decrease may have been because of a dilution effect as bile flow increased. The same effect on calcium concentration was seen in continuous feeding experiments 2a, 2b and 2c where calcium concentrations decreased significantly as flow rate of bile increased.

Phosphate (figure 23)

Phosphate concentrations decreased significantly from .22 ± .02 mM/1. to .14 ± .02 mM/1. from 2 hours prior to feeding to 2 hours after feeding respectively. This pattern follows that observed with increased levels of continuous feeding which were accompanied by increased bile flow.

Pancreatic Juice (Table 20)

Volume

Once a day feeding at a rate of 1200 g. resulted in an increase of pancreatic juice secretion rate from a mean of 0.15 ± .03 ml./min. before feeding to 0.54 ± .04 ml./min. at 1½ hours after feeding. Sheep fed continuously at 1300 g. feed/24 hrs. had a significantly lower pancreatic juice flow rate at .22 ± .03 ml./min. than that observed at 1½ hours after feeding 1200 g. feed to sheep at one time. The mean flow rate following feeding peaked at 1½ hours but remained above pre feeding values for 5 hours following feeding (figure 24).

TABLE 20 Means and standard errors for pancreatic secretion volume and electrolyte concentrations before and after feeding 1200 g. of alfalfa pellets. (n=2)

Time	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
10:00 A.M.	0.15 ± .03	145 ± 1	4.5 ± .1	1.6 ± .2	.24 ± .03
11:00 A.M.	0.21 ± .01	146 ± 1	4.4 ± .1	1.7 ± .2	.26 ± .01
Feed Giuen 12:00	0.22 ± .01	146 ± 1	4.6 ± .1	1.6 ± .2	.30 ± .04
12:30 P.M.	0.31 ± .02	145 ± 1	4.5 ± .1	1.8 ± .2	.28 ± .02
1:00 P.M.	0.35 ± .02	145 ± 1	4.4 ± .2	1.9 ± .2	.26 ± .04
1:30 P.M.	0.54 ± .04	146 ± 1	4.6 ± .2	2.0 ± .2	.27 ± .03
2:00 P.M.	0.52 ± .05	147 ± 1	4.6 ± .1	2.0 ± .2	.29 ± .03
3:00 P.M.	0.52 ± .07	146 ± 1	4.4 ± .1	2.0 ± .3	.26 ± .03
4:00 P.M.	0.40 ± .03	146 ± 1	4.4 ± .1	2.3 ± .5	.25 ± .03
5:00 P.M.	0.29 ± .06	146 ± 1	4.5 ± .1	2.3 ± .4	.26 ± .03

FIGURE 22 Means and standard errors for bile calcium concentration in two sheep before and after feeding I200 g. alfalfa pellets.

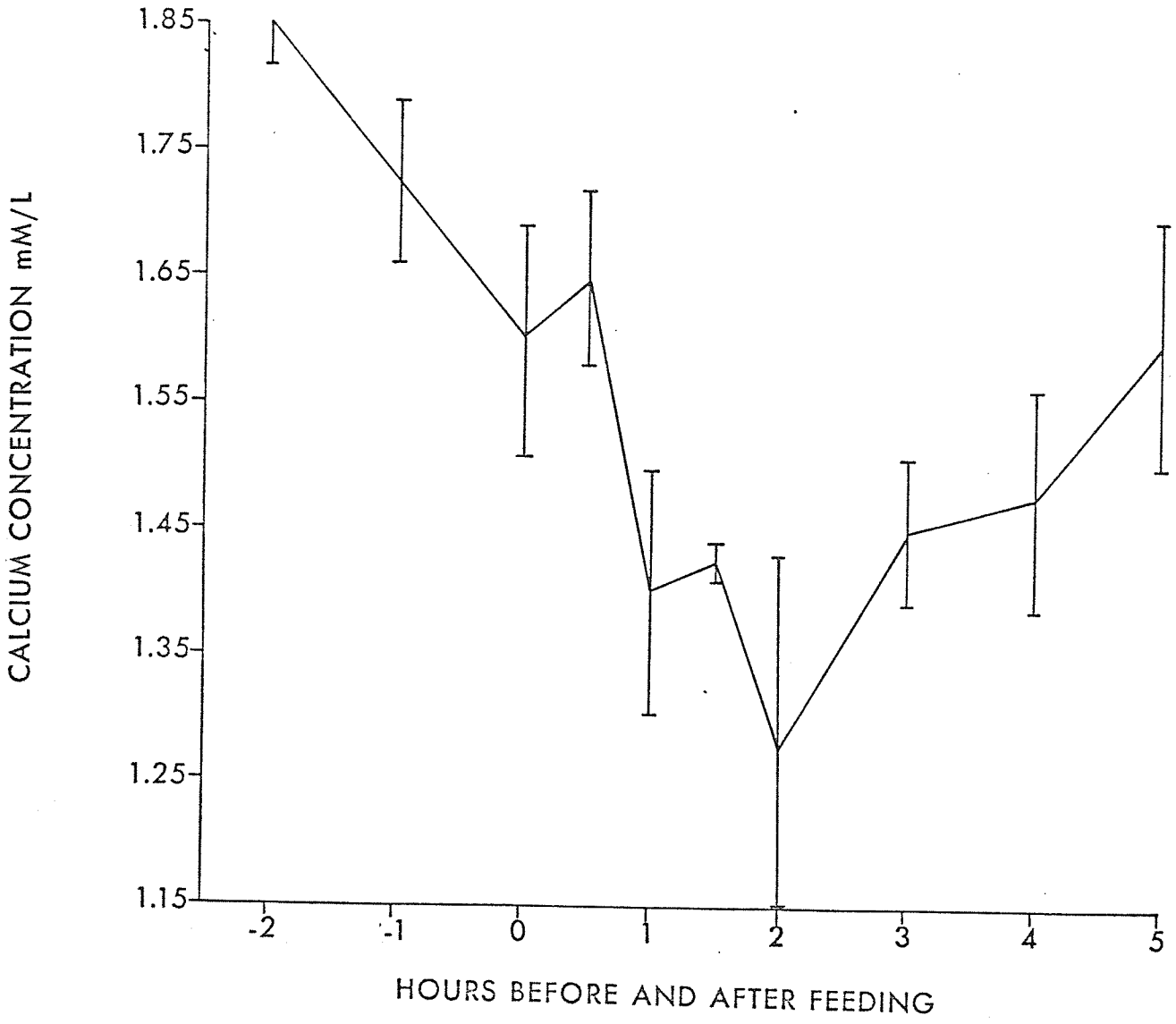


FIGURE 23 Means and standard errors for bile phosphate concentration in two sheep before and after feeding I200 g. alfalfa pellets.

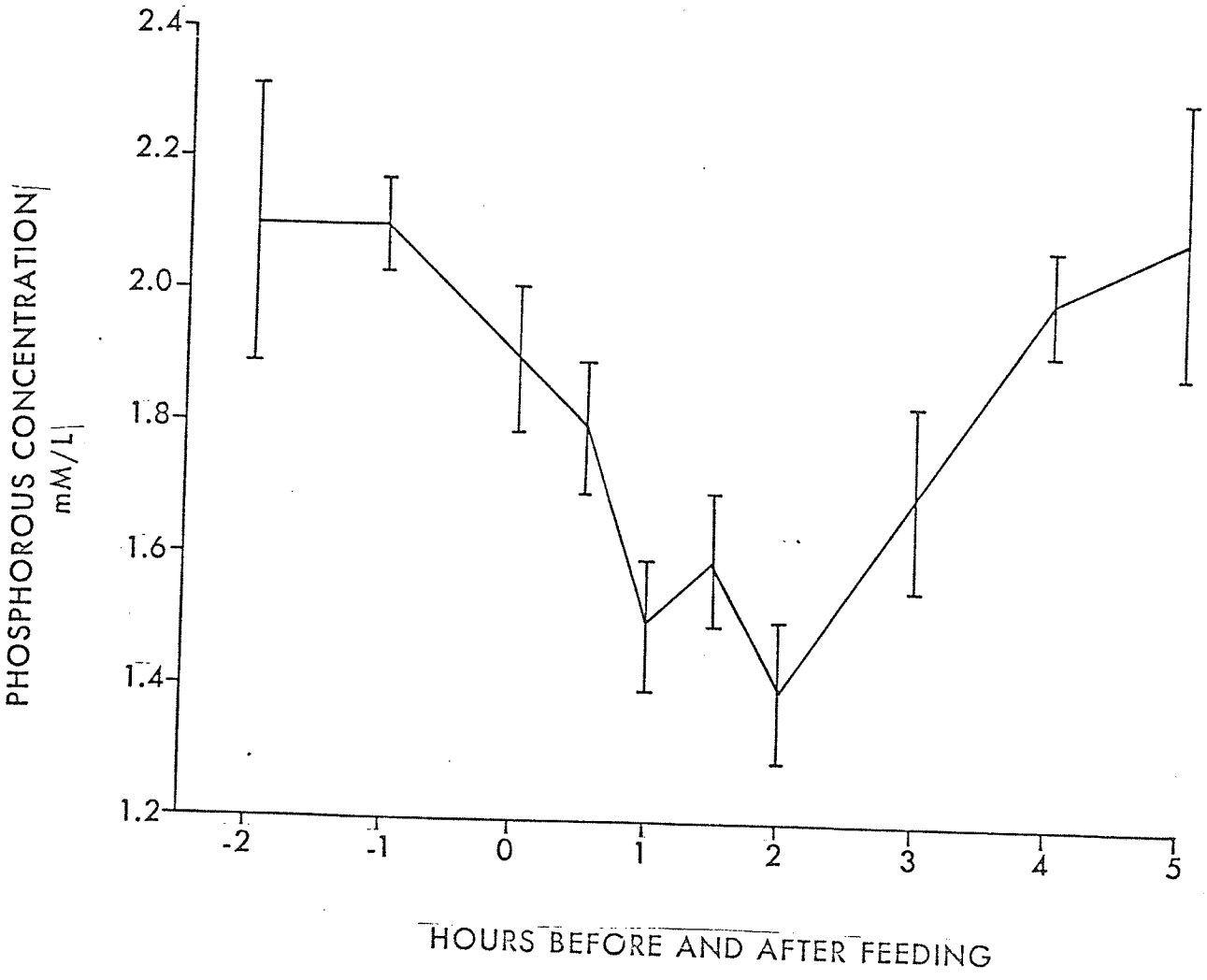
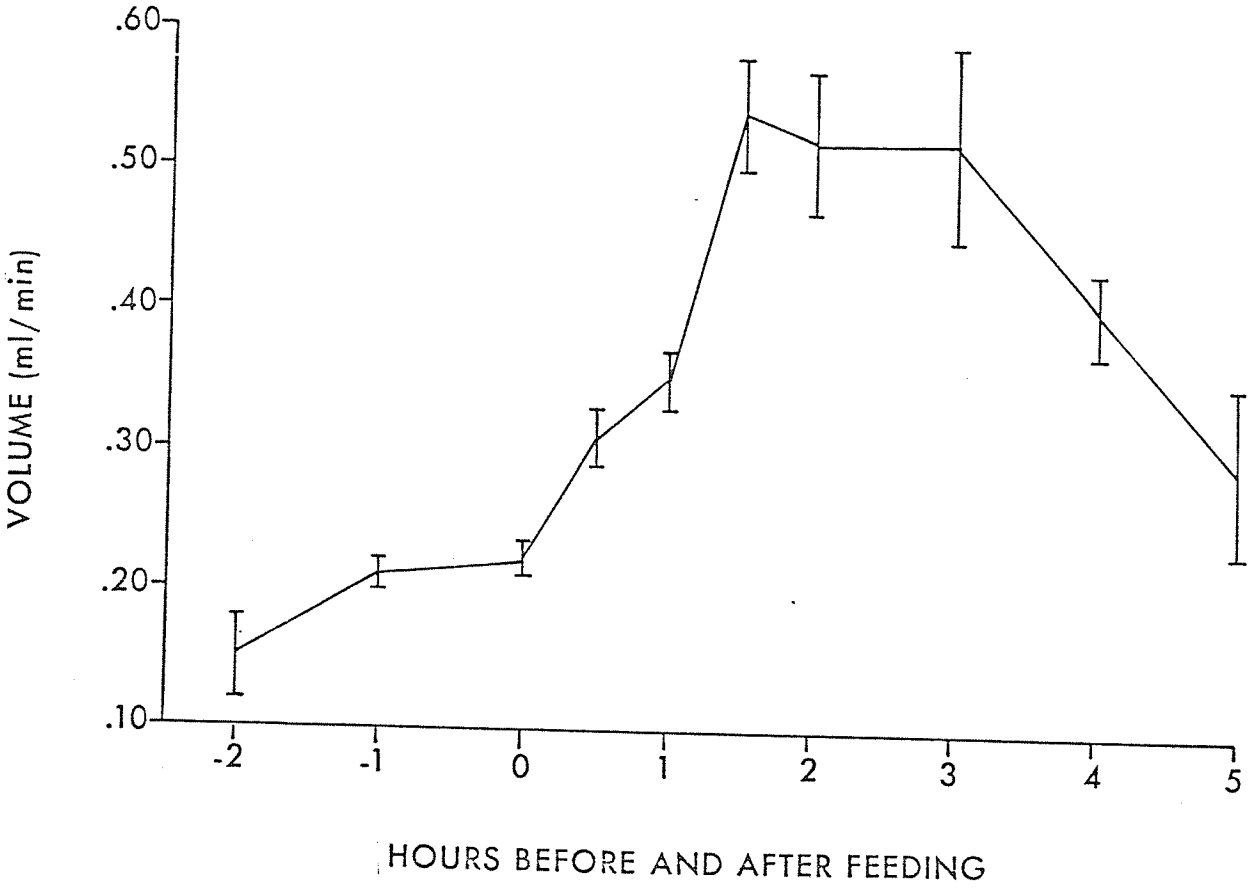


FIGURE 24 Means and standard errors for pancreatic juice volume in two sheep before and after feeding I200 g. alfalfa pellets.



Pancreatic juice electrolyte concentrations

The results for electrolyte concentrations in pancreatic juice indicate (table 20, figures 25 through 28) that no significant changes in sodium, potassium or phosphate concentrations occurred following feeding 1200 g. of feed. Calcium concentrations increased, however not significantly, from a pre feeding value of 1.6 ± .2 mM/l. to 2.0 ± .2 mM/l. at 1½ hours following feeding. The calcium concentration increase unlike pancreatic volume did not reach a peak after feeding within the 5 hours after feeding. The 5 hour sample after feeding had a mean calcium concentration of 2.3 ± .3 mM/l.

Pancreatic enzyme secretion (table 21, figures 29 through 32).

There were significantly increased concentrations of the enzymes trypsin, chymotrypsin, lipase and amylase following the eating of 1200 g. feed. Trypsin concentration increased from $49 \times 10^3 \pm 1.6 \times 10^3$ TAME units/ml. at 2 hours prior to feeding to a peak value of $136 \times 10^3 \pm 1 \times 10^3$ TAME units/ml. at 1½ hours after feeding. Chymotrypsin followed a similar significant increase from $32 \times 10^3 \pm 1 \times 10^3$ BTEE units/ml. at 2 hours prior to feeding to a peak value of $53 \times 10^3 \pm 4 \times 10^3$ BTEE units/ml. at 1½ hours post feeding. Figures 29 and 30 indicate that a second rise in both trypsin and chymotrypsin occurred subsequent to the first peak following feeding. This second rise continued until the end of the sampling schedule at 5 hours after feeding and may only indicate a sustained rise in enzyme concentrations.

Lipase concentration increased significantly from 1700 ± 150 Sigma Tietz units/ml. at 2 hours prior to feeding to a first peak of 2600 ± 100

Sigma Tietz units/ml. at 1½ hours after feeding, followed by a further rise to 2700 ± 100 Sigma Tietz units/ml at 3 hours after feeding (figure 32).

Amylase concentration increased significantly from 1350 ± 200 Somogyi units/ml. at 2 hours prior to feeding to 3900 ± 850 Somogyi units/ml. at 2 hours after feeding. Due to large variability between sheep it was difficult to determine if a second peak occured. Unlike the previously mentioned enzymes which reached peak concentrations at 1½ hours following feeding the highest concentration of amylase occured at 2 hours post feeding.

FIGURE 25 Means and standard errors for pancreatic juice sodium concentration in two sheep before and after feeding I200 g. alfalfa pellets.

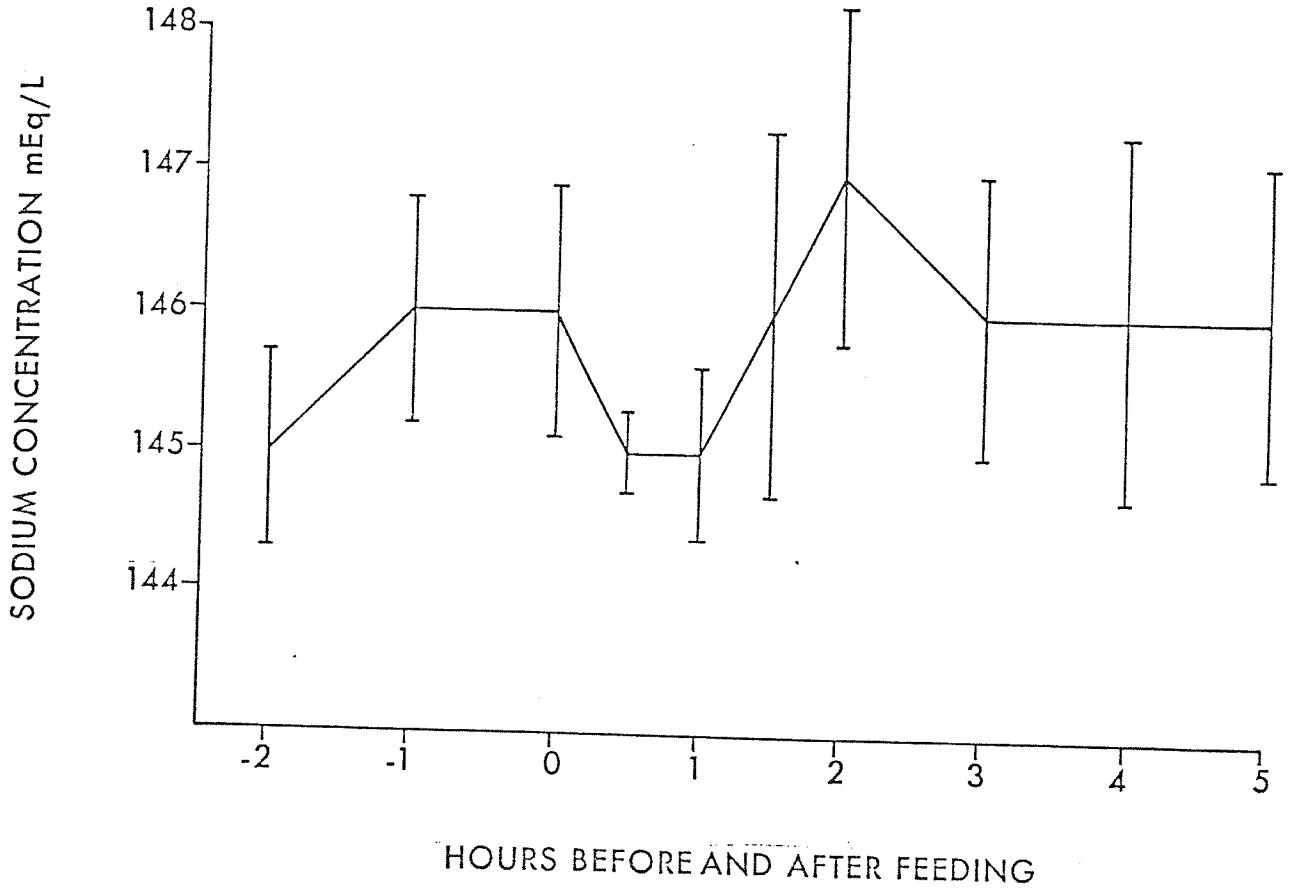


FIGURE 26 Means and standard errors for pancreatic juice potassium concentration in two sheep before and after feeding I200 g. alfalfa pellets.

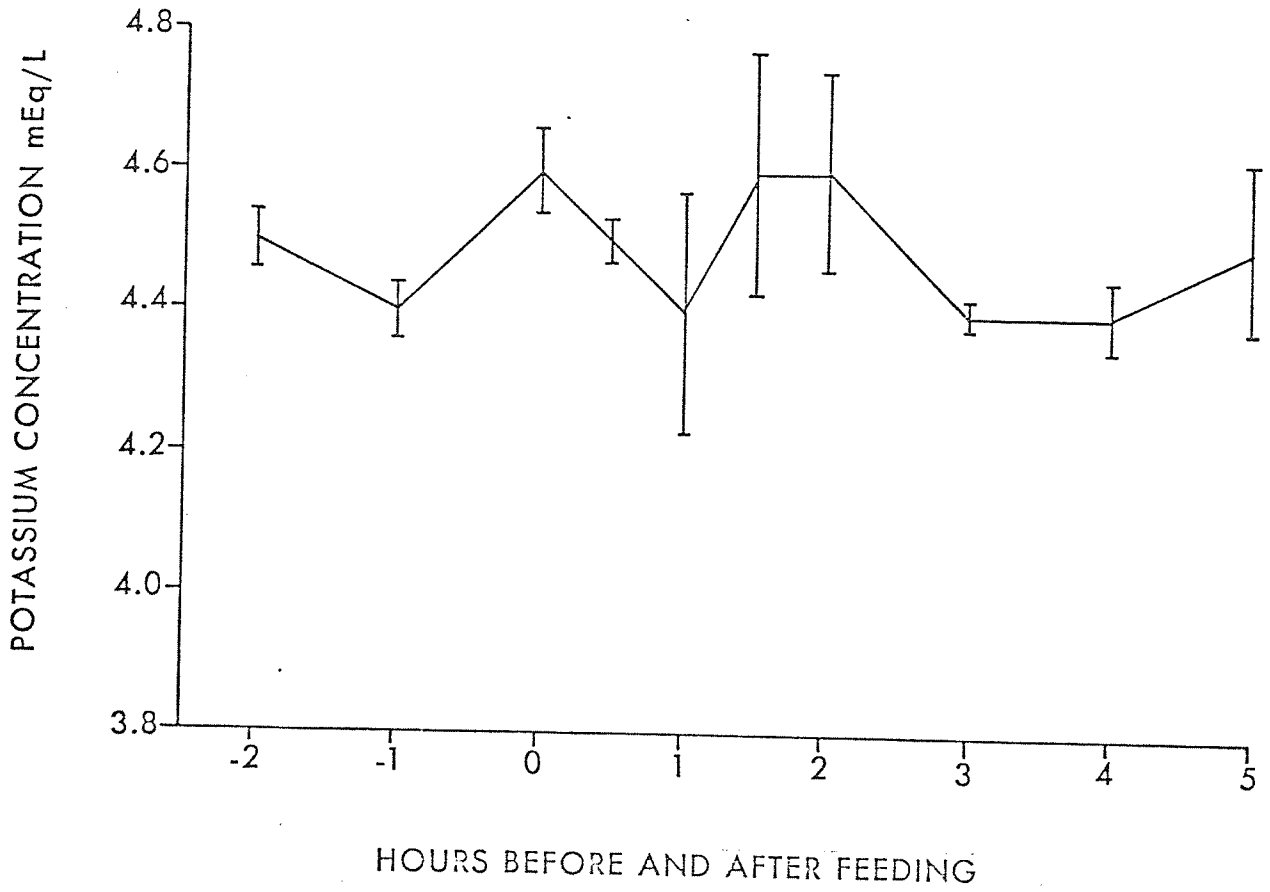


FIGURE 27 Means and standard errors for pancreatic juice calcium concentration in two sheep before and after feeding I200 g. alfalfa pellets.

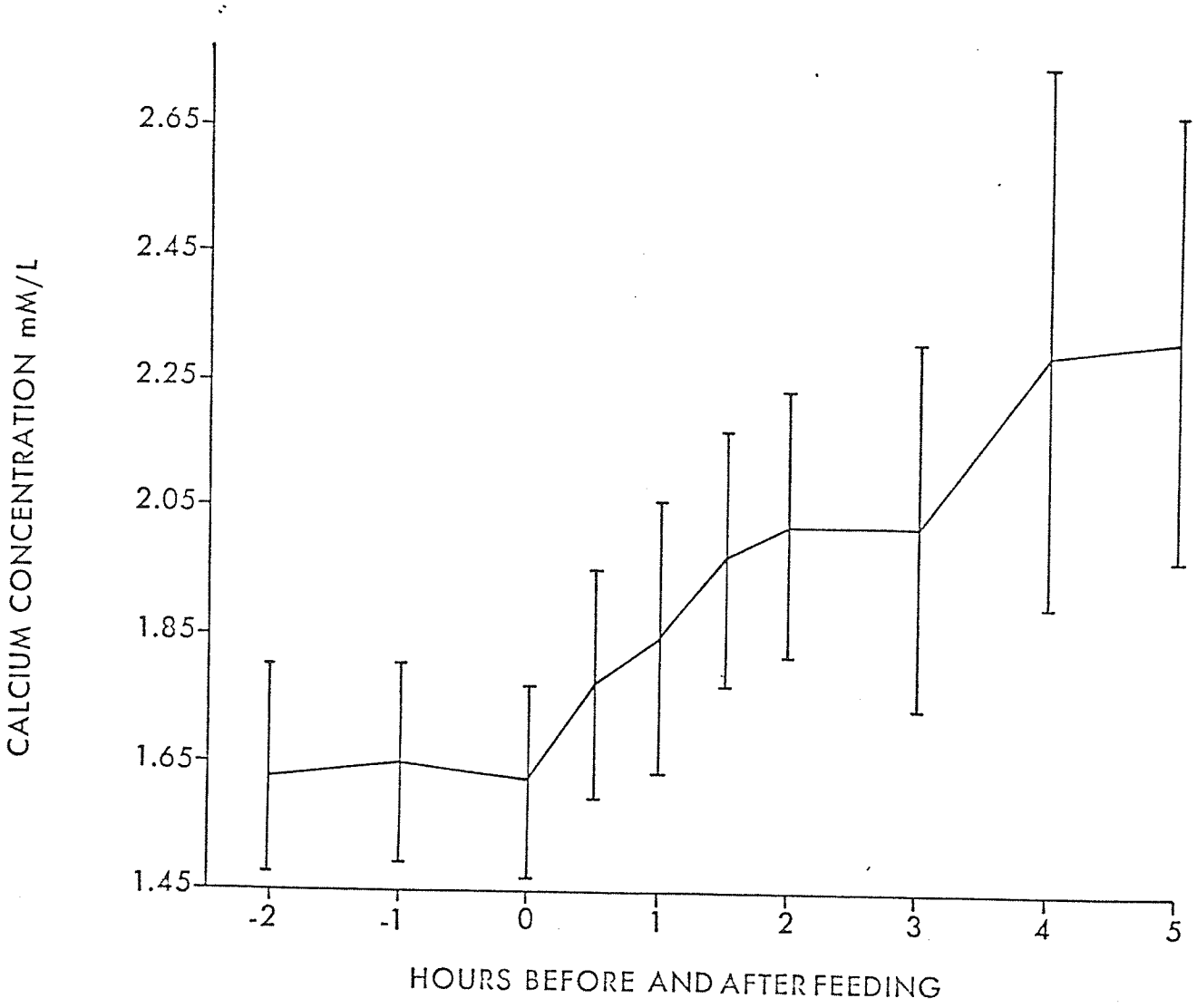


FIGURE 28 Means and standard errors for pancreatic juice phosphorous concentration in two sheep before and after feeding I200 g. alfalfa pellets.

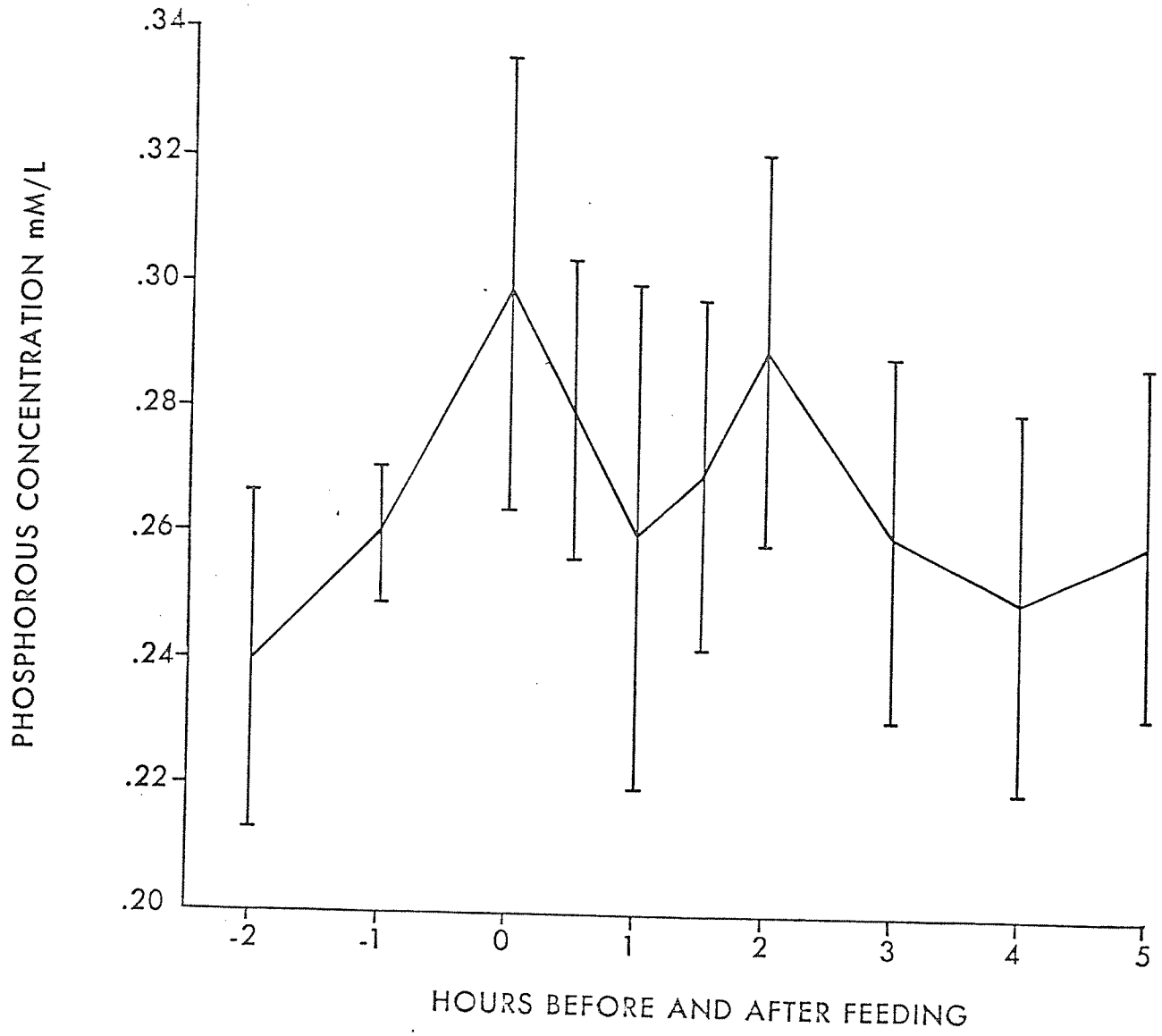


TABLE 21 Means and standard errors for pancreatic enzymes before and after feeding 1200 g. of alfalfa pellets. (n=2)

Time	Volume ml./min.	Trypsin TAME U/ml. x 10^3	Chymotrypsin BTEE U/ml. x 10^3	Lipase SIGTIETZ U/ml.	Amylase SOMOGYL U/ml.
10:00 A.M.	0.15 ± .03	49 ± 2	32 ± 1	1700 ± 150	1350 ± 200
11:00 A.M.	0.21 ± .01	56 ± 1	29 ± 2	1700 ± 50	1600 ± 200
Feed Giuen 12:00	0.22 ± .01	63 ± 2	28 ± 3	1750 ± 100	1400 ± 150
12:30 P.M.	0.31 ± .02	86 ± 17	31 ± 3	2200 ± 150	2300 ± 700
1:00 P.M.	0.35 ± .02	126 ± 5	47 ± 3	2400 ± 50	2000 ± 59
1:30 P.M.	0.54 ± .04	136 ± 1	53 ± 4	2600 ± 100	2700 ± 550
2:00 P.M.	0.52 ± .05	126 ± 9	45 ± 4	2450 ± 150	3900 ± 850
3:00 P.M.	0.52 ± .07	112 ± 23	39 ± 5	2700 ± 100	2750 ± 650
4:00 P.M.	0.40 ± .03	119 ± 19	51 ± 5	2700 ± 250	3650 ± 1500
5:00 P.M.	0.29 ± .06	124 ± 12	54 ± 3	2500 ± 100	1800 ± 300

FIGURE 29 Means and standard errors for pancreatic juice trypsin concentration in two sheep before and after feeding I200 g. alfalfa pellets.

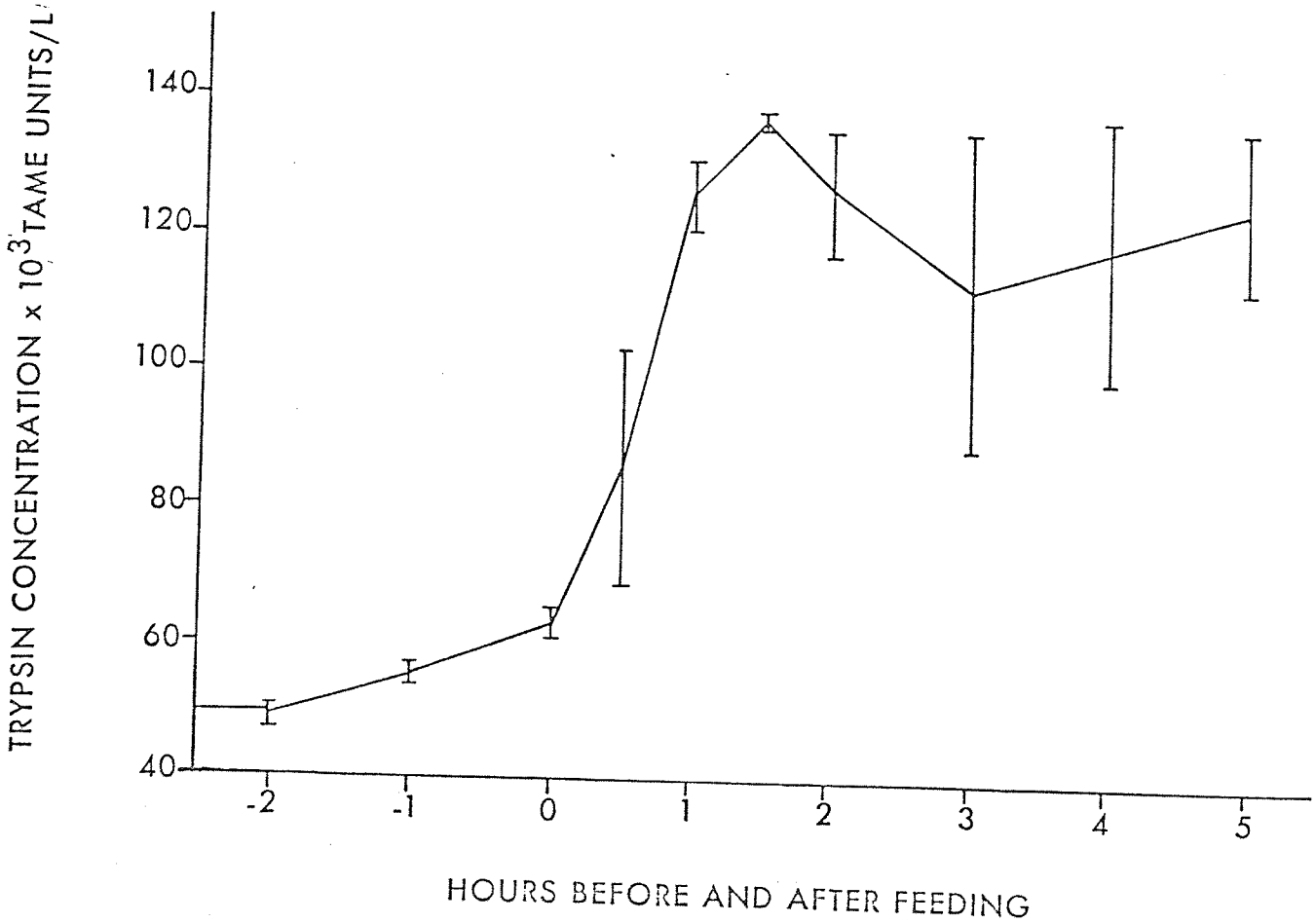


FIGURE 30 Means and standard errors for pancreatic juice chymotrypsin concentration in two sheep before and after feeding I200 g. alfalfa pellets.

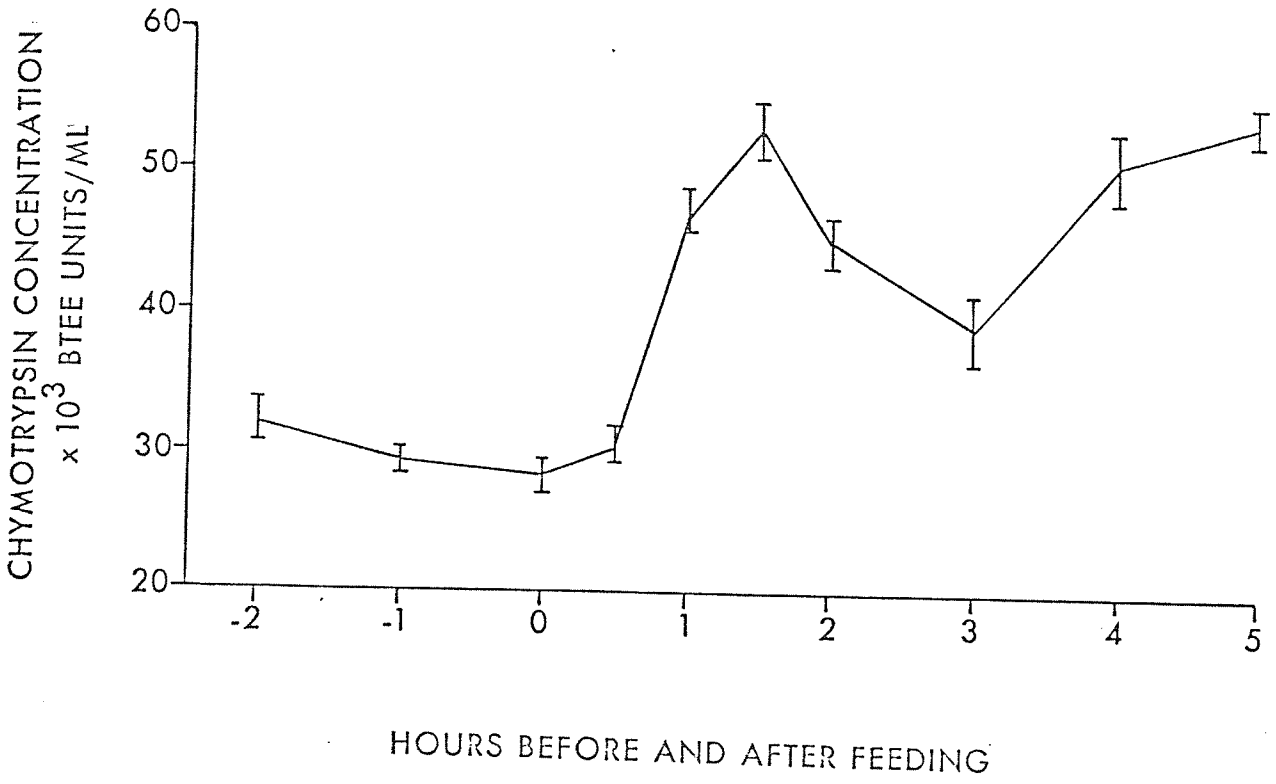


FIGURE 3I Means and standard errors for pancreatic juice lipase concentration in two sheep before and after feeding I200 g. alfalfa pellets.

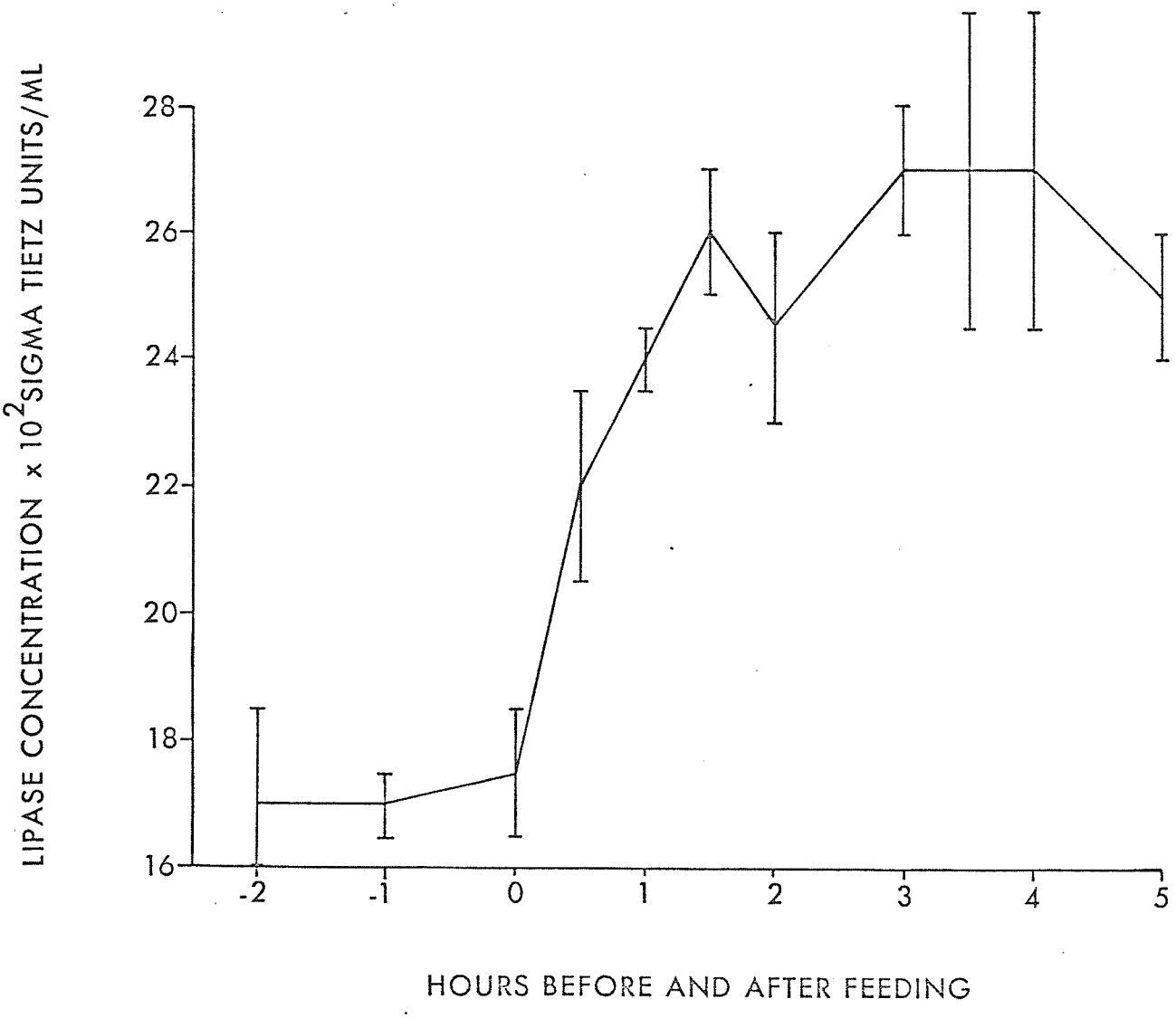
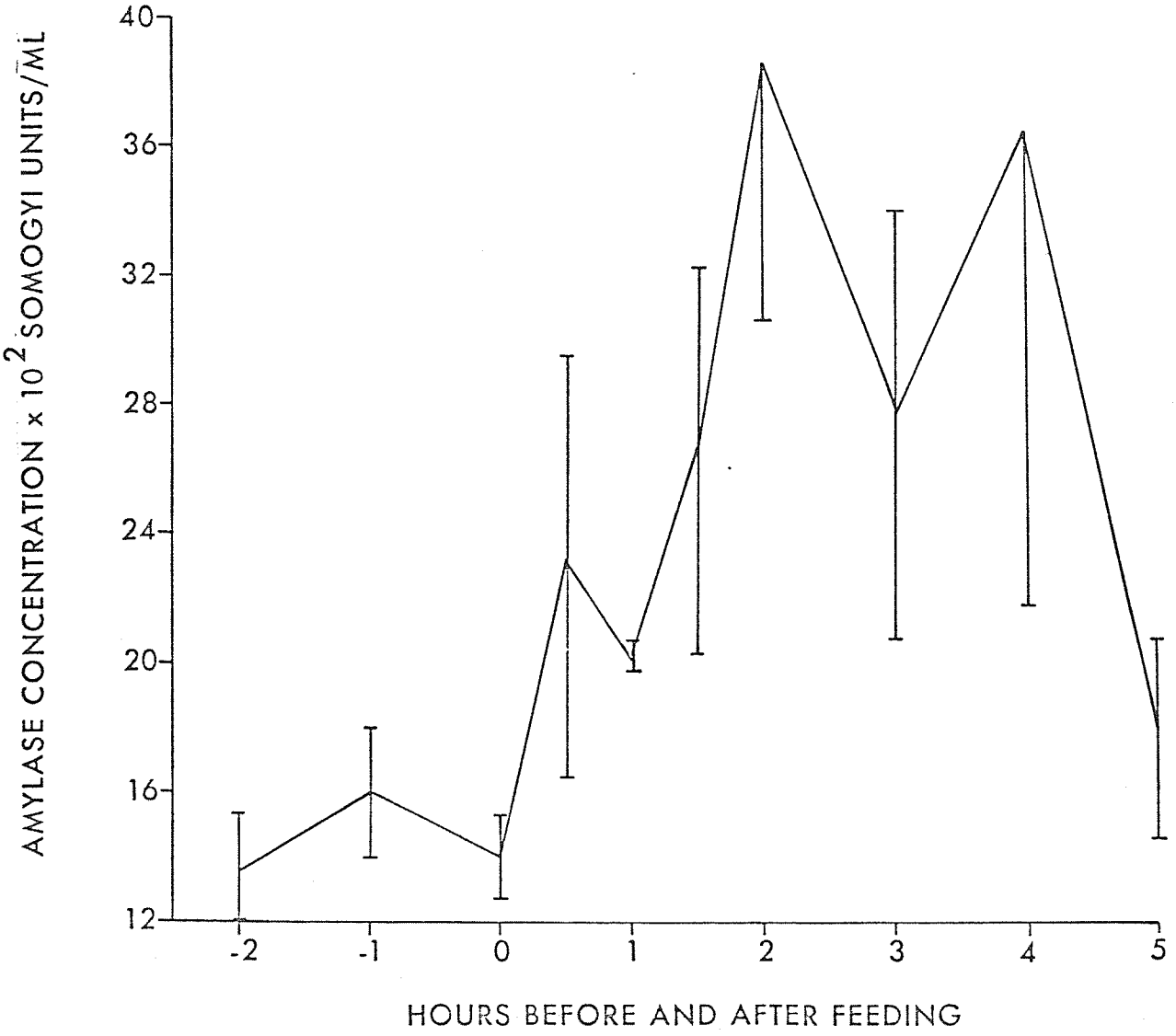


FIGURE 32 Means and standard errors for pancreatic juice amylase concentration in two sheep before and after feeding I200 g. alfalfa pellets.



EXPERIMENT 5

Bile

Volume (Table 22)

Following injection of 2.5 C.U./kg. secretin I.V. no significant increase of bile flow occured in the 5 minute samples up to 30 minutes. However during this experiment no samples from the fasting sheep prior to injection of secretin were taken. Comparing the mean bile flow from fasted sheep in experiment 3 (table 16) of 0.59 ± .02 ml./min., to the mean bile flow of 1.31 ± .12 ml./min. following secretin injection (table 22) a significant increase was observed. Since no pre injection values were obtained for this experiment, the values for fasted sheep from experiment 3 were used as pre injection values.

Electrolytes in Bile (table 22)

The mean value of sodium concentration in bile of fasted sheep was not significantly different from fasted sheep following secretin injection. Following secretin injection no demonstrable change in sodium concentration was observed over the six samples taken after secretin injection.

Similar results were obtained for potassium, and calcium with respect to significant changes in concentration following secretin injection. The mean potassium concentration was 4.5 ± .1 mM/1. prior to secretin injection and 5.0 ± .1 mM/1. following secretin injection, while calcium concentrations were 1.3 ± .1 mM/1. and 1.4 ± .1 mM/1. before and after secretin injection respectively.

The concentration of phosphate increased significantly from .09 ± .01 mM/1. for fasting sheep to .31 ± .01 mM/1. following secretin injection.

TABLE 22 Means and standard errors for bile volume and electrolyte concentrations after 2.5 C.U./kg secretin injection at time 0 in fasting sheep. (n=2)

Time minutes	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
0-5	0.93 ± .09	153 ± 1	5.0 ± .1	1.4 ± .1	.32 ± .02
5-10	1.39 ± .38	153 ± 2	5.0 ± .1	1.4 ± .1	.31 ± .02
10-15	1.46 ± .45	154 ± 1	5.0 ± .1	1.4 ± .1	.30 ± .02
15-20	1.50 ± .07	151 ± 3	4.9 ± .1	1.4 ± .1	.30 ± .01
20-25	1.66 ± .83	155 ± 1	5.0 ± .1	1.4 ± .1	.30 ± .01
25-30	0.94 ± .82	154 ± 1	5.0 ± .1	1.4 ± .1	.30 ± .03
$\bar{X}$ ± S.E.	1.31 ± .12	153 ± 1	5.0 ± .1	1.4 ± .1	.31 ± .01

Phosphate was the only bile electrolyte to change concentration following secretin injection.

Pancreatic Secretion (table 23)

Volume

Following 2.5 C.U./kg secretin injection a significant increase in pancreatic secretion was observed during the second 5 minute sample (table 23). The mean pancreatic juice flow rate over the 30 minute period after secretin injection was .68 ± .20 ml./min. which was significantly higher than the flow rate of 0.16 ± .01 ml./min. observed in fasting sheep (table 17).

Pancreatic electrolytes (table 23)

Mean sodium concentration following I.U. injection of 2.5 C.U./kg secretin was 153 ± 1 mM/l. which was significantly higher than the mean fasting sodium concentration of 149 ± 1 mM/l.

The mean values for potassium indicate a significant increase from 4.4 ± .1 mM/l. during fasting to a mean of 5.5 ± .3 mM/l. following secretin injection.

Calcium concentration also increased significantly from 1.4 ± 0 mM/l. to 1.6 ± .1 mM/l., the means during fasting and following secretin injection respectively. Phosphate concentration decreased from .25 ± .01 mM/l. to .12 ± .01 mM/l. following secretin injection.

The pattern of electrolyte secretion observed following secretin injection was similar in pancreatic juice to bile secretion with the exception of phosphate ion.

Pancreatic enzymes (table 24)

Table 24 contains the mean values for enzyme concentration following

TABLE 23 Means and standard errors for pancreatic secretion volumes and electrolyte concentrations after 2.5 C.U./kg. secretin injection at time 0 in fasting sheep. (n=2)

Time minutes	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
0-5	0.71 ± .35	153 ± 1	5.3 ± .1	1.5 ± .1	.13 ± .01
5-10	1.47 ± .13	153 ± 1	5.8 ± .5	1.7 ± .1	.13 ± .01
10-15	0.57 ± .19	153 ± 1	5.3 ± .1	1.6 ± .1	.12 ± .01
15-20	0.41 ± .32	154 ± 1	5.5 ± .3	1.6 ± .1	.11 ± .01
20-25	0.59 ± .06	152 ± 1	5.5 ± .3	1.6 ± .1	.12 ± .01
25-30	0.32 ± .12	152 ± 1	5.4 ± .3	1.6 ± .1	.11 ± .01
$\bar{X}$ ± S.E.	0.68 ± .20	153 ± 1	5.5 ± .3	1.6 ± .1	.12 ± .01

TABLE 24 Means and standard errors for pancreatic enzymes after 2.5 C.U./kg. secretin injection at time 0 in fasting sheep. (n=2)

Time minutes	Volume ml./min.	Trypsin TAME U/ml. X 10^3	Chymotrypsin BTEE U/ml. X 10^3	Lipase SIGTIETZ U/ml.	Amylase SOMOGYL U/ml.
0-5	0.71 ± .35	36 ± 2	31 ± 2	1100 ± 150	750 ± 300
5-10	1.47 ± .13	40 ± 2	31 ± 1	1000 ± 200	850 ± 300
10-15	0.57 ± .19	44 ± 5	30 ± 1	850 ± 50	250 ± 150
15-20	0.41 ± .32	38 ± 1	34 ± 1	1250 ± 500	600 ± 150
20-25	0.59 ± .06	41 ± 4	30 ± 1	1000 ± 250	350 ± 150
25-30	0.32 ± .12	42 ± 6	30 ± 1	750 ± 10	300 ± 150
$\bar{X}$ ± S.E.	0.68 ± .20	40 ± 3	31 ± 1	990 ± 190	520 ± 200

secretin injection. No significant peaks occur for any of the enzymes following secretin injection. These results were compared to enzyme concentrations in fasting sheep (table 18). The comparison indicated that no significant changes in enzyme concentration occurred from fasting to post injection periods with the exception of chymotrypsin. Chymotrypsin concentration decreased from a mean fasting value of $38 \times 10^3 \pm 1 \times 10^3$ BTEE units/ml. to post secretin injection mean of $31 \times 10^3 \pm 1 \times 10^3$ BTEE units/ml.

EXPERIMENT 6

Bile (table 25)

Volume

Following an I.V. injection of 2.5 C.U./kg. CCK bile flow rate increased significantly to 2.48 ± 0.28 ml./min in the first 5 minutes as compared to a flow rate of 0.59 ± .02 ml./min for fasting sheep. The mean flow rate of 0.97 ± 0.13 ml./min. for the 30 minutes after CCK injection was also significantly greater than the flow rate observed for fasting sheep although flow rates steadily decreased to a value of 0.45 ± .05 ml./min. at 30 minutes after CCK-PZ injection.

Electrolytes

Sodium and potassium concentrations in bile did not change significantly following CCK injection and were comparable to the concentration found for fasting sheep in experiment 3 (table 16). The mean sodium concentration for bile over 30 minutes following CCK injection was 154 ± 2 mM/l. while the mean potassium concentration was 4.7 ± .3 mM/l.

Both calcium and phosphate concentrations in bile increased significantly above those observed for fasting sheep. Calcium concentration increased from 1.3 ± .1 mM/l. to 1.7 ± .3 mM/l. and phosphate from .09 ± .01 mM/l. to .44 ± .05 mM/l. (table 25).

Pancreatic Secretion

Volume (table 26)

Following CCK injection pancreatic secretion increased to a peak value of .58 ± .06 ml./min. within the first five minutes declining steadily to .20 ± .06 ml./min. at 20 minutes after injection. This increase

TABLE 25 Means and standard errors for bile volume and electrolyte concentrations after 2.5 C.U./kg. CCK injection at time 0 in fasting sheep (n=2)

Time minutes	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
0-5	2.48 ± .28	153 ± 1	4.7 ± .3	1.5 ± .2	.46 ± .04
5-10	1.25 ± .05	154 ± 1	4.9 ± .1	1.5 ± .1	.44 ± .06
10-15	0.80 ± .01	153 ± 2	5.0 ± .1	1.5 ± .1	.44 ± .04
15-20	0.38 ± .22	154 ± 4	4.3 ± .7	2.0 ± .5	.44 ± .04
20-25	0.44 ± .17	154 ± 4	4.5 ± .3	1.8 ± .4	.43 ± .05
25-30	0.45 ± .05	155 ± 4	4.5 ± .1	1.8 ± .3	.43 ± .05
$\bar{X}$ ± S.E.	0.97 ± .13	154 ± 2	4.7 ± .3	1.7 ± .3	.44 ± .05

was significantly above the flow rate 0.16 ± .01 ml./min. observed in fasting sheep (table 17).

Electrolytes (table 26)

Of the electrolytes studied, only calcium concentrations increased significantly following CCK injection. The concentration for calcium in pancreatic secretion of fasting sheep was 1.4 ± .1 mM/l. and following CCK injection the mean for 20 minutes was 2.8 ± 1.3 mM/l.

Enzymes (table 27)

Following injection of 2.5 C.U./kg CCK trypsin concentrations had a mean of $54 \times 10^3 \pm 6 \times 10^3$ TAME units/ml. over the first 20 minutes. This mean concentration was significantly higher than $39 \times 10^3 \pm 2 \times 10^3$ TAME units/ml. observed for fasting sheep (table 18).

Chymotrypsin concentrations increased to a mean of $42 \times 10^3 \pm 10 \times 10^3$ BTEE units/ml. but this was not significantly greater than $38 \times 10^3 \pm .6 \times 10^3$ BTEE units/ml. observed in fasting sheep.

Lipase concentrations increased from 790 ± 25 Sigma Tietz units/ml. observed for fasting sheep to a mean of 1330 ± 310 Sigma Tietz units/ml over 20 minutes following CCK injection.

Amylase concentrations increased significantly following CCK injection from 400 ± 20 Somogyi units/ml observed for fasting sheep to a mean of 3220 ± 430 Somogyi units/ml. (table 27).

TABLE 26 Means and standard errors for pancreatic secretion volume and electrolyte concentrations after 2.5 C.U./kg. CCK injection at time 0 in fasting sheep. (n=2)

Time minutes	Volume ml./min.	Sodium mM./l.	Potassium mM./l.	Calcium mM./l.	Phosphate mM./l.
0-5	0.58 ± .06	151 ± 2	4.4 ± .3	2.4 ± .7	.30 ± .09
5-10	0.40 ± .01	154 ± 1	5.1 ± .8	3.4 ± 1.6	.26 ± .09
10-15	0.28 ± .12	150 ± 1	4.4 ± .6	3.3 ± 2.3	.31 ± .07
15-20	0.20 ± .06	151 ± 1	4.7 ± .3	2.1 ± .5	.29 ± .07
20-25	<0.02				
25-30	<0.02				
$\bar{X}$ ± S.E.	0.37 ± .06	152 ± 1	4.6 ± .5	2.8 ± 1.3	.29 ± .08

Missing values resulted from a lack of adequate sample obtained for electrolyte analysis.

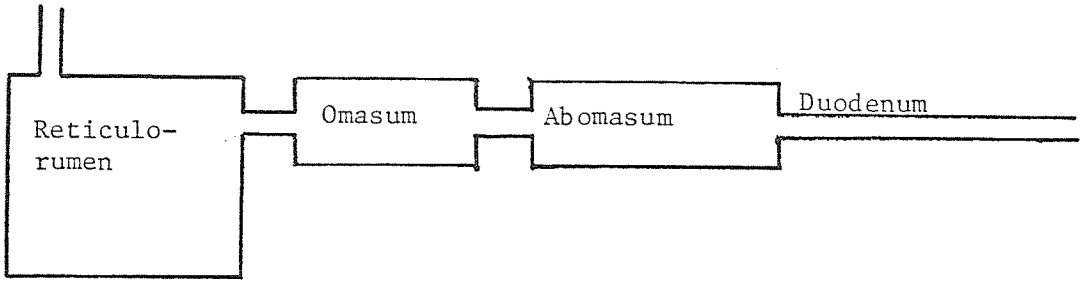
TABLE 27 Means and standard errors for pancreatic enzymes after 2.5 C.U./kg. CCK injection at time 0 in fasting sheep . (n=2)

Time minutes	Volume ml./min.	Trypsin TAME U/ml. X 10^3	Chymotrypsin BTEE U/ml. X 10^3	Lipase SIGTIETZ U/ml.	Amylase SOMOGYL U/ml.
0-5	0.58 ± .06	67 ± 13	47 ± 13	1230 ± 210	2950 ± 280
5-10	0.40 ± .01	65 ± 4	44 ± 10	1450 ± 260	3130 ± 220
10-15	0.28 ± .12	45 ± 3	44 ± 10	1350 ± 350	3460 ± 550
15-20	0.20 ± .06	40 ± 2	34 ± 8	1300 ± 410	3320 ± 680
20-25	<0.02				
25-30	<0.02				
$\bar{X}$ ± S.E.	0.37 ± .06	54 ± 6	42 ± 10	1330 ± 310	3220 ± 430

Missing values resulted from a lack of adequate sample obtained for electrolyte analysis.

DISCUSSION

Immunoreactive serum gastrin concentrations were shown to be dependent on a number of factors which regulate the release of gastrin in monogastrics. Increased gastrin release was demonstrated with antral distention in dogs (Grossman, 1967) and this response was dependant on neural reflexes. Factors such as amino acids (Blair, et. al., 1975), and alcohols (Grossman, 1967) were also shown to increase release of gastrin from antral "G" cells. Both neural and chemical factors appear to play a significant role in the release of gastrin. We examined the effect of various fed states on the concentration of immunoreactive serum gastrin in sheep. Our results indicated that when sheep fasted, gastrin levels fell to values of around 20 pg/ml. Following feeding these values increased to around 30 pg/ml. within one hour. These values were lower than those observed for sheep fed continuously. Assuming that an antral "G" cell which releases gastrin is present in the ruminant abomasum it is suggested that for sheep on continuous feed a constant stimulus is present in the abomasum which may be both mechanical (via distension) and chemical (via substances such as proteins in chyme). When fasted sheep were fed some increase in gastrin release was seen which may be because of a neural reflex to the abomasum. This increase however was not large enough to reach gastrin levels seen in the continuous fed state situation which suggests that the feed consumed in the fasting state did not stimulate the abomasum directly but rather entered the rumen. To illustrate this, a model is presented below.



The reticulo rumen acts as a receiving organ for all feed consumed. As the rumen fills and microbial digestion takes place some of the digested material enters the omasum and then abomasum. The amount of digesta that enters the abomasum is dependent on the amount of feed in the rumen and the digested state of the feed. Therefore in the fasting animal the rumen would be relatively empty with little digesta entering the abomasum and hence little stimulation of gastrin secreting cells. After feeding fasting sheep, most feed would have entered the rumen& as expected this would result in a small but significant increase in gastrin concentration, which remained below animals on continuous feed. In the continuously fed state the abomasum would be constantly exposed to a continuous flow of digesta from the rumen. This would be as the relatively constant serum gastrin levels observed in sheep on continuous feed (figure 14).

When sheep were fed once daily the serum gastrin levels increased to values around 65 pg/ml. from pre fed values of 50 to 57 pg/ml. The rumen prior to feeding would not be expected to be empty on a once daily feeding schedule and would therefore empty some of its contents soon after rapid feed consumption. This should suddenly increase the flow of digesta to the abomasum and stimulate gastrin release which

would explain the observed spike in immunoreactive serum gastrin. The spike is followed by a decline, but the fasting level did not reach serum gastrin concentrations found for sheep fed continuously. Of interest is the serum gastrin values for sheep prior to feeding once daily. These values remained above 50 pg/ml. during the 2 - hours prior to feeding at which time expected values would be lower than those observed in continuously fed animals. Unfortunately we do not have data for the gastrin concentrations over 24 hours. Data of this type may indicate that gastrin levels actually began increasing prior to feeding when animals were adjusted to a certain feeding schedule. The increase prior to feeding may be associated with vagal release of gastrin in the "cephalic" phase.

Following feeding in both the fasted sheep and those fed once daily, a spike in gastrin concentration occurred within 1½ hours following feeding. The concentration of gastrin then diminished which may be because of inhibition of gastrin release by other hormones as secretin (Konturek, et.al., 1977) or VIP (Barbezat and Grossman, 1971) or that acid release was actively inhibiting gastrin release as suggested by Walsh et. al. (1975) for monogastrics.

Further studies are required to determine whether gastrin release is directly stimulated by abomasal distention, similar to the antral distention response observed in monogastrics (Grossman, 1967).

Experiments Ia, Ib and Ic demonstrate a relationship of serum gastrin concentration to the fed state of sheep and that serum gastrin concentractions were dependent on time following feeding. The results support the hypothesis of gastrin release associated with

abomasal distension but other factors should also be considered.

CCK-PZ inhibits gastric acid secretion in dogs but weakly stimulates gastric acid secretion in cats (Stening and Grossman, 1969a). Odori, et. al. (1970) also demonstrated that CCK-PZ inhibited gastric acid production which had been stimulated by gastrin, probably because of competition for the gastrin receptor. To determine whether or not gastrin release is inhibited by CCK-PZ, 2.5 C.U./kg. were injected intravenously ½ hour after feeding. Experiment 1b demonstrated that serum gastrin concentrations increased following feeding and therefore injection of CCK-PZ should demonstrate inhibition of gastrin release if it exists. Due to the variable serum gastrin concentrations observed following CCK-PZ injection we were unable to clearly demonstrate an inhibitory action on gastrin but the data do suggest that CCK-PZ weakly inhibited gastrin release. It appears that CCK-PZ may inhibit the action of gastrin by competing for a gastrin receptor although this has not been demonstrated for ruminants.

Secretin was shown to inhibit gastrin release following a meal in the dog (Konturek, et. al., 1977). In the dog the capacity for neutralization of acid chyme entering the duodenum is much less than the gastric capacity to secrete acid and therefore secretin may be expected to act as a strong inhibitor of gastrin release, as is the case. The capacity to neutralize acid chyme may be linked to secretin's ability to inhibit gastrin release. In man the capacity to neutralize acid chyme by bile and pancreatic juice is high, but for the dog it is low (Johnson, 1974). In sheep the neutralizing capacity of acid chyme by

pancreatic juice and bile is also low(Caple and Heath,1974). Therefore it was expected that secretin would inhibit gastrin release. The same rationale was applied before, that secretin would demonstrate inhibition on gastrin release following feeding. The results demonstrated a reduction in serum gastrin concentrations following secretin injection although this change was not large. However the increase of serum gastrin to values of 67 pg./ml. observed after feeding in experiment 1b was definitely eliminated by secretin injection. It was concluded that secretin plays an important physiological role in the inhibition of gastrin release. This mechanism would prevent over acidification of the small intestine in ruminants by reducing abomasal secretion of acid.

To examine factors regulating bile and pancreatic juice volume and electrolyte composition we used sheep with surgically implanted catheters in the bile and pancreatic ducts (figure C). The experiments (2a, 2b, 2c, 3, 4, 5 and 6) examined the effects of fasting, continuous feeding at 3 levels of feed intake, once a day feeding, intravenous secretin infusions and intravenous CCK-PZ infusions, on the flow rate and composition of bile and pancreatic juice. The data for these experiments are summarized for purposes of this discussion in figures 33, 34 and 35.

Volume

Bile

Caple and Heath (1972) demonstrated that bile flow rate was dependent

on the enterohepatic circulation of bile salts (mainly sodium taurocholate) and secretin concentration in the serum. When bile was sampled for 48 hrs. with no return of bile to the duodenum a significant decline in bile production occurred after 4 hours of sampling, resulting in a mean flow rate of 0.31 ± .02 ml./min. over the 12 to 48 hour collection period. This reduction was attributed to the interuption of the enterohepatic circulation of bile salts. Intraduodenal infusion of 55 umol/minute sodium taurocholate increased the basal flow rate to 0.66 ± .04 ml./min. The data were obtained within a maximum of 45 minutes of continuous sampling without return of bile to the duodenum. No significant change in bile volume or ion composition occured over this period which was taken to indicate that our sampling procedure did not significantly alter the enterhepatic circulation of bile salts sufficiently to reduce bile flow rates.

Fasting sheep had flow rates similar to those for sheep fed continuously at 600 g. feed/24 hrs. When feed levels were increased to 1300 g./24 hrs., 1700 g./24 hrs. or the sheep were fed once daily at 1200 g., the bile flow rate increased 3 fold over the fasting values. These data clearly demonstrated that increased feed intake or once a day feeding results in peak bile flow rates between 1.6 to 2.0 ml./min.

Secretin increased bile flow rate in man (Konturek, 1970; Domschke et. al., 1976) in dogs (Konturek, 1971) in monkeys (Gardiner and Small, 1976) and in sheep (Magee, 1961; Heath, 1970; Caple and Heath, 1972; Caple and Heath, 1974; Harrison and Hill, 1962). Our data are in agreement with the general finding of increased bile flow following secretin

infusion. The previous studies on secretin infusions into sheep used a constant infusion of secretin, but we used a bolus injection. This method resulted in a peak bile flow rate of 1.66 ± .83 at 20-25 minutes following injection. This value compared closely to that obtained by Caple and Heath (1972) who used both intravenous taurocholate infusion and 0.88 C.U. per min. of secretin. The mean bile flow rate over 30 minutes following secretin injection in our experiments was 1.31 ± .12 ml./min. which is significantly greater than the flow rate of .59 ± .02 ml./min. obtained for fasting sheep but significantly less than the values obtained for sheep fed continuously at 1300 and 1700 g. feed/24 hrs. or sheep fed once daily at 1200 g. of feed.

Infusion of 2.5 C.U./kg of CCK-PZ increased bile flow rate within the first 5 minutes to a peak value of 2.48 ± .28 ml./min. A significant decline followed over the rest of the 30 minute sampling period. The 30 minute period mean was 0.97 ± .13 ml./min. which is similar to that obtained for secretin infusion. The finding that CCK-PZ increased bile flow rate is interesting in light of the fact that the sheep we used were cholecystectomized. Caple and Heath (1971) noted an increased frequency in gall bladder contractions following feeding and that, in sheep, the contraction response could be produced by intravenous infusion of CCK-PZ. Meyer et. al., (1971) demonstrated that CCK-PZ could weakly stimulate water and bicarbonate secretion into both bile and pancreatic juice. Similar information exists for monkeys (Gardiner and Small, 1976) and rabbits (Esteller, et. al., 1977).

The data demonstrate an increased bile flow rate following administration of CCK-PZ which is not due to gall bladder emptying, thus suggesting a direct action for CCK-PZ on the ovine hepatocytes. These data do not exclude the possibility of CCK-PZ potentiating the choleretic response to secretin as is the case for pancreatic responses in man (Wormsley, 1969), cats (Brown, et. al., 1967) and dogs (Henriksen and Worning, 1967).

Bile flow rates following either secretin or CCK-PZ injection were significantly less than those obtained for sheep on 1300 and 1700 g. feed/24 hr. or for sheep fed once daily (1200 g. feed). Assuming that increased feeding increases the volume of digesta passage from abomasum to the duodenum, we would expect greater stimulation of both secretin and CCK-PZ release from the duodenal mucosa. Since both secretin and CCK-PZ appear to increase the rate of bile flow we would expect their simultaneous action to be greater than either hormone's action alone.

Pancreatic Juice

Volume

The rate of pancreatic juice flow was shown in previous studies to be dependent on vagal activity (Taylor, 1962), secretin concentrations in the serum (Horn and Huber, 1975; Magee, 1961; Caple and Heath, 1972) and serum concentrations of CCK-PZ (Stening and Grossman, 1969). The present studies examined the effect of fasting, continuous feeding at 3 levels of intake, once a day feeding and intravenous injections of secretin and CCK-PZ on flow rates of pancreatic juice. The data are summarized in figure 34 which will be referred to for comparisons between treatment effects on pancreatic juice flow rates.

During fasting and continuous feeding of 600 g. feed/24 hrs. the flow rate of pancreatic juice was .16 ml./min. Increasing the amount of feed intake to 1300 g. feed/24 hrs. and 1700 g. feed/24 hrs. significantly increased pancreatic juice flow rates to 2.2 and 2.8 ml./min. respectively. The mean peak flow rate at 2 hrs. following feeding of 1200 g. feed was .52 ml/min. This value was significantly higher than the pancreatic juice flow rates observed for continuous feeding treatments, but was similar to that observed for the mean following injection of secretin. These data may be explained by an evaluation of the flow rates of digesta in the duodenum, during the different treatments. Sheep fed continuously would be expected to have a continuous flow rate of digesta into the duodenum whereas sheep fed once daily might be expected to have a sudden increase in chyme entering the duodenum. This type of increased digesta flow rate may stimulate a large release of secretin which produced an increase in pancreatic juice flow. CCK-PZ which may also be released from the ovine duodenum may also stimulate pancreatic juice flow to increase. The data demonstrate a significant increase of pancreatic juice flow following CCK-PZ injection, to a mean flow of .37 ml./min. Following CCK-PZ injection the pancreatic juice flow rate was not as great as that observed after once a day feeding, but greater than that in sheep fed continuously.

The results for secretin's action on pancreatic flow rate confirm the trend of experiments done previously by Magee (1961), Horn and Huber (1975), and Caple and Heath (1974). Caple and Heath (1974) suggested that pancreatic juice production over a 24 hr. period should be

300-400 ml. which is in agreement with values of 200-250 ml./24 hr. for fasting sheep and of 275-360 ml./24 hr. in sheep fed 1200 g. feed/ 24 hr. observed in the present experiments.

Bile

Sodium

Little information is available for electrolyte composition in bile or pancreatic juice of sheep particularly under different treatments. The present studies demonstrate a significant increase in bile sodium concentration in sheep fed continuously, over sodium concentrations in bile of fasting sheep, 2 hrs. after feeding 1200 g. or sheep injected with secretin or CCK-PZ. Sheep fed continuously had bile sodium concentrations of 184 - 199 mM/l. These values are considerably above values of approximately 155 mM/l. seen in sheep on the other treatments previously mentioned. Caple and Heath (1972) demonstrated sodium concentrations of around 150 mM/l. and that this was not significantly changed by secretin infusions, which is in agreement with the current findings. It was also noted that injections of CCK-PZ did not significantly change concentrations of sodium in the bile of sheep.

At 2 hr after feed the sodium concentrations in bile dropped significantly to 145 ± 2 mM/l. This might be explained by the "wash out" effect (Wormsley, 1968), a term used to describe flushing out of the enzymes in pancreatic ducts. The sudden increase in bile flow observed after feeding 1200g.feed may have acted to dilute the sodium, and reduce the concentration. Sheep fed continuously would not show sudden increases in bile flow and would therefore have higher

sodium concentrations.

Pancreatic Juice

Sodium

Sodium concentrations in pancreatic juice did not increase significantly from sheep during fasting to sheep fed continuously at 600 or 1700 g. feed/24 hr. Sheep fed continuously at 1300 g. feed/24 hr. showed a small but significant increase in sodium concentration compared to fasting sheep or those fed continuously at 600 or 1700 g. feed/24 hr. These results were taken to indicate that continuous feeding at different levels of feed intake do not change sodium concentration in pancreatic juice of sheep.

Sheep receiving injections of secretin or CCK-PZ did not have higher pancreatic juice sodium concentrations compared to fasting sheep or those on continuous feed. Secretin and CCK-PZ may have a stimulating action on sodium transport into pancreatic juice but this action is coupled with an increased pancreatic juice flow rate. These data do not clearly demonstrate whether increased sodium transport into pancreatic juice obligates an increased flow rate or whether the flow rate increase is independent of sodium transport. The data for sheep fed once a day at 1200 g. feed show a significant drop in sodium concentration at 2 hr. following feeding. This coupled with the fact of an increased pancreatic juice flow rate suggests that sodium transport was not a limiting factor in pancreatic juice production.

Bile

Potassium

Potassium concentrations in bile appear to follow a similar pattern

to that observed for sodium, Caple and Heath (1974) obtained a mean potassium concentration of 4.4 ± 0.9 mM/l. in bile of sheep fasted for 48 hrs. The present studies found a value of 4.5 .1mM/l. for fasting sheep and levels of between 6.5 and 7.0 mM/l. for sheep fed continuously. At 2 hours following feeding of 1200 g. feed the potassium concentration was 5.3 ± .3 mM/l. which was significantly lower than values for continuously fed sheep but higher than values for fasted sheep. This observation differs from that for sodium where a dilution effect was used to explain the low values observed 2 hr after feeding 1200 g. feed. Unlike the results for sodium, a significant stimulation of potassium secretion was seen after secretin injection. This would explain why a drop in potassium concentrations below those for fasting sheep was not observed, since secretin release after feeding should increase potassium transport.

The results for CCK-PZ show that this hormone does not increase potassium concentration in bile, but this does not mean that it has no effect on potassium transport, merely that these data do not demonstrate such an effect.

Pancreatic Juice

Potassium

Caple and Heath (1974) obtained a mean potassium concentration of 4.6 ± 0.4 mM/l. in pancreatic secretions of sheep deprived of feed for 48 hrs. Their results are in agreement with the potassium concentration of 4.4 ± .1 mM/l. obtained for fasting sheep in these experiments. Sheep fed continuously showed significant increases in potassium concentrations over those for fasting sheep. Peak potassium concentrations of 5.5 ± .1

mM/l. were obtained for sheep fed continuously at 1700 g. feed/24 hr, As the level of continuous feed intake increased from 600 to 1700 g. feed/24 hr, a significant increase in potassium concentration occurred. This increase was attributed to increased release of secretin from the duodenal mucosa which possibly increased potassium secretion into pancreatic juice. This was supported by the significant increase in potassium concentration in pancreatic juice following intravenous injection of secretin. CCK-PZ injection did not increase potassium concentration significantly over fasting levels. Since an increase in pancreatic juice secretion was observed following CCK-PZ it was impossible to suggest that stimulation of potassium transport by CCK-PZ does not occur.

At 2 hr. after feeding 1200 g. feed potassium concentrations did not change significantly from concentrations in fasting sheep. If secretin release was stimulated following the feeding of 1200 g. feed a rise in potassium concentration would be expected since secretin administration definitely increased potassium transport into pancreatic juice. Further evidence for serum concentrations of secretin following feeding would be required to clarify this point.

Bile

Calcium

The concentration of calcium in the bile of sheep appears to be dependent on whether the animals are fed continuously or not. During the fasted state, at 2 hr. following feeding and following secretin injection the calcium concentration was between 1.2 and 1.5 mM/l.

Administration of CCK-PZ intravenously produced a rise in calcium concentration to 1.7 ± .3 mM/l. but this was not significantly different from the previously mentioned levels because of large standard error.

Sheep fed continuously had bile calcium concentrations of 2.25 ± 2.70 mM/l. with no significant differences between levels of feed intake. Continuously fed sheep had bile calcium concentrations significantly greater than all other groups studied. An explanation for the high calcium concentrations in bile of continuously fed animals is presently lacking. Information on the calcium concentrations in bile of ruminants under different treatment conditions was unavailable, thus precluding comparison with previous work.

Pancreatic Juice

Calcium

Sheep fed continuously from 600 to 1700 g. feed/24 hrs. had pancreatic juice calcium concentrations between 1.8 and 3.1 mM/l. The calcium concentration for sheep fed continuously at 1300 g. feed/24 hrs. was significantly lower than that for sheep fed continuously at either 600 or 1700 g.feed/24 hr. This difference cannot be explained.

All of the calcium concentrations in continuously fed sheep were significantly greater than those for fasting sheep or sheep receiving secretin injections. It was concluded that secretin does not stimulate increased calcium concentration in pancreatic juice of sheep. Goebell, et. al., (1972, 1973) demonstrated that CCK-PZ increased calcium concentrations in both dogs and humans and that this increase paralleled protein secretion. The present studies also demonstrate an increase in

pancreatic juice calcium concentration following CCK-PZ injection which was above those observed for fasting or secretin injected sheep. The difference between these groups was not statistically significant because of large variability between sheep but was taken to suggest physiological significance. Calcium concentrations in pancreatic juice of sheep 2 hrs. after feeding 1200 g. alfalfa pellets were significantly above those for fasting sheep and sheep receiving an injection of secretin. Assuming that feeding may stimulate release of CCK-PZ from intestinal mucosa it is likely that the increased calcium concentrations following feeding was because of CCK-PZ stimulation.

Bile

Phosphate

Phosphate concentrations in bile of fasted sheep were significantly lower than for other groups studied. At 600 g. feed/24 hrs. in continuously fed sheep the phosphate concentrations were higher than the fasting concentrations. Increasing the level of continuous feed intake reduced bile phosphate concentrations but the concentrations remained significantly above those observed in fasting sheep. Both secretin and CCK-PZ increased the phosphate concentration in bile, with CCK-PZ having the greater effect. Since these two hormones appear to strongly stimulate phosphate secretion into bile it was expected that for the sheep fed once daily at 1200 g. feed, a rise in phosphate concentration would occur, if feeding does stimulate the release of secretin and CCK-PZ. A possible explanation is that gastrin which was shown to increase following feeding in sheep may inhibit phosphate secretion into bile. This hypothesis needs further study to examine the role of gastrin in not

only phosphate secretion but all electrolytes secreted into bile and pancreatic juice. Jones and Grossman (1970) demonstrated that gastrin stimulates water and bicarbonate secretion by the liver. Gastrin's actions on the regulation of other electrolyte secretions by the liver or pancreas are currently not known.

Pancreatic Juice

Phosphate

Phosphate concentration in pancreatic juice decreased significantly from the fasted to the continuously fed state. A further decrease was observed as the level of continuous feed intake was increased from 600 to 1700 g. feed/24 hrs. These results suggest that an inverse relationship exists for rate of digesta flow through the duodenum and phosphate concentration in pancreatic juice. Intravenous injection of secretin did not increase phosphate concentrations in pancreatic juice, but it may have an inhibitory action on phosphate secretion. CCK-PZ however stimulated an increase in pancreatic phosphate concentration. At 2 hrs after feeding 1200 g. feed, phosphate concentrations rose to levels similar to those observed following stimulation with CCK-PZ, and support the possibility that CCK-PZ released by a sudden rise in duodenal flow rate following feeding does stimulate phosphate secretion. However during the fasted state, pancreatic juice phosphate concentration was also high at a time when CCK-PZ levels were expected to be low. The actual controlling factors for phosphate secretion are probably both neural and hormonal, with the interactions of gastrin, CCK-PZ and secretin being of importance for the later. Further information regarding

hormonal and neural control of all electrolyte secretions is required.

Pancreatic Enzyme Secretion

Pancreatic enzyme secretion appears to be regulated largely by the hormone CCK-PZ (Harper and Raper, 1943; Robberecht, et. al., 1975; Vaysse, et. al., 1974; Laval, et. al., 1977; Shaw and Heath, 1972; Horn and Huber, 1975). Goebell et. al. (1973) demonstrated that CCK-PZ increased parallel enzyme output in humans while Robberecht, et. al. (1977) suggested that more than one pool of intracellular enzymes exists in pancreatic acinar cells and therefore a stimulus may be specific for specific enzyme pools which would produce non parallel enzyme secretion.

The present studies examined the effects of fasting, once a day feeding, secretin and CCK-PZ injections on the pancreatic juice enzyme concentration of trypsin, chymotrypsin, lipase and amylase in sheep. The results indicate that CCK-PZ injections significantly increased the concentrations of all enzymes studied with the exception of chymotrypsin. Lipase, trypsin, chymotrypsin and amylase increased to varying percentages above fasting concentrations observed. Amylase increased the most followed by lipase then trypsin and finally chymotrypsin although the latter increase was not significantly above the chymotrypsin concentration for fasting sheep. These variable increases support the non parallel enzyme secretion hypothesis (Robberecht,et.al.,1977).

The greatest increase for all enzyme secretion observed was at two hours following feeding of 1200 g. feed in sheep fed once daily. All the enzyme concentrations increased significantly above those for fasting sheep or those receiving an injection of secretin. Trypsin

and lipase concentrations were also significantly higher than those recorded following injection of CCK-PZ. Since secretin did not significantly increase enzyme concentrations, the effect of feeding on enzyme concentration increase was suggested to be because of CCK-PZ and gastrin release following a meal. Gastrin levels which increase following a meal have been shown to strongly stimulate pancreatic enzyme secretion in dogs (Stening and Grossman, 1969a).

Further study into the action of gastrin in pancreatic enzyme secretion of ruminants is required. Whether secretin can potentiate CCK-PZ's actions on pancreatic enzyme secretion is also not known for the ruminant animal.

Figure 33 Comparison of means and standard errors for bile volume and electrolyte concentrations in sheep on 7 different treatments

A - Sheep fasted over a period of 3 days

B - Sheep fed continuously 600 g. feed/24 hrs.

C - Sheep fed continuously 1300 g. feed/24 hrs.

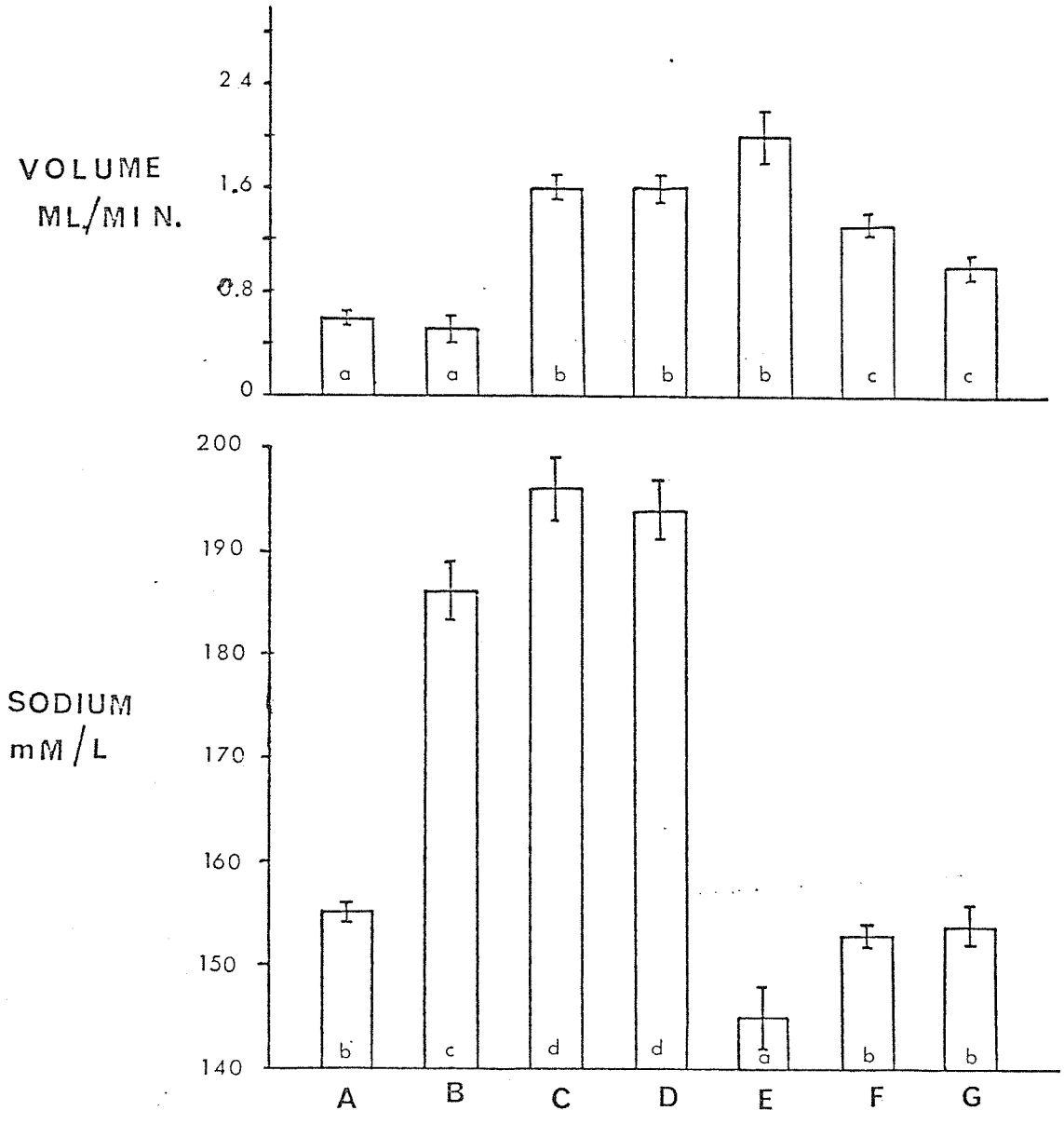
D - Sheep fed continuously 1700 g. feed/24 hrs.

E - Sheep fed once daily 1200 g. feed/24 hrs. The values indicate means at 2 hrs. after feeding.

F - Sheep injected with 75 C.U./kg. secretin. The values indicate means for 30 minutes after injection.

G - Sheep injected with 75 C.U./kg. CCK-PZ. The values indicate means for 30 minutes after injection.

Superscripts indicate significant differences between groups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b
b
b
c
c
SODIUM
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190
180
170
160
150
140
b
c
d
d
a
b
b
A
B
C
D
E
F
G

Figure 33 Comparison of means and standard errors for bile volume and electrolyte concentrations in sheep on 7 different treatments

A - Sheep fasted over a period of 3 days

B - Sheep fed continuously 600 g. feed/24 hrs.

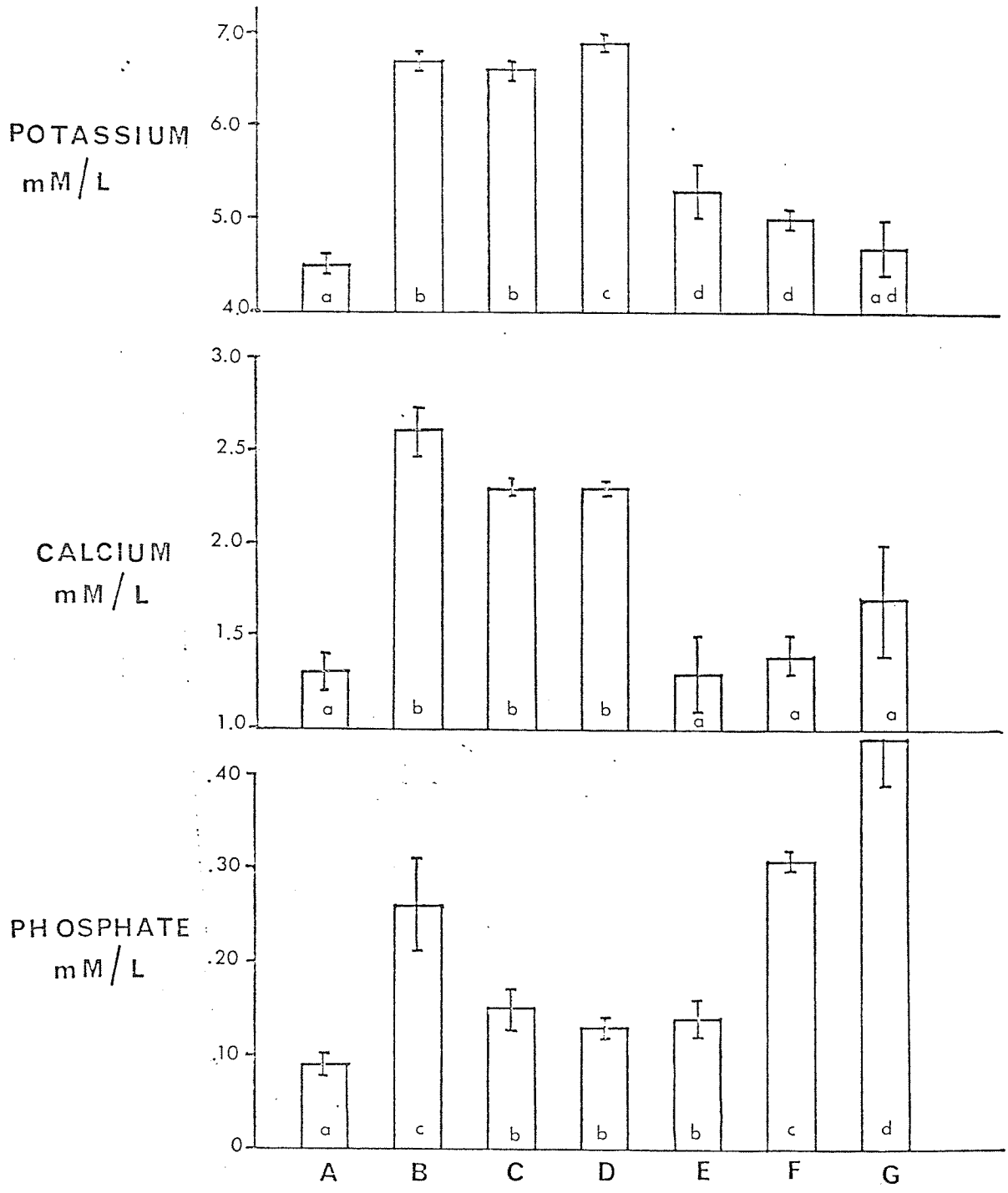
C - Sheep fed continuously 1300 g. feed/24 hrs.

D - Sheep fed continuously 1700 g. feed/24 hrs.

E - Sheep fed once daily 1200 g. feed/24 hrs. The values indicate means at 2 hrs. after feeding.

F - Sheep injected with 75 C.U./kg. secretin. The values indicate means for 30 minutes after injection.

G - Sheep injected with 75 C.U./kg. CCK-PZ. The values indicate means for 30 minutes after injection.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b
b
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d
d
a d
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mM/L
3.0
2.5
2.0
1.5
1.0
a
b
b
b
a
a
a
PHOSPHATE
mM/L
.40
.30
.20
.10
0
a
c
b
b
b
c
d
A
B
C
D
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F
G

Figure 34 Comparison of means and standard errors for pancreatic volume and electrolyte concentrations in sheep on 7 different treatments.

A - Sheep fasted over a period of 3 days

B - Sheep fed continuously 600 g. feed/24 hrs.

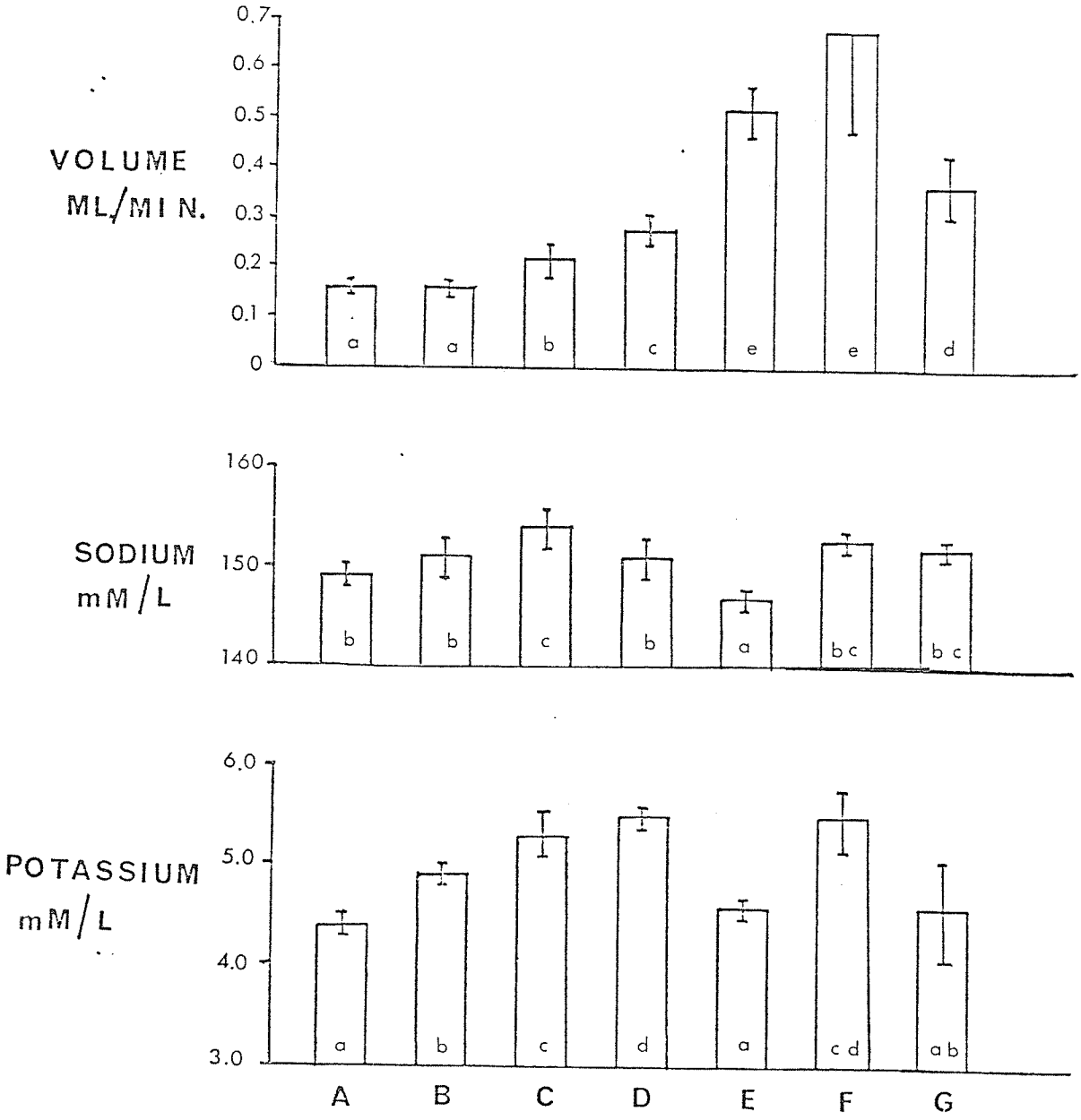
C - Sheep fed continuously 1300 g. feed/24 hrs.

D - Sheep fed continuously 1700 g. feed/24 hrs.

E - Sheep fed once daily 1200 g. feed/24 hrs. The values indicate means at 2 hrs. after feeding.

F - Sheep injected with 75 C.U./kg. secretin. The values indicate means for 30 minutes after injection.

G - Sheep injected with 75 C.U./kg. CCK-PZ. The values indicate means for 30 minutes after injection.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e
d
SODIUM
mM/L
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150
140
b
b
c
b
a
bc
bc
POTASSIUM
mM/L
6.0
5.0
4.0
3.0
a
b
c
d
a
cd
ab
A
B
C
D
E
F
G

Figure 34 Comparison of means and standard errors for pancreatic volume and electrolyte concentrations in sheep on 7 different treatments.

A - Sheep fasted over a period of 3 days

B - Sheep fed continuously 600 g. feed/24 hrs.

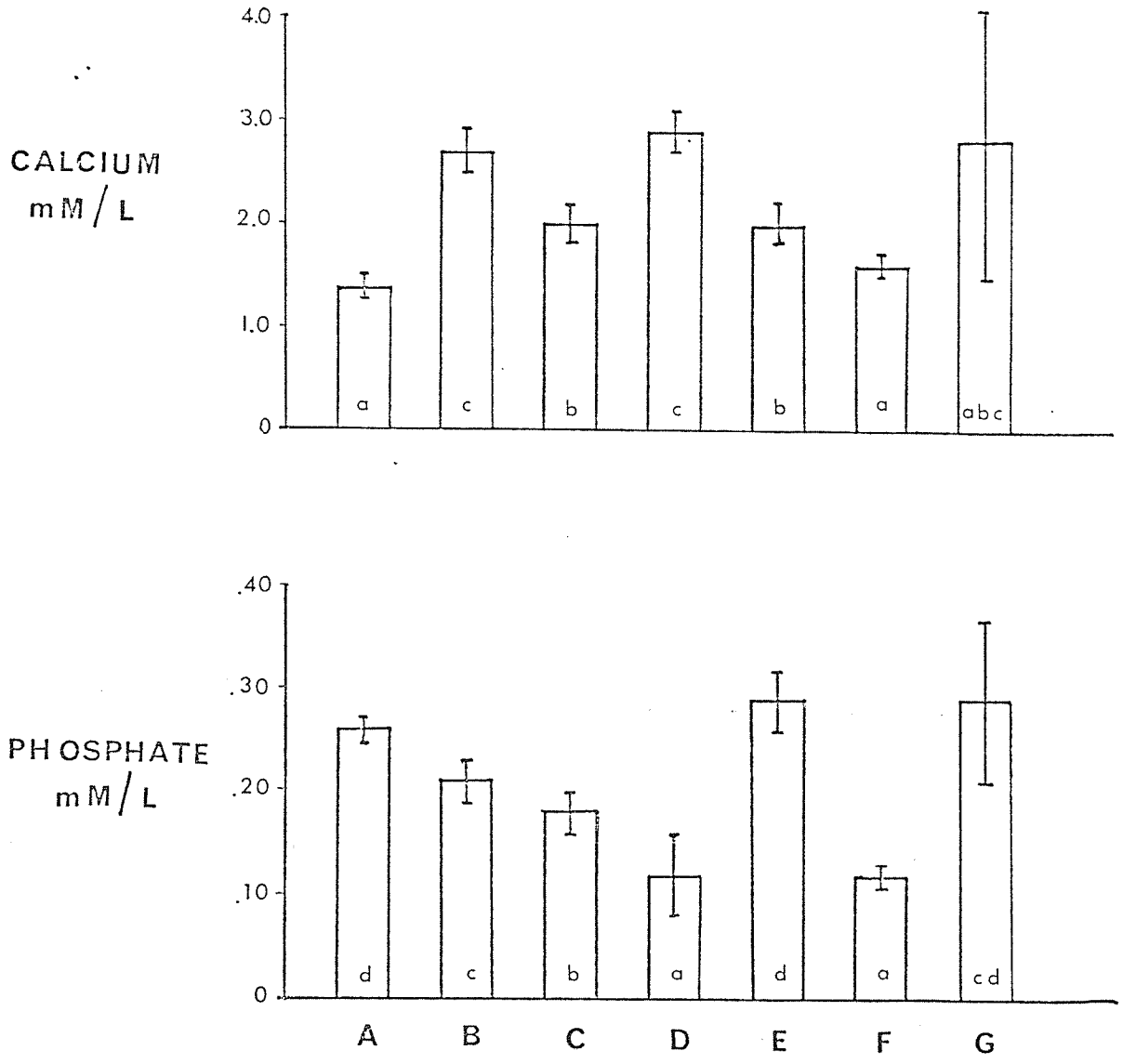
C - Sheep fed continuously 1300 g. feed/24 hrs.

D - Sheep fed continuously 1700 g. feed/24 hrs.

E - Sheep fed once daily 1200 g. feed/24 hrs. The values indicate means at 2 hrs. after feeding.

F - Sheep injected with 75 C.U./kg. secretin. The values indicate means for 30 minutes after injection.

G - Sheep injected with 75 C.U./kg. CCK-PZ. The values indicate means for 30 minutes after injection.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mM / L
.40
.30
.20
.10
0
d
c
b
a
d
a
cd
A
B
C
D
E
F
G

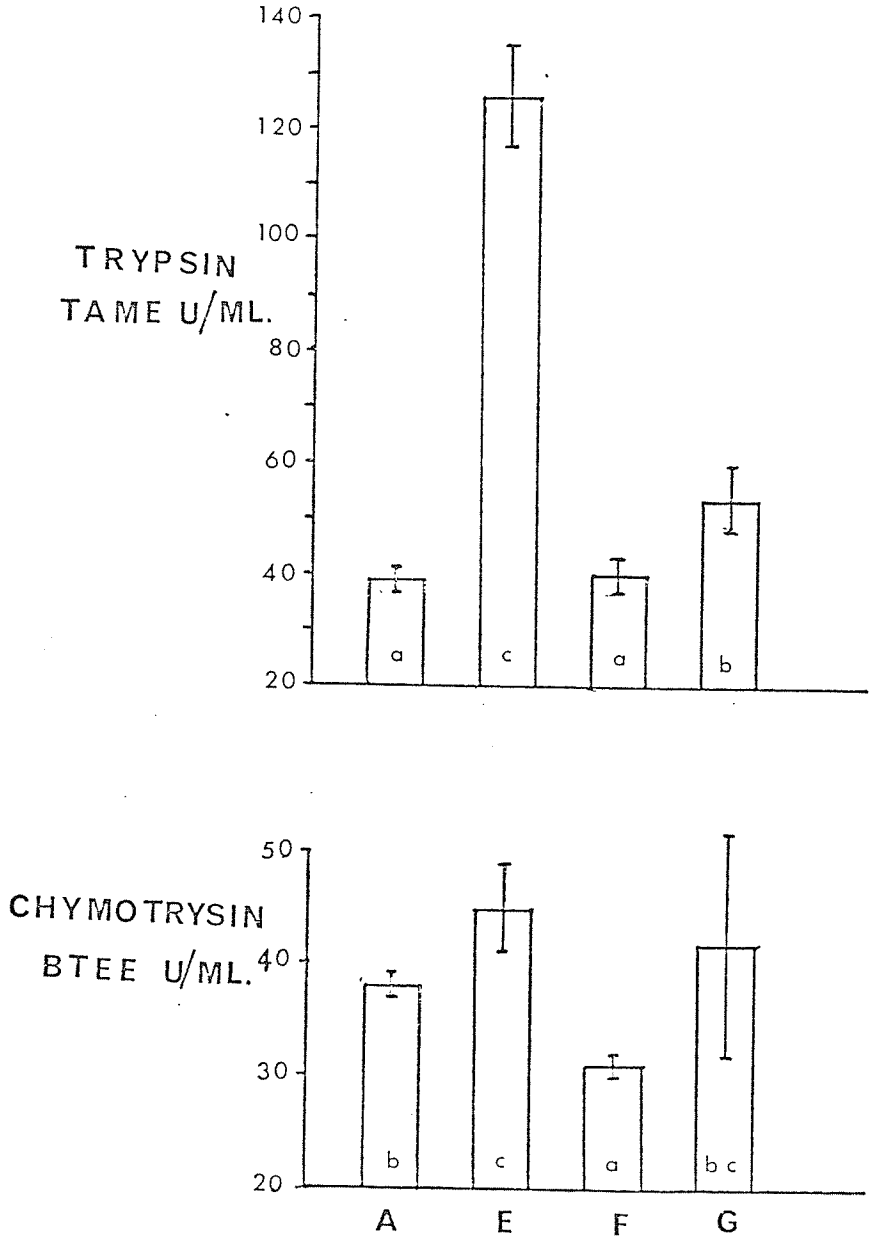
Figure 35 Comparison of means and standard errors for pancreatic juice enzyme concentrations in sheep on 4 different treatments.

A - Sheep fasted over a period of 3 days

E - Sheep fed once daily 1200 g. feed/24 hrs. The values indicate means at 2 hrs. after feeding.

F - Sheep injected with 75 C.U./kg. secretin. The values indicate means for 30 minutes after injection.

G - Sheep injected with 75 C.U./kg. CCK-PZ. The values indicate means for 30 minutes after injection.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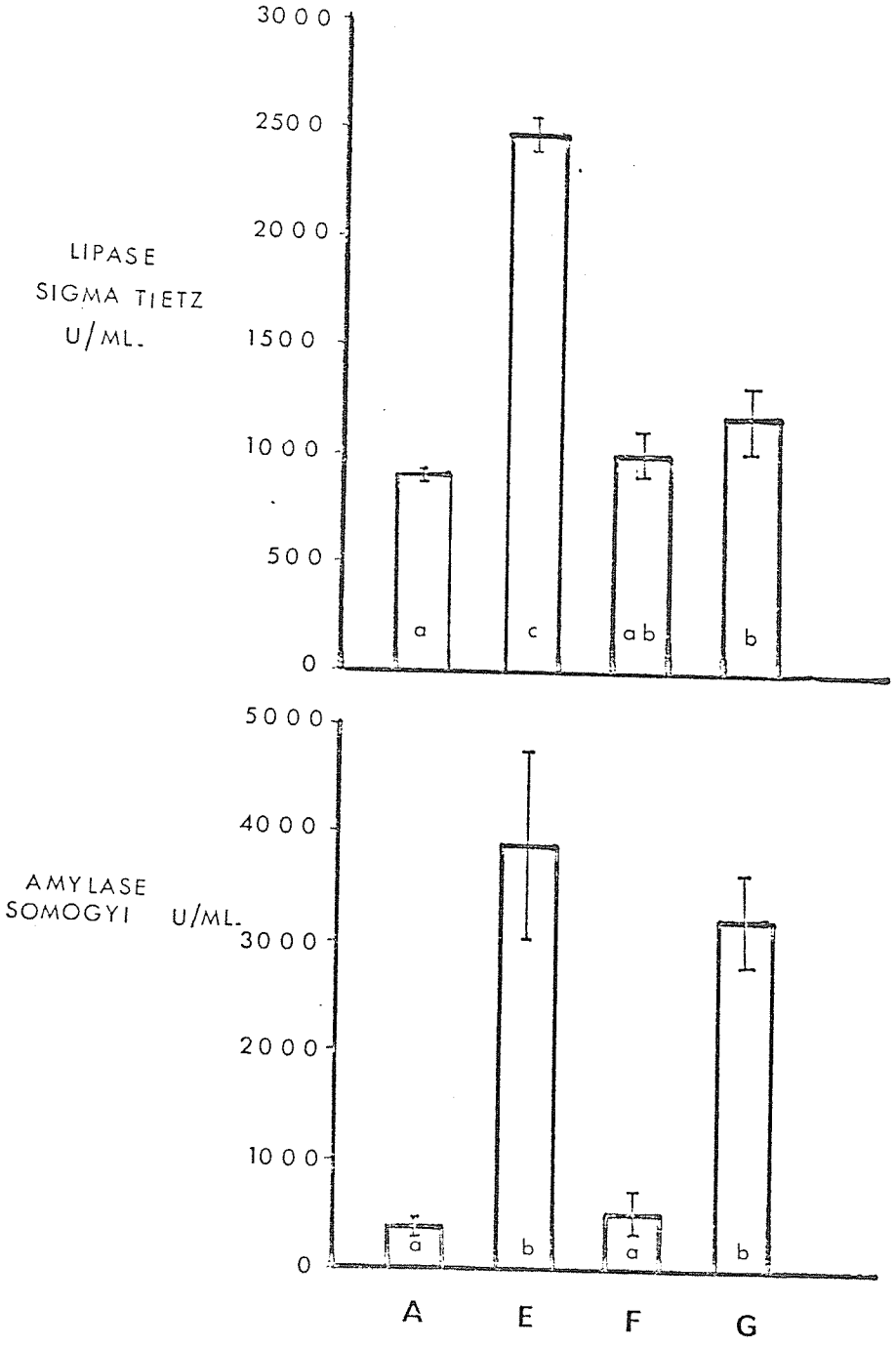
E
F
G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2000
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a
b
a
b
A
E
F
G

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