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Some Aspects of Histamine in the Sheep Stomach

Summary.

In this investigation, experiments have been designed to assess the significance of histamine as a factor affecting gastric motility in sheep.

Responses of smooth muscle from the stomach have been examined using isolated strips of reticulum, rumen, omasum and abomasum suspended in oxygenated Tyrode solution at 37°C. The response of these preparations to acetylcholine, adrenaline, histamine and 5-hydroxytryptamine (5HT) have been investigated.

All preparations contracted in response to acetylcholine. 5HT contracted all strips except omasum which were usually insensitive to this drug. The action of adrenaline varied according to both the dose and the site from which preparation were taken. In general, strips of rumen and abomasum were contracted by high concentrations and relaxed by low concentrations of adrenaline.

Histamine did not contract any of the preparations, but relaxed strips of abomasum and of rumen. It was also found that contractions of strips from these compartments in response/

SOME ASPECTS OF HISTAMINE IN THE SHEEP STOMACH

by

John Sanford B.V.Sc., M.R.C.V.S.

Thesis submitted for the Degree of Doctor of
Philosophy in the University of Glasgow.

October 1962.

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response to acetylcholine were inhibited by histamine.

The isolated tissue experiments were followed by an investigation of the effects of histamine on the stomach movements of conscious sheep fitted with ruminal and abomasal cannulae. Intravenous injections of histamine inhibited movements of the reticulum, rumen and abomasum. The reticulum recovered rapidly from inhibition but the other compartments often showed a more gradual recovery. The inhibitory action of histamine was readily prevented by mepyramine.

The action of histamine on blood pressure and respiration was examined in anaesthetised sheep. Intravenous injections caused a fall in blood pressure which was proportional to the dose given. Respirations were depressed and with larger doses a period of apnoea sometimes occurred. Larger doses of histamine given intravenously to conscious sheep provoked salivation, facial oedema, defaecation and marked temporary respiratory distress.

Free histamine may originate in the sheep either by formation and absorption in the alimentary tract or by release from the tissues. The formation of histamine by ruminal/

ruminal fluid was examined in a series of incubation experiments. Incubation of ruminal fluid containing 2% added glucose led to the formation of histamine after 2 - 3 days. This was accompanied by a fall in pH to 4.0. Histamine was not destroyed either by incubation with ruminal fluid or when put into the rumen of a cannulated sheep. Histamine put into the rumen started to pass to the abomasum within one hour of dosing.

Sheep were unaffected by dosing with histamine per cannula and it was concluded that little absorption of free histamine occurred in the rumen. None of the evidence obtained supported previous suggestions that formation of histamine in the rumen was, of itself, toxic to the animal concerned.

The distribution and content of histamine and mast cells in the sheep stomach was next examined. The abomasal mucosa contained more histamine and mast cells than any other tissue of the stomach. Histamine profiles of abomasal mucosa revealed evidence of some non mast cell histamine in the pyloric region. A close correlation between histamine and mast cells was also found in many other sheep tissues.

Acknowledgements

The early part of this work was carried out in the Department of Pharmacology, Liverpool University and I am very grateful to Professor Andrew Wilson for his advice at that time.

I am glad to acknowledge the continued advice, encouragement and criticism of Professor W.L. Weipers at each stage of this investigation. Surgical facilities were made freely available by Mr. D.D. Lawson of the Department of Small Animal Surgery for whose assistance I am most grateful.

Technical Assistance was given by Miss Doreen Fraser and Miss Christine Lawlor. I am greatly indebted also to Mr. A. Finnie who prepared the photographs and to Miss Noreen Millar who typed the manuscript.

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"La multiplicité des estomacs dans les bêtes à cornes et dans les bêtes à laine semblerait devoir garantir ces animaux d'être affectés d'indigestion, ou du moins devroient-ils par cette raison y être infiniment moins exposés; cependant, l'expérience prouve la contraire: la maladie dont il s'agit est, en effect, une de celles qui enlèvent le plus de ces animaux".

(M. Chabert: from "Instructions Vétérinaires". 1808)

Chabert, writing thus at the beginning of the nineteenth century, seemed to feel that it was rather unfair that a ruminant should suffer from indigestion, despite the complexity of its stomach. He went on to suggest, however, that this might not be a failing on the part of nature, but might in fact be a result of domestication.

There is no account, in Chabert's treatise, of any experimental studies of the movements of the ruminant stomach but Youatt in 1844 described how "some cruel experiments have been instituted in order to ascertain the nature of this muscular action/

action of the coats of the rumen, so necessary to produce this revolution of the food through its compartments. A considerable opening was cut into the flank, immediately over the paunch, and a swinging or balancing motion of that stomach, both upwards and downwards, and forwards and backwards, was plainly seen" (Youatt, 1844). Although Youatt realised the importance of these stomach movements, he was not aware that they occurred in any sequence. Colin (1871) created rumen fistulae and studied the stomach movements but he too failed to observe any definite pattern. It was not until the early part of the present century that a cycle of movements was described by Wester (1926) and by Schalk and Amadon (1928).

Disturbances of digestion are of considerable economic importance in the dairy cow for in this animal any impairment of function is rapidly reflected by a fall in milk yield. Although a few experimental observations have been made on cows most investigations of gastric motility in the ruminant have been carried/

carried out for reasons of economy and convenience in the sheep or goat.

Whatever part disturbances of motility may play in the development of acute conditions such as bloat, there is little doubt that persistent stasis of the stomach compartments must both disrupt the mixing of food in the reticulorumen and delay its ultimate passage to the small intestine (Duncan 1953). Stasis of the abomasum with dilatation is now also recognised in the bovine. So far, however, the causes of spontaneous abomasal stasis are not clearly understood.

Factors affecting motility of the ruminant stomach have been the subject of studies for the past thirty years. Such studies have been of two kinds - those dealing with the mechanism of initiation and control of stomach movements, and those concerned with substances occurring in foodstuffs which inhibit gastric motility and which may be capable of producing digestive disturbances. The evidence presented in these past investigations will be reviewed in the first part of this thesis.

In/

In several previous studies of ruminal function it has been noted that histamine, injected intravenously, caused paralysis of both the reticulo-rumen and the abomasum. (Dougherty 1942, Clark 1950 and Duncan 1954). This finding has been extended by reports of experimentally-induced digestive disturbances in which symptoms have been attributed either to the absorption or to the release of histamine (Dougherty & Cello 1949, Dain, Neal & Dougherty 1955).

In the present investigations experiments have been designed to assess the significance of histamine as a factor affecting gastric motility in the sheep. This experimental work will be reported and discussed in three sections.

In the first section the action of histamine on isolated smooth muscle preparations from each compartment of the sheep stomach was examined. The responses of such preparations to histamine were compared with those caused by acetylcholine, adrenaline and 5-hydroxytryptamine. Each of these compounds acts on smooth muscle and all are/

are known to occur in the body.

The isolated tissue experiments were followed by an investigation of the effect of histamine on the stomach movements of conscious sheep. Sheep were fitted with cannulae to permit recording of movements of the reticulum rumen and abomasum and histamine and other drugs were given by intravenous injection or infusion. In other acute experiments, the effect of similar doses of histamine on blood pressure and respirations was examined.

The formation and absorption of histamine in the rumen were next studied. Two aspects of the problem were examined; the formation of histamine by ruminal contents and its absorption from the alimentary tract. The decarboxylation of histidine by intestinal and faecal bacteria has been repeatedly demonstrated (Mellanby and Twert 1912, Hanke & Koessler 1924a, Gale 1940, Epps 1945), and more recently the formation of histamine by ruminal micro-organisms has also been described (Dain et al. 1955, Van der Horst 1961). As yet, however, the ruminal organisms responsible/

responsible have not been identified.

Experiments were designed to demonstrate decarboxylation of histidine by ruminal fluid. This was assessed by determination of the amount of histamine formed rather than by measuring carbon dioxide production in the presence of the amino-acid substrate (Gale 1940). In further experiments an attempt was made to discover whether histamine could be absorbed from the rumen, a possibility suggested by Dougherty and Cello (1949). The rate of passage of histamine from the rumen to the abomasum was also examined.

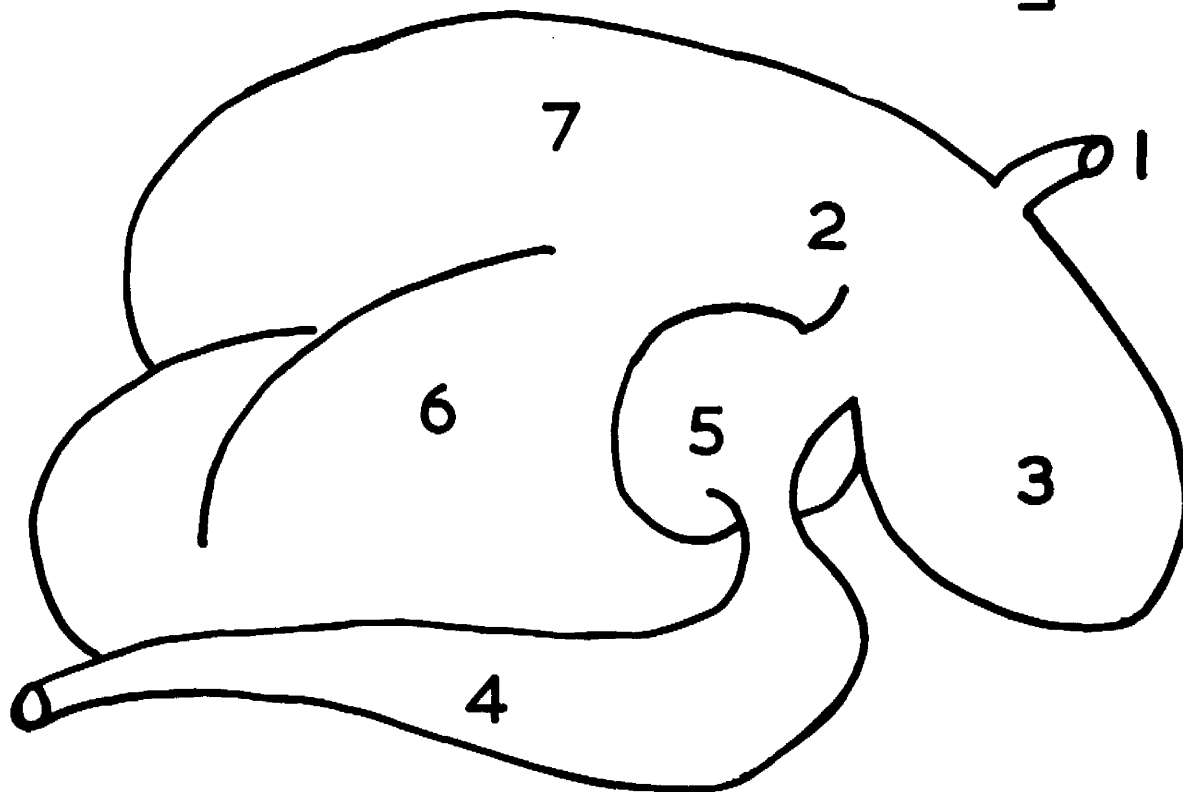
In the third part of this investigation the distribution of endogenous histamine in the sheep has been studied. As Riley and West (1953) have shown that, in many tissues, histamine is associated with the presence of mast cells, the field of investigation was widened to include a study of mast-cell population in each tissue. The histamine content of the stomach was examined in considerable detail, whilst other organs were considered in more general terms.

PART I

REVIEW OF THE LITERATURE RELATING TO
THE FUNCTION OF THE SHEEP STOMACH

Fig. 1. Regions of the sheep stomach.

- | | |
|--------------|---------------|
| 1 OESOPHAGUS | 2 VESTIBULE |
| 3 RETICULUM | 4 ABOMASUM |
| 5 OMASUM | 6 VENTRAL SAC |
| | 7 DORSAL SAC |
- } RUMEN



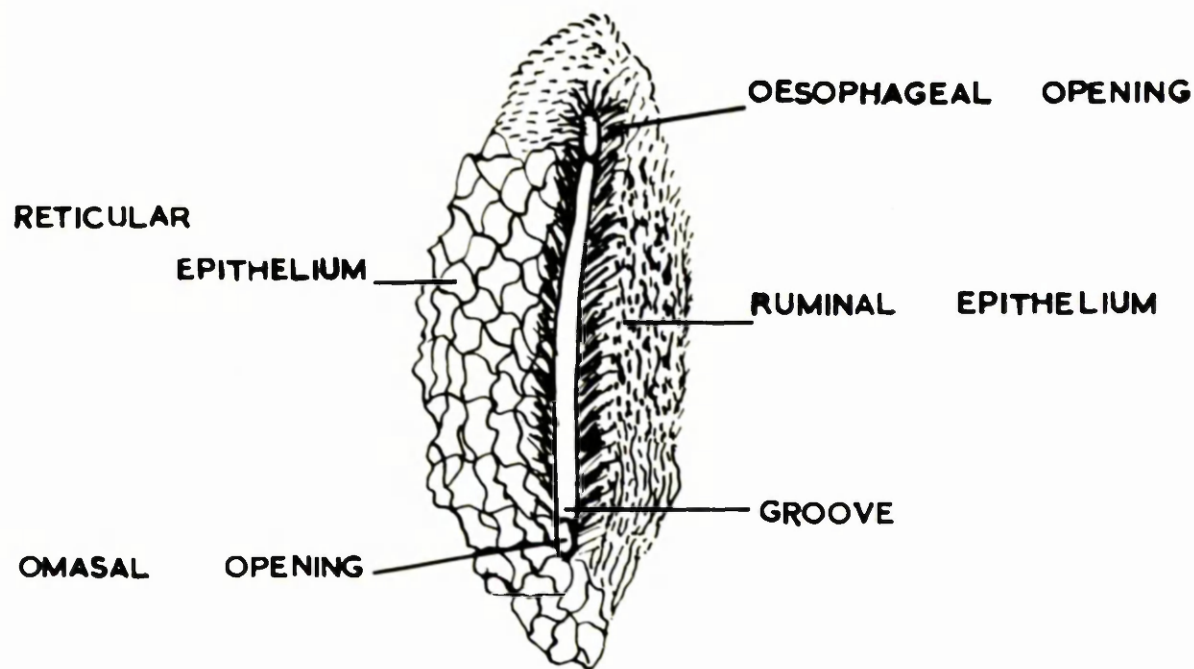
1. Anatomy of the Ruminant Stomach

The ruminant differs anatomically from other mammals principally in the possession of a complex stomach, having four compartments: reticulum, rumen, omasum and abomasum. The arrangement and relative proportions of these four compartments are shown in Figure 1.

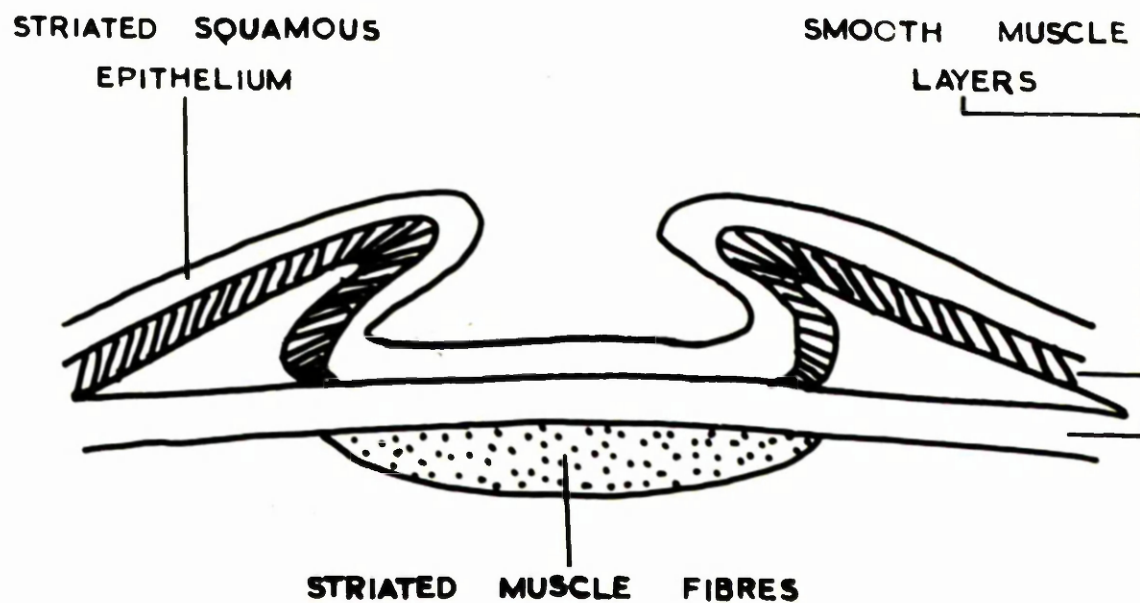
The rumen is the largest of the compartments of the fore-stomach, and is partially divided by pillars of muscle into dorsal and ventral sacs. These sacs are subdivided by other muscular pillars to give anterior and posterior dorsal and ventral sacs. Anteriorly, the rumen joins the reticulum. The junction between these two compartments is denoted on the ventral surface by the rumino-reticular fold, whilst dorsally, they meet at the vestibule where there is no clear line of demarcation. The wall of the rumen consists of a layer of stratified squamous epithelium with numerous papillae and two layers of smooth muscle. In the outer muscle layer, the fibres run essentially antero-posteriorly, although they become more oblique in many places. The fibres of the inner layer/

Fig. 2.

THE OESOPHAGEAL GROOVE



CROSS SECTION THROUGH GROOVE



FROM PHILLIPSON

1946

layer have in general a circular arrangement.

The reticulum lies anterior to the rumen and is a small sac-like structure. The opening of the oesophagus is situated dorsally in the region of the vestibule. A groove extends downwards from the cardia of the oesophagus on the right wall of the reticulum. This structure is termed the oesophageal groove and terminates at the reticulo-omasal orifice. The anatomy of the structure has been described by Phillipson (1946).

"The floor of the oesophageal groove consists of a single transverse layer of unstriated muscle fibres continuous with the external fibres of the reticulum and rumen and outside which is a thin layer of striated fibres continued from the oesophagus and running longitudinally with the groove. The muscular pillars are formed from the internal muscle fibres of the reticulum and rumen which end at the groove and turn upon themselves. (See Figure 2.) These fibres run longitudinally with the groove and continue round the cardia so that this orifice is encircled on three sides. A similar arrangement is found at the entrance to the omasum, except that the posterior pillar tends to overlie the anterior pillar".

The mucous membrane of the reticulum is raised into folds, enclosing 4, 5 or 6-sided cells. These cells are subdivided by smaller folds/

folde and are studded with pointed papillae. The two muscular layers of the reticulum follow an oblique course and cross at right angles.

The omasum is situated to the right of the rumen and in the sheep it has the form of a kidney-shaped sac. It communicates with the reticulum above and with the abomasum below. These two openings are situated at either end of the lesser curvature of the organ and are connected by a groove-like passage. The lumen of this compartment is occupied by a large number of projecting folds or laminae. These vary considerably in the extent to which they project. The muscular tunic of the omasum is composed of an outer thin longitudinal layer and an inner thicker circular layer which is continuous internally with the intermediate muscle sheets of the omasal laminae.

The abomasum is located to the right of the rumen and rests upon the abdominal floor. It is roughly similar in shape to the simple stomach of other animals and communicates anteriorly with the omasum and posteriorly with the duodenum. It/

It possesses a glandular mucous membrane which is clearly divisible into two parts. The anterior or peptic part is marked by large folds of mucous membrane running parallel to the long axis, whilst the posterior or pyloric part is almost entirely smooth, except for one or two longitudinal folds near the pylorus. Acid and pepsin are secreted only in this compartment of the stomach where typical gastric secretory cells of both acid and peptic types are found (Hill, 1951). The muscular wall of the abomasum consists of outer longitudinal and inner circular layers.

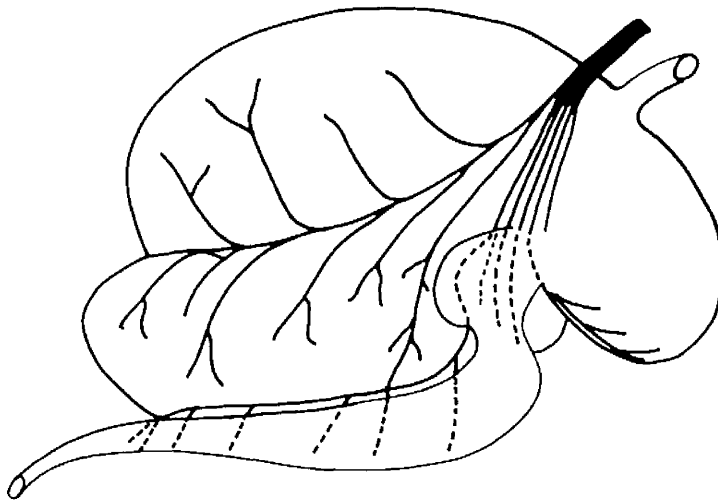
Nerve Supply.

Studies on the innervation of the ruminant stomach have been reviewed in great detail in a paper by Habel (1956). The ensuing descriptions are largely taken from this paper.

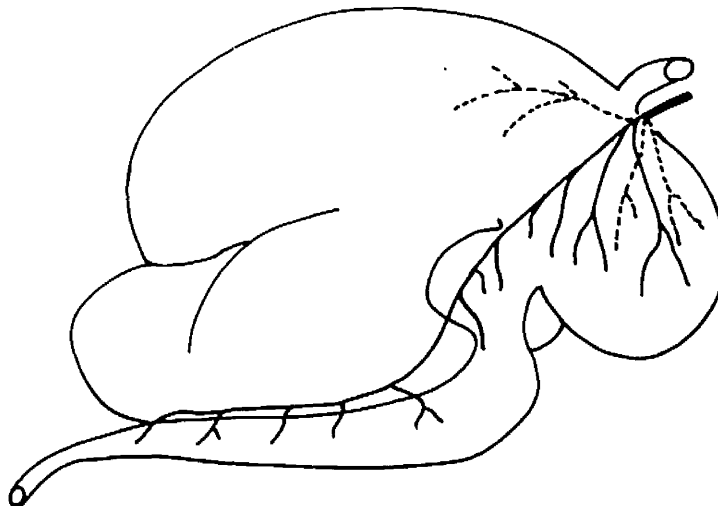
All compartments of the ruminant stomach receive a parasympathetic nerve supply from the branches of the vagus nerves. The left and right vagus nerves divide over the heart into dorsal and ventral branches which unite with their counterparts/

Fig. 3.

DISTRIBUTION OF DORSAL VAGUS NERVE
RIGHT ASPECT
(diagrammatic)



DISTRIBUTION OF VENTRAL VAGUS NERVE
RIGHT ASPECT
(diagrammatic)



----- indicates branches on left
side of organs.

counterparts to form the dorsal and ventral vagal trunks. These nerve trunks appear to receive approximately equal numbers of fibres from both left and right vagi, and continue through the diaphragm in close association with the oesophagus. The distribution of these branches of the vagus nerves is shown diagrammatically in Figure 3.

The dorsal vagal trunk gives a small branch to the reticulum and also supplies the oesophageal groove. Other branches descend on the left of the reticulo-omasal junction and supply the posterior surface of the reticulum. This trunk also gives many branches to the omasum and to the left side of the abomasum. The rumen also receives branches from the dorsal vagus and is almost entirely supplied by this nerve.

The ventral trunk gives off some branches to the rumen and also supplies the anterior and lateral surfaces of the reticulum. Further branches supply the right side of the reticulo-omasal orifice. The trunk continues across the surface of the omasum and gives numerous branches to/
to/

to the right surface of the abomasum. The long pyloric nerve which is a branch of the ventral vagal trunk supplies the pyloric region of the abomasum.

The sympathetic nerve supply to all compartments is derived from the coeliac plexus. Sympathetic nerves are found accompanying the left and right ruminal arteries, the reticular and splenic arteries and both branches of the left gastric artery.

2. Normal Function of the Stomach.

The reticulum and rumen undergo a co-ordinate cycle of movements which is more clearly defined than the peristalsis of a simple stomach. Schalk and Amadon (1921) pointed out that it was quite inadequate to describe ruminal movements in terms of the contractions shown by the simple-stomached animal. In their treatise, entitled "Physiology of the Ruminant Stomach" (Schalk and Amadon 1928), they described a sequence of stomach movements based on results obtained both by direct inspection and palpation through large gastric fistulae and by recording changes of pressure in inflated balloons placed in various positions within the stomach. The description given by Schalk and Amadon in that paper has provided a useful starting point for many subsequent studies of rumen motility.

The sequence of movements which occurs in the ruminant stomach has been clearly described by Phillipson (1946) as follows:-

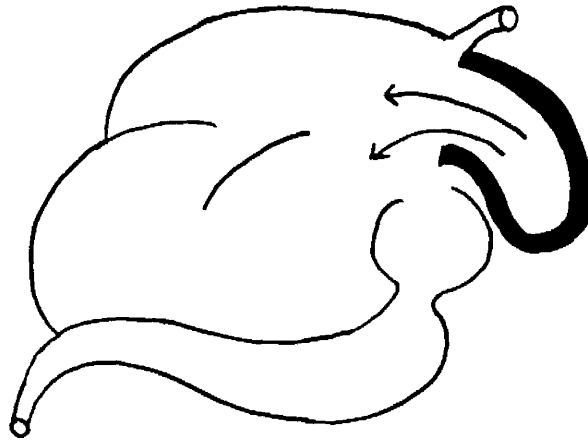
"The reticulum contracts about once a minute in a brisk two-stage movement sending a wave of liquid ingesta backwards into the rumen. Relaxation occurs about/

Fig. 4. Sequence of movements of reticulum and rumen.

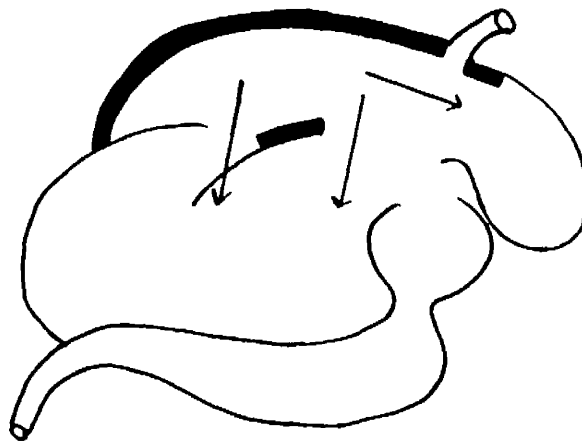
- denotes contraction.

← denotes direction of movement of contents.

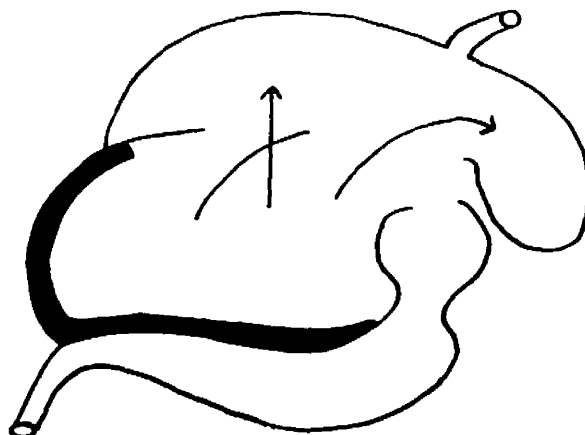
1. RETICULUM CONTRACTS TWICE



2. RUMEN-DORSAL SAC CONTRACTS



3. RUMEN-VENTRAL SAC CONTRACTS



about five seconds after the commencement of contraction and the reticulum again becomes filled with liquid material which runs back from the rumen. The movements of the rumen are slow and sustained compared to those of the reticulum. The dorsal sac contracts as the reticulum relaxes and compresses the ingesta so that the liquid is squeezed from the dorsal to the ventral sac which in turn relaxes. The rumen returns to its resting position and finally, the ventral sac contracts as the dorsal sac relaxes, a movement that drives fluid ingesta in the ventral sac in an upwards and forwards direction".

This sequence is illustrated in Figure 4. Phillipson also describes common variations of this pattern. These are:-

- a) two contractions of each sac of the rumen . . . between each contraction of the reticulum.
- b) a typical cycle alternating with one in which only contraction of the dorsal sac occurs.
- c) a typical cycle alternating with the first variation.

Movements of the omasum in cattle were first studied by Wester (1926) and by Schalk and Amadon (1928). The latter authors observed pronounced waves of increased tonus with slow contractions which increased in frequency when food/

food was eaten. Furthermore, a sudden fall in pressure in the lumen of the omasum occurred during the second contraction of the reticulum. Phillipson (1939) studied changes in the outline of the omasum of the sheep radiographically. A sequence of movements was seen in some cases, but this was an inconstant finding. In this sequence, the dorsal pole of the omasum first relaxed after contraction of the reticulum. This was followed by elongation of the whole organ which then returned to its original shape. Phillipson suggested that other secondary contractions might also occur at irregular intervals. Balch (1960) considered that most of the digesta pass only through the neck of the omasum and that movements of the muscular pillars serve to press fluid material against the omasal leaves. This would permit water to pass between the leaves and so be absorbed.

Movements of the abomasum have been described by Hill (1951). He found that body of the abomasum normally showed only small movements but that larger contractions occurred when food was eaten/

eaten. The prepyloric region of the abomasum showed typical peristaltic contractions.

The normal cycle of ruminal movements is dependent upon an intact vagal nerve supply (Mangold & Klein 1950, Duncan 1953, Bell & Lawn 1955, Iggo 1956, and Titchen 1958). After total vagotomy, Duncan found that the normal rhythmic activity and propulsive effect of the movements of reticulum and rumen were completely abolished, although abnormal movements were sometimes seen two to three weeks after operation. After partial (unilateral) vagotomy, however, propulsive motility remained. The abomasum continued to show contractions after section of both vagus nerves, although the propulsive effect was reduced, leading to delayed emptying and dilatation of this compartment. Iggo (1956) observed a similar cessation of stomach movements in decerebrate sheep when transmission of impulses through the vagi was blocked in the cervical region by cooling with saline at 4°C. He found also that stimulation of the vagus nerve supply to the stomach gave rise to contractions. Under suitable conditions contractions could be induced which/

which resembled those occurring in the normal animal. Further evidence for the importance of the vagus nerve supply to this organ is provided by Dougherty (1942¹) and by Duncan (1954) who found that contractions of the rumen were inhibited by the administration of atropine. Titchen (1958) obtained similar evidence of the action of atropine in inhibiting contractions of the reticulum in decerebrate sheep.

The complete dependence of the cycle of rumen movements upon impulses received via the vagus nerves suggests the existence of a "centre" from which the appropriate motor stimuli are transmitted. Such an area has been identified by Bell and Lawn (1955) who stimulated localised areas of the medulla oblongata by means of carefully placed electrodes and noted the responses produced in the reticulum rumen and oesophagus. A repetitive stimulus of 10 V at 50 pulses per second produced contractions which could still be elicited after transection of the rhombencephalon at the pons and of the cord at the level of the first cervical vertebra. The positions/

positions of the sites stimulated were carefully checked by subsequent histological study of the brain tissue.

Dussardier (1957) has also presented evidence in support of a discrete medullary centre in the ruminant from which motor impulses travel via the vagus nerves to initiate contractions of the stomach. He found that the potential produced in certain areas of the hind-brain by antidromic stimulation of the vagus nerve was diminished or abolished when a contraction of the reticulum was about to occur. He suggested that this was due to a rhythmic grouped discharge of neurons giving rise to the typical rhythm of the cycle of contractions of reticulum and rumen.

Several factors have been discovered which influence the normal contractions of the stomach reflexly. In a review of results of extensive experiments in decerebrate preparations Comline and Titchen (1960) have concluded that typical contractions of the reticulum represent a reflex response. The efferent limb of the reflex arc consists of cholinergic parasympathetic fibres in the/

the vagus whilst the fibres of the afferent limb are also situated in the vagus nerves. The evidence on which their conclusions are based must now be briefly considered.

Phillipson (1939) has shown that the introduction of fluid into the abomasum by means of a fistula, increased the interval between reticular contractions. If the abomasum was grossly distended by the inflation of a balloon within its lumen, reticular contractions ceased, but the rumen continued to contract in an irregular manner. More recently, Titchen (1958) has demonstrated that after division of the splanchnic nerves distension of the abomasum no longer resulted in inhibition but rather stimulated contractions of the reticulum.

In decerebrate sheep and goats in which movements of the reticulum and rumen were absent, these could be elicited by distension of the reticulum. Increasing distension led to an increase in the number of impulses travelling in the afferent vagal nerve fibres and shortened the interval between contractions (Iggo 1956).

The factors which act reflexly have been investigated in detail by Titchen (1958 and 1960). In experiments on decerebrate sheep and goats he has established a number of clearly defined stimuli which are summarised below.

Contractions of the reticulum were stimulated by:-

- a) Stretch of the reticulum and/or the reticulo-ruminal fold.
- b) Distension of a balloon in the omasal canal.
- c) Increase of the acidity of the abomasal contents to pH 0.9 - 1.0.
- d) Touching the lower part of the thoracic oesophagus or the abomasal mucosa.
- e) Stretching the abomasum after section of the splanchnic nerves.

Contractions were inhibited by:-

- a) Distension of the abomasum or manipulation of the pylorus.
- b) Distension of the rumen.

The most effective stimulus was found to be distension of the reticulum and reticulo-ruminal fold which was achieved by means of a water-filled balloon. In contrast to the observations of Phillipson/

Phillipson (1939) referred to above, Titchen found that in decerebrate sheep inhibition of contractions was more marked in the rumen than in the reticulum. Marked inhibition was also caused by surgical interferences involving the rumen, reticulum or abdominal wall. This effect was abolished by section of the spinal cord in the anterior thoracic region but preparations treated in this way rarely survived for more than 45-60 minutes in spite of attempts to restore blood pressure by intravenous infusion of solutions of dextran or polyvinyl-pyrrolidone.

Eructation.

Gases produced by the fermentation of the contents of the reticulum and rumen are expelled by repeated eructations. The events involved in this process and the factors which influence it have been studied in some detail by Weiss (1953), Dzuik and Sellers (1955), Dougherty and Meredith (1955) and Dougherty, Habel and Bond (1958) and have been reviewed recently by Dougherty (1960).

From his observations, Weiss concluded that pressure of gas in the rumen was the main stimulus to/

to eructation, and that the process was entirely dependent on the ability of the reticulum and rumen to contract. He described a contraction of the rumen moving in a postero-anterior direction accompanying each eructation. This usually followed within 10 - 15 seconds of an antero-posterior movement, although the interval between these two movements might be extended up to 60 seconds. Failure of eructation, however, was not necessarily due to a loss of ruminal motility, as records from animals where the pressure of gas in the rumen had become very great showed evidence of powerful contractions. Apart from mechanical factors, such as oesophageal obstruction and the posture of the animal, Weiss believed that any factor tending to cause ruminal stasis would also inhibit eructation.

On the other hand, Clark & Quin (1945) found that eructation could occur in the absence of ruminal movements. Dougherty and Meredith (1955) also noted that motility of the reticulum and rumen was not essential for eructation, provided that/

that the cardia was not covered by ingesta.

Stevens and Sellers (1959) have also described an association between eructation and a definite ruminal contraction and Dougherty (1960) in reviewing this subject reaches no conclusions regarding the significance of such contractions.

Dougherty, et al. (1958) investigated the eructation reflex in acute experiments on decerebrate sheep. These authors described receptors for this reflex in the region of the cardia and noted that eructation was stimulated by the presence of gas at the cardia, but was inhibited by liquids in that region. They believed that failure to eructate was due primarily to the retention of ingesta at the cardia. Dougherty and Habel (1955) had previously shown that this region was normally cleared of ingesta by contraction of the reticulum and contraction and elevation of the rumino-reticular fold and anterior pillar.

Regurgitation

Regurgitation is the process by which portions of rumen contents are returned to the mouth for further/

further chewing and mixing with saliva. The complete sequence of regurgitation chewing and swallowing constitutes the phenomenon of rumination, a masticatory process associated with the development of the fore-stomach as a fermentation chamber. This process is not apparently essential to the animal although sheep normally occupy about eight hours each day in rumination. (Bell and Lawn 1957).

Although regurgitation is in some ways similar to vomiting, experiments by Bell (1958a) have not demonstrated a very close connection. Apomorphine, a powerful emetic in animals which are able to vomit, did not produce regurgitation in young ruminants although the associated symptoms of salivation and distress were seen.

That regurgitation may result from a mechanical stimulus was shown by Wester (1926), who found that rumination could be started by tactile stimulation of the oesophageal groove. Bell (1960) considered that the series of reflex responses involved in rumination were related to the cycle of contractions of the reticulum and rumen, and that these reflexes were integrated in the brain stem. Bell and Lawn (1955) demonstrated that/

that stimulation of an area in the medulla near to the respiratory centre gave rise to many of the responses involved in rumination. In a recent review Bell (1960) discussed further evidence in support of the existence of such an integrating centre.

There have been several suggestions as to the means by which solid material can pass from rumen to mouth and the problem still remains partially unresolved. Wester and later Bergman and Dukes (1926) considered that entry of food into the oesophagus followed the production of a negative pressure within its lumen, by a special inspiratory effort with closed glottis. Wester also considered that the reduction in intra-oesophageal pressure was partly due to a change in shape of the oesophagus itself.

Bergman and Dukes found that regurgitation was not accompanied by contraction of either the rumen or the abdominal musculature, although contractions of the reticulum occurred just before and just after this process. Downie (1954) considered that the pressure difference between the/

the contracted reticulum and relaxed oesophagus caused rumen contents to be forced into the oesophagus. He suggested that the force of this movement gave rise to the typical jerk seen at each regurgitation. Bell (1958b) has re-emphasised the importance of contractions of the reticulum by demonstrating that regurgitation was not affected when the diaphragm was immobilised by section of the phrenic nerves and that regurgitation was still possible, though somewhat impaired, when tracheotomy was performed in addition to the nerve section. Nevertheless, he was unable to explain how regurgitation could still occur when reticular contractions had been abolished by the administration of atropine, an observation made by Wester (1926) and confirmed by Duncan (1954).

Closure of the Oesophageal Groove.

The peculiar structure of the oesophageal groove has given rise to much speculation as to its precise functions and several of the earlier/

earlier theories were discussed at some length by Colin (1871). He attributed the first description of this structure to Faber, who, although incorrect in his idea of its function termed it "la voie lactee".

More recently it has been shown experimentally that in young suckling ruminants, milk does pass directly from the oesophagus by way of the oesophageal groove and omasum to the abomasum. Schalk and Amadon (1928) described the closing of this groove to form a firm tube. Groove closure in calves suckling milk was also described by Wester (1926) who found that this was a reflex action, as it still occurred in animals with an oesophageal fistula when milk passed only through the mouth and pharynx.

Further evidence for the reflex nature of this process in calves has been provided by Watson (1944) who found that the application of a local anaesthetic solution to the mucous membrane of the mouth and pharynx inhibited closure of the groove. More recently, the mechanism/

mechanism of this reflex has been demonstrated by Comline & Titcher (1951a). In decerebrate preparations of calves and lambs, stimulation of the superior laryngeal nerve consistently caused contraction of the groove. The efferent fibres concerned in this reflex were confined to the dorsal vagus nerve. The reflex was inhibited by stimulation of the central end of the glossal branch of the glosso-pharyngeal nerve. Stimulation of the central end of the abomasal nerve, or manipulation of the abomasum or reticulum also produced an inhibition which was slow in onset and which was considered by the authors to be due to the release of adrenaline. It was also shown that when adrenaline was given intravenously a similar inhibition occurred. Contraction of the musculature of the groove was abolished by small doses of atropine and, in this respect, the smooth muscle situated in this rather specialised region appears to behave in a similar manner to that of the rest of the stomach.

A number of factors have been found to initiate closure of the groove in young animals.

Watson/

Watson (1944) noted that the act of suckling provided an effective stimulus, whereas drinking from a bucket did not. The nature of the fluid taken was also important, since milk provided a greater stimulus than water. Watson has also found that closure of the groove occurred in animals trained to expect milk, even when they were given water.

Clunies Ross (1934) found that when fluid was given to lambs by means of a tube passing into the oesophagus, it went into the rumen, but if the mouth was swabbed with a solution of copper sulphate, the fluid passed straight into the abomasum. In calves, on the other hand, Riek (1954) found that a 10% solution of sodium bicarbonate was the most effective stimulus causing closure of the groove although other sodium salts were also effective.

3. Factors Affecting Movements of the Stomach.

1. Effects of Drugs.

Some mechanical factors such as distension, which reflexly affect the normal movements of the reticulum and rumen, have already been considered in the previous section. Chemical and humoral factors which may modify these movements have received a sporadic study during the past twenty years. The results obtained in these investigations have been, in many instances, perplexing and this has led to some speculation as to their real significance. Much of the difficulty has been related to the experimental methods adopted. In some cases, the routes of injection chosen for the administration of drugs have led to widespread systemic effects, which might well have masked a more direct action.

It is clear from the consideration of the mechanism of the stomach movements that the vagus nerves are responsible for the transmission of stimuli to maintain the normal cycle of contractions. It would seem probable, therefore, that/

that acetylcholine acts as the mediator between vagus nerve endings and smooth muscle, as is generally accepted to be the case for other organs supplied by this nerve. Dougherty (1942) administered acetylcholine both subcutaneously and intravenously to cows. Using the former route of injection there was no noticeable effect, but when given intravenously acetylcholine led to paralysis of the rumen, and, if the dose was large enough, to the ultimate collapse of the cow. These findings were confirmed in sheep by Duncan (1954) who found that 3 - 5 mg. of acetylcholine given intravenously inhibited rumen contractions for five minutes, and depressed subsequent movements for thirty minutes. This action was potentiated by eserine. This author attributed the anomalous response to acetylcholine to its systemic action, particularly in causing the release of adrenaline. Other cholinergic agents, such as carbachol and arecoline, have been found to increase the force of contractions (Dougherty 1942, Duncan 1954). The administration of atropine always depresses or inhibits normal contractions/

contractions (Dougherty 1942, Clark 1950, Comline and Titchen 1951, Duncan 1954).

A more direct route of administration, by injection into the coeliac artery, has been used by Dussardier (1954). He found that acetylcholine given in this way caused a contraction of the rumen and not an inhibition. He also showed that the intravenous injection of very large doses in an atropinised animal would also cause a contraction. For this apparent paradox, Dussardier offered the explanation that atropine may have a more potent action on the heart and circulatory system than on the stomach. If this is so, then doses of atropine sufficient to prevent collapse of the animal when acetylcholine is given are insufficient to inhibit contractions of the stomach. This explanation is not altogether satisfactory, as it might be expected that the nicotinic actions of acetylcholine, including stimulation of the sympathetic nervous system and the release of adrenaline, would be more marked under these conditions. It is possible, therefore, that the contraction of the rumen seen by Dussardier/

Dussardier was in reality a response to adrenaline.

The intravenous injection of adrenaline or nor-adrenaline causes inhibition of ruminal contractions (Dougherty 1942, Duncan 1954, Titchen 1958). Dussardier (1954) obtained similar results, but found that sometimes adrenaline caused a biphasic contraction of the reticulum in sheep similar in appearance to a normal spontaneous movement. Furthermore, in the spinal animal, adrenaline more often gave rise to a tonic contraction. Similarly in vagotomised sheep, where normal movements of the stomach were absent, the administration of adrenaline caused a single, strong contraction (Duncan 1954).

Comline and Titchen (1951b) have examined the action of adrenaline given intravenously to young ruminants and have found some apparent species differences in response to this drug. In kids, adrenaline produced a contraction of the reticulum, but in calves no contraction was seen following either injection of adrenaline or splanchnic stimulation.

The/

The action of adrenaline on the reticulum and rumen may in part be due to the effect produced on the blood flow in these organs. From measurements of the flow in the posterior vein of the rumen, Dobson and Phillipson (1956) found that the injection of a single dose or continuous infusion of adrenaline caused an initial increase followed by a slowing in blood flow. This was, in turn, followed by a further marked increase. This response was related to the pH of the rumen contents. At pH 7.5, the changes were small, but as the contents were made more acid, the secondary increase phase was particularly marked. With acetate buffers at pH 4, blood flow was very fast, and the slowing effect was sometimes absent, being replaced by an increased blood flow, corresponding to the rise in systemic blood pressure (Using acetate buffers at pH 3.6 - 5.0, Ash (1959) has shown that contractions of reticulum and rumen are completely inhibited under these conditions without the administration of adrenaline). Thus it would appear that the action of adrenaline is complex and variable, although it can be said that its injection into an intact animal will usually result/

result in temporary ruminal stasis.

It has also been shown that intravenous injection of glucose solutions is followed by a progressive reduction in the amplitude of contractions. (Le Bars, Nitescu and Simmonet 1953). Immediately following injection the blood glucose concentration rose to 300 mg. per cent and did not return to its pre-injection value for 4 - 5 hours. Inhibition of contractions of the reticulum and rumen reached a maximum 60 - 90 minutes after the injection and persisted for as long as the hyperglycaemia. Conversely when insulin was injected intravenously in doses of 0.5 - 2.0 I.U./kg. both the amplitude and frequency of movements were increased. This effect was first observed about 50 minutes after the injection of insulin and persisted for $2\frac{1}{2}$ - 3 hours. This stimulatory action of insulin has been confirmed by Hill (1954) who found that abomasal secretion was also increased. Thus the force of ruminal contractions appears to be related to the blood glucose level and Dussardier (1954) has suggested that the inhibitory action of adrenaline may, in part, be due to the hyperglycaemia which follows injection of this compound/

compound.

The inhibition of movements of the reticulum and rumen which follows the intravenous administration of histamine has been described by several authors (Dougherty 1942, Clark 1950, Duncan 1954). Clark noted that this effect was accompanied by hypermotility of the rectum, and that both these actions could be prevented by the previous administration of anti-histaminic drugs. Clark has also found that, after section of the left vagus nerve, histamine no longer produced any effect. The rumen was still able to contract, however, if the distal end of the cut nerve was stimulated, so that the inhibitory effect of histamine did not seem to be due to a direct action on the muscle of the rumen.

11. Contents of the Rumen and Metabolites.

Changes in the pH of the ruminal contents have also been shown to have an effect on ruminal motility. Clark and Lombard (1951) found that administration of alkaline solutions (pH 7.5) to sheep caused ruminal stasis. The solutions used contained either sodium carbonate or $\frac{N}{5}$ sodium hydroxide/

hydroxide. Stasis did not occur until 10 - 20 minutes after the rise in alkalinity of the ruminal contents and normal motility could be restored either by feeding or by the injection of carbachol. If intravenous injections of the alkaline solutions were made, however, an immediate stasis occurred. Attempts made by these authors to increase the acidity of the rumen contents failed as the normal solutions of hydrochloric acid which they used did not lower the pH below 6.7 and no effect was observed. From their results, Clark and Lombard concluded that changes in pH exerted a central rather than a peripheral action upon ruminal movements.

Ash (1959) introduced buffered alkaline solutions (pH 7.9 - 10.1) into the empty rumen in concentrations of 0.1 molar. The effects on contractions were slight and transient and led Ash to conclude that the presence of an alkaline solution in the rumen does not of itself have any direct action on motility.

The action of urea on ruminal motility is also related to changes in pH. Clark (1951) gave/

gave urea by fistula to Merino sheep and obtained stasis, accompanied by a rise in pH from which recovery took 3 - 4 hours. He concluded that the effect produced was due to increased alkalinity (pH 8.15) with the formation of ammonia. Le Bars, Molle and Simmonnet (1957) gave urea orally and obtained a similar effect. They found, however, that if urea was given by slow intravenous injection, an increase in the frequency of contractions occurred. On the other hand, the slow, intravenous injection of ammonium acetate resulted in a stasis which persisted for almost three hours. It seems from these results that the effect of feeding urea to ruminants in any quantity is to cause an increase in the alkalinity of the contents of the rumen, which in turn leads to stasis. It must be noted, however, that Ash has not been able to confirm that alkalinity, of itself, can exert this effect.

Appreciable quantities of acetic, propionic and butyric acids are produced in the fermentation of ruminal contents and Ash (1959) has made an/

an extensive study of the effects of these acids on the motility of the fore-stomach of sheep. He found that buffered solutions of acetate, propionate and butyrate at pH 3.6 - 5.0, put into the emptied rumen through a cannula, inhibited contractions of both the reticulum and rumen. Buffered solutions of lactate, HCl or citric acid at similar pH values did not produce this effect although movements were inhibited by a solution of lactic acid at pH 2.5. From these results Ash concluded that the inhibitory action depended on the presence of fatty acid in the rumen rather than upon a solution of any particular acidity.

Ash then attempted to determine the site of action of the fatty acids. He demonstrated that contractions of the reticulum of an anaesthetised sheep produced by stimulation of the peripheral end of the cut left cervical vagus were not depressed when a buffered solution of sodium acetate was put into the rumen. Furthermore, he observed that although the presence of fatty acid solutions in the rumen/

rumen led to a fall in both the pH value and the CO_2 concentration of arterial blood, inhibition of contractions could still be obtained by blowing fatty acid vapour into the rumen, which did not cause any appreciable change in arterial blood. From these observations Ash concluded that the inhibitory action was probably caused reflexly by stimulation of acid-sensitive receptors in the wall of the fore-stomach. He suggested that the failure of other acids to produce this action was probably due to their relatively slow penetration of the epithelium of the fore-stomach.

4. Toxic Factors in Food and Ruminal Contents.

It has long been known that inhibition of ruminal contractions may follow dietary changes or excesses. Many such conditions have been described vaguely as indigestion although this term has not been restricted to these disorders of the alimentary tract. Several substances have been extracted from the ingesta of ruminants suffering from stasis of the reticulum and rumen, and these have been tested for potential toxicity in a number of ways.

Dougherty and Cello (1949) reported the presence of a toxic factor in ruminal contents. They stated that an extract, injected intravenously in dogs, depressed the blood pressure, whilst in sheep it inhibited ruminal motility and stimulated defaecation. Some extracts also depressed spontaneous motility of the isolated rabbit ileum. The active material was heat-stable, non-volatile and dialyzable. The rumen contents were found to be markedly acid (pH 4.9 - 5.4) and the content of volatile fatty acids was greater than that of normal ingesta. In view of their findings, the authors/

authors suspected that the toxic effects might be due to the presence of histamine, although this substance caused contraction of the rabbit ileum. On this assumption, they sprayed histamine on to the ruminal epithelium through a cannula and observed that stasis and an increase in respiratory rate occurred. They suggested that these findings indicated that histamine was absorbed, although they obtained no direct evidence of this.

The experimental feeding of excess grain or glucose to sheep has been found to cause severe indigestion with ruminal stasis. In some cases, the condition has eventually proved fatal (Hungate, Dougherty, Bryant and Cello 1952). Examination of the contents of the rumen revealed that there was a marked increase in lactic acid, associated with an increase in one type of gram positive organism, streptococcus bovis. At the same time, a decrease in the volatile fatty acid content was noted. A somewhat similar picture has been described by Scarisbrick (1954). He observed the occurrence of acute indigestion in sheep fed an excess of mangolds./

mangolds. The ruminal contents were markedly acid (pH 4.7) and this was associated with increased lactate production and a transient increase in volatile fatty acid content. Searisbrick noted also the occurrence of laminitis in the animals, which might be associated with the presence of histamine or some other vaso-dilator substance.

The association between a high carbohydrate diet and entero-toxaemia of sheep has been discussed by Bullen & Batty (1957). *Clostridium welchii* type D was able to multiply rapidly and produce toxin in the small intestines of sheep fed an excess of carbohydrate, whilst no entero-toxaemia occurred in sheep on normal diets. Bullen and Batty attributed this lack of susceptibility to the absence of a suitable fermentable carbohydrate substrate and to the relatively rapid flow of intestinal contents. The latter factor was thought to be important in view of the slow absorption of the toxin.

Enterotoxaemia, in which high concentrations of epsilon toxin were produced in the gut/

gut could be distinguished symptomatically from acidosis which might also result from a high carbohydrate diet. In enterotoxaemia death was very rapid and was usually preceded by convulsions. Acidosis, however, was characterised by a period of dullness and inappetence followed either by sudden collapse or by prolonged prostration with hyperventilation and excessive salivation.

Prier (1954) examined ruminal contents from spontaneously occurring cases of indigestion. Extracts had either of two effects. With some, he observed a depression of blood pressure, whilst with others, an elevation occurred.

In 1955, Dain et al. reported the presence of histamine and tyramine in considerable quantities in the rumen contents of overfed sheep. Amounts of histamine present were of the order 90 $\mu\text{g/ml}$ but if the acidity of the rumen contents was greater than pH 4.5, much higher concentrations of histamine were found. Both histamine and tyramine/

tyramine were identified by paper chromatographic techniques, and the concentration of histamine was determined colorimetrically. The histamine content of ingesta from normal sheep was found to be very low.

Dain et al. also reported the presence of another potentially toxic factor in the non-dialyzable fraction of the ingesta. This fraction caused a fall in blood pressure when injected intravenously but subsequent injections produced no effect. They drew attention to the similarity of the effect produced by this factor to that of polyvinylpyrrolidone described by Halpern and Briot (1953). Dain et al. suggested that the factor might act in a similar way by liberating histamine. Fraser (1959) has discussed the changes occurring in "rumen overload" produced by feeding excess wheat to ruminants. He considered that stasis resulted from the increased acidity of the ruminal contents, which was largely due to the production of lactic acid. He found also that the ruminal contents became more fluid and/

and increasingly hypertonic with respect to tissue fluid. As a result of this haemoconcentration occurs as water is lost from the plasma into the rumen.

A study of the effects of ruminants of over-eating of grain has recently been presented by Broberg (1960). He noted that overeating led to an increase in the acidity of rumen contents to pH 4.0 to 4.9 and this was due largely to the formation of lactic acid. He found also that the osmotic tension of rumen contents rose from the normal value of 6 - 9 osmoles to 20 - 25 osmoles, following an excessive intake of carbohydrate. These findings, together with the observation that the normal stable redox potential of ruminal contents is not maintained at values of pH < 5.0 , has led Broberg to conclude that normal fermentation does not occur under these conditions.

Although lactic acid is absorbed to some extent from the rumen, Broberg does not consider that such absorption is responsible for the toxic/

toxic effects observed. Evidence of absorption from duodenal infusions was much more convincing and it was found that the subsequent rise in blood lactic acid levels could be reduced by adding thiamine to the infusion. Further evidence for the importance of thiamine was found in the fall in urinary excretion of this vitamin from 70 - 105 $\mu\text{g/hr.}$ to 10 $\mu\text{g/hr.}$ after overeating.

This evidence has led Broberg to propose that the toxic effects produced by the overeating of grain may be due to the absorption of lactic acid coupled with an acute lack of thiamine, caused by the inability of the abnormal ruminal contents to continue synthesis of this vitamin.

In contrast to studies of ruminal contents, other workers have examined various dietary constituents and have paid particular attention to forage which was known to cause bloat. Ferguson, Ashworth and Terry (1949 and 1950) have shown that lucerne juice contained a substance which inhibited the activity of rabbit ileum in vitro. This substance was eventually identified as tricin (5'7'4' trihydroxy 3'5' dimethoxyflavone). More recently/

recently, Parsons, Neumann, Whitehain and Sampson (1955) have investigated the actions of extracts prepared from bloat-producing forage and also from rumen ingesta of cattle which had died from an acute attack of bloat. These extracts inhibited the movements of the isolated rabbit ileum, a property which was not impaired by freezing, heating nor storing for four weeks at -10°C . Parsons et al. also prepared an extract of clover juice from a pasture known to cause bloat and examined its effects on a Jersey cow prepared with a rumen fistula. When the extract was introduced directly into the rumen, it inhibited eructation in this cow, whilst, when it was administered to a sheep, the clover extract produced acute and rapidly fatal bloat.

Evans and Evans (1949) reported that an extract of white clover inhibited movements of the isolated rabbit ileum. They attributed this action to the presence of hydrocyanic acid in the extract.

Although the absorption of toxic substances from the rumen was at one time considered to be a potential/

potential cause of bloat (Clark and Weiss 1952), more recent work has indicated that the condition may be entirely due to the physical state of the ruminal contents. Johns (1958) has reviewed the evidence on which this approach to the problem is based. He noted that rumen movements often continued in the bloating animal so that ruminal stasis was not in itself a cause of bloat. The persistence of ruminal motility during bloat has also been recorded by Lindahl, Davis, Jacobson and Shaw (1957) who found that contractions increased in frequency during the onset of bloat.

Johns stressed that typical bloat is associated with the formation in the rumen of a stable foam of high viscosity which inhibits eructation. Mangan (1959) has suggested three factors which may all play a part in the development of stable foam. These are plant saponins, leaf proteins and salivary mucoprotein and the conditions in which these will act as foaming agents have been further investigated.

Individual differences in the susceptibility
of/

of cattle fed on a bloat-inducing diet, have been emphasised by Lindahl et al. (1957). This variation has been studied by Mendel and Boda (1961), who found that susceptible cattle secreted less saliva and that this contained abnormally high amounts of bicarbonate. There was no difference, however, in the content of other electrolytes nor was the rate of gas production or buffering capacity of rumen liquor from susceptible cows in any way abnormal.

PART II

SECTION I - THE ACTION OF HISTAMINE AND OTHER DRUGS
ON ISOLATED MUSCLE FROM THE SHEEP STOMACH

Factors affecting isolated smooth muscle.

It is clear from the evidence previously considered that many of the factors which influence ruminal motility do not appear to act directly on the muscle, or may, in some cases, act simultaneously at several sites in the body. For this reason, it is desirable to study how the isolated muscle responds to various chemical compounds. Such information may then provide a basis for analysing the results observed in the live animal.

Few reports have been published of investigations of the responses shown by isolated muscle strips taken from the various compartments of the ruminant stomach. There are certain difficulties associated with the preparation of such strips which may account for the paucity of information. Nevertheless, a suitable method has the practical advantage that large numbers of observations can be made, and that the results so obtained may facilitate interpretation of the results of "in vivo" experiments.

Studies/

Studies of isolated smooth muscle from the ruminant stomach have previously been carried by Von Graf & Delak (1951), Dussardier and Navarro (1953) and Duncan (1954). In the two latter reports, the authors described some difficulty in obtaining active preparations and found that abomasal strips gave the most satisfactory responses. Most of the conclusions reached by these authors were based, therefore, on results obtained from strips taken from the abomasum. They reported that in every case acetylcholine caused contraction of strips of muscle and that these contractions were potentiated by eserine and reduced or abolished by atropine. In addition, Dussardier and Navarro noted that acetylcholine caused a slight increase in the rate of spontaneous movements of the strips.

Adrenaline also contracted abomasal strips. Both Duncan and Dussardier and Navarro described the action of adrenaline as an initial, brief inhibition of movements, followed by a contraction lasting for several minutes. If the dose of/

of adrenaline was reduced, only the inhibitory phase was seen (Duncan). Similar results were also obtained by Dussardier and Navarro with strips taken from the rumen. The latter have stressed that the contraction produced by adrenaline is a true motor effect and not merely a reaction to the previous inhibition. They found that an adrenaline antagonist (883F) increased the inhibition and decreased the subsequent contraction, while atropine abolished the motor effect. On washing out the atropine, the strip again responded normally to adrenaline, but was insensitive to acetylcholine.

Von Graf and Delak made preparations of isolated strips of dorsal sac of ox rumen. They found that both acetylcholine and histamine caused transient contractions of these preparations. Duncan (1954) using strips of sheep stomach found that histamine never caused contraction and seemed to have little effect. Apart from these reports, there appears to be no further information on the action of drugs on isolated muscle from the ruminant stomach.

Experimental Studies of Isolated Tissue.

In the present investigation, it was found that the abomasum gave a suitable response in almost every case, whilst a proportion of the strips taken from other compartments were inactive. There was some evidence, however, that the time interval between killing the sheep and suspending the strips in the tissue bath determined the sensitivity of the tissue. When the intervals were less than thirty minutes, active strips from all compartments were usually obtained. Nevertheless, in spite of these precautions, it was sometimes not possible to obtain active strips of reticulum and omasum. Most of the findings to be described have been confirmed subsequently using strips removed from the stomach of healthy sheep killed at the laboratory for this purpose.

Methods

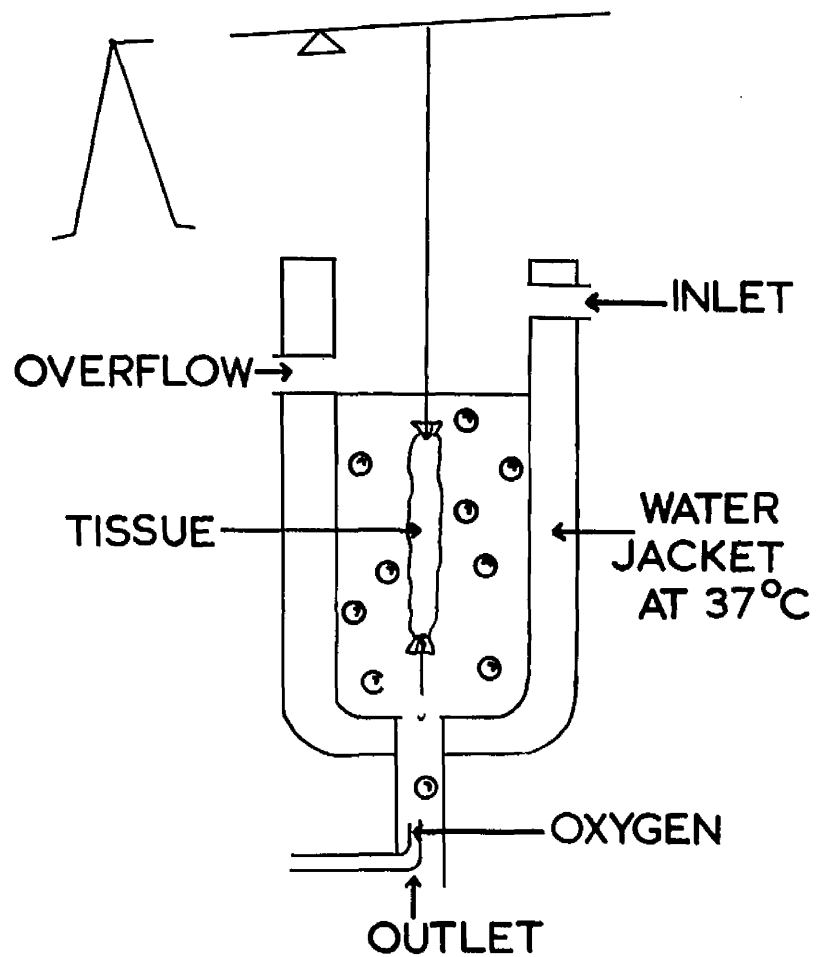
The specimens used in these experiments were obtained from sheep killed at the local abattoir/

abattoir. Sheep were stunned by means of a captive-bolt humane killer and were then bled. The entire stomach with part of the oesophagus and the duodenum was removed as soon as possible after death, and the contents of the rumen and reticulum were released by making a small slit in the wall of the rumen. The stomach was then placed in Ringer-Locke's solution at approximately $0 - 10^{\circ}\text{C}$ according to the method described by Duncan (1954). It took 15 - 30 minutes to transport the stomachs from the abattoir to the laboratory.

On arrival at the laboratory, the interior of the stomach was rinsed in Ringer-Locke's solution, and the portions of tissue required were quickly removed and washed again in Ringer-Locke's solution. Strips of tissue 35 - 50 mm. x 4 mm. were then cut and the mucosa or epithelium was detached.

The strips were suspended in oxygenated Tyrode's solution in a tissue bath of 10 ml. capacity, maintained at $37 \pm 0.1^{\circ}\text{C}$. One end of/

Fig. 5. Isolated tissue bath. 10 ml. capacity.



of the tissue was attached by a thread to an isotonic writing lever giving a magnification of 12 : 1. Movements of the lever were recorded on a smoked kymograph. A tension of 1 - 5g was usually applied to the tissue, although strips from the abomasum required a greater tension (up to 10g) than those from other compartments. A diagram of the apparatus is shown in Fig. 5.

A strict time schedule was adopted for changing the fluid in the tissue bath. Drugs were allowed to act for 1 - 5 minutes, but the time was constant for each drug. The fluid in the bath was changed at least once every six minutes as longer intervals allowed changes to occur in the tonus of the muscle strip. Strips which failed to respond consistently to acetylcholine after they had been set up for one hour were rejected. Spontaneous motility was not a useful criterion for assessing the activity of a preparation, as movements often appeared and disappeared during the course of/

of an experiment. It was found, however, that sensitive preparations usually showed some spontaneous movement.

Drugs.

Drugs were diluted in Tyrode's solution and were added to the tissue bath by means of a 1 ml. tuberculin syringe. Dilutions were prepared so that the amount added to the bath did not exceed 0.25 ml., although it was found that up to 0.4 ml. of Tyrode's solution at room temperature could be added without affecting the preparation. When an antagonist was allowed to act for a long period, it was diluted in 0.5 litres of Tyrode's solution and the apparatus was so arranged that solution at 37°C containing the antagonist could be run into the tissue bath.

Solutions of acetylcholine were prepared from acetylcholine chloride (Roche Products Ltd.), and solutions of histamine were prepared from histamine acid phosphate (British Drug Houses Ltd.). A stock solution of L-adrenaline hydrochloride/

hydrochloride containing 2 mg/ml. adrenaline base was prepared by dissolving L-adrenaline (Wellcome Foundation Ltd.), in a dilute hydrochloric acid solution at pH 2.0. This was diluted as required, but, as adrenaline is unstable in neutral or alkaline solution, dilutions were not prepared until required for use. 5-hydroxytryptamine solutions were prepared from 5-hydroxytryptamine creatinine phosphate (Sandoz Products Ltd.).

The following drugs were also used, diluted in Tyrode's solution:-

Atropine sulphate (Evans Medical Supplies Ltd.)
Neostigmine methylsulphate (0.25% aqueous solution) Roche Products Ltd.).
Mepyramine malate (May & Baker Ltd.).
D-lysergic acid diethylamide tartrate (Sandoz Products Ltd.).

Legend of figures 8 - 27

1. Drugs were allowed to act for two minutes unless otherwise stated.
2. All concentrations of drugs are expressed in terms of metric quantities. e.g. 1 mg/ml = 1×10^{-3} .
3. Concentrations of adrenaline and histamine are given as the base, but concentrations of all other drugs are expressed as the salt.

Fig. 6. Regions of stomach examined.

↔ denotes sites from which strips were taken.

A = body.

B = pylorus.

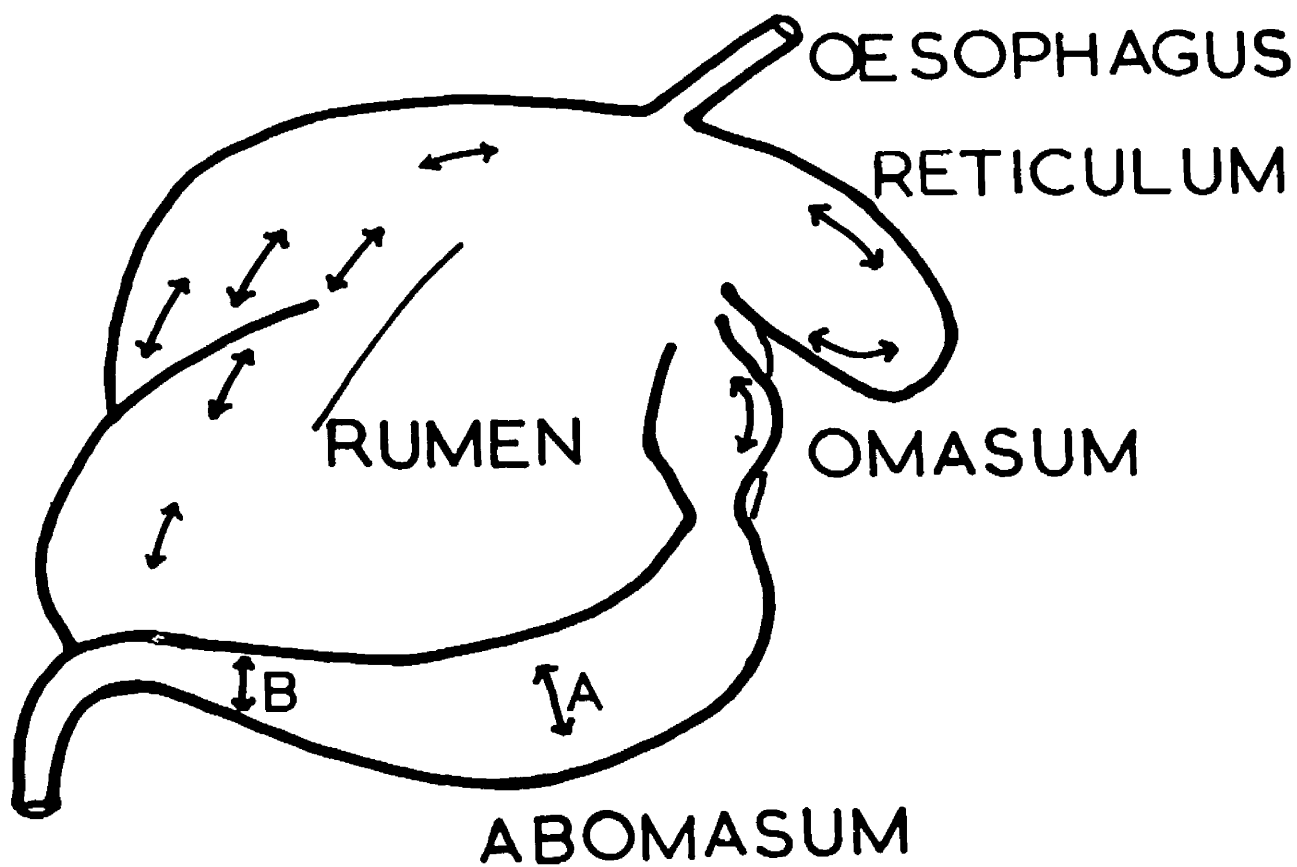
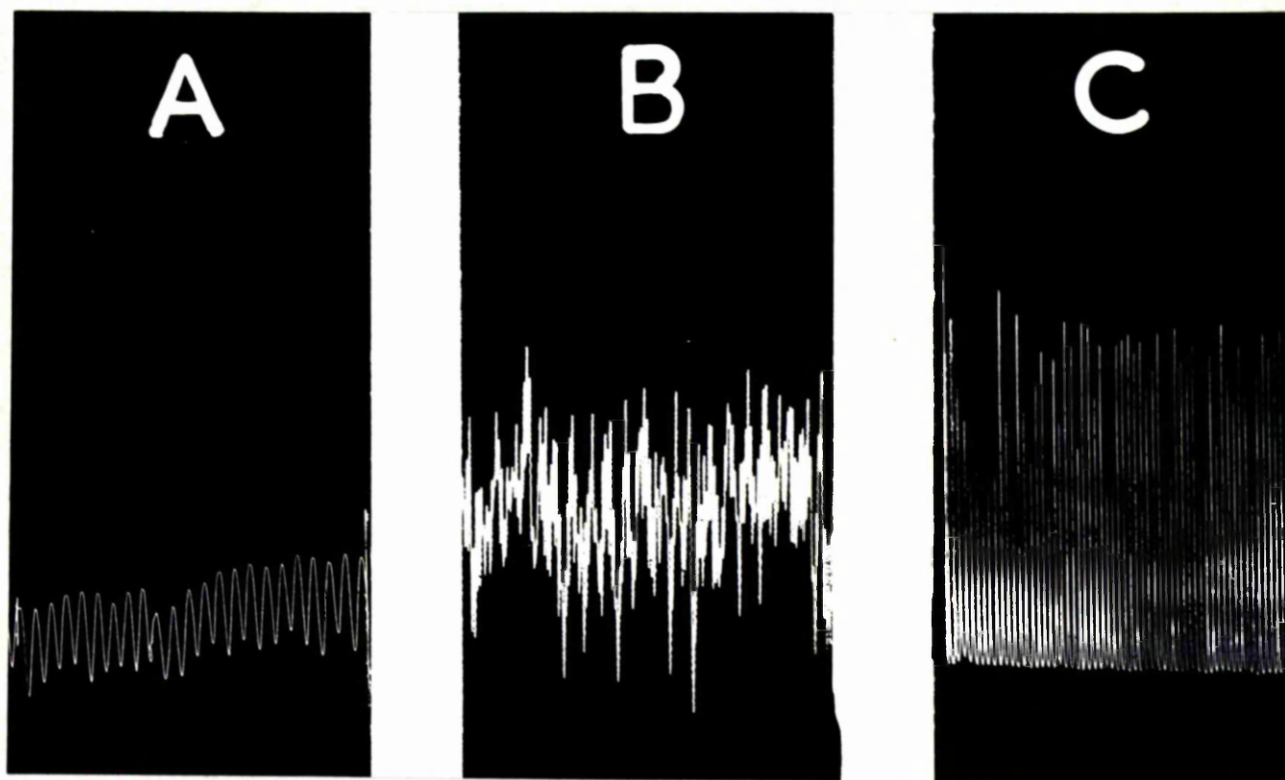


Fig. 7. Spontaneous movement of abomasal strips.

A and B = body. C = pylorus.



1 MIN.

Results.

Regions of Stomach examined.

Strips were taken from the various sites in each compartment indicated in Fig. 6. In general, it was found that the frequency and magnitude of spontaneous movements of abomasal strips did not conform to any uniform pattern. Fig. 7 illustrates some of the types of spontaneous movement shown by abomasal strips.

Whilst responses of the pyloric muscle were qualitatively similar to those of other parts of the abomasum, the large spontaneous movements tended to mask the contraction or relaxation produced by drugs. For this reason, preparations of the body of the abomasum rather than the pylorus were used in these experiments.

Strips of the omasum cut at right angles to the long axis of the organ were less sensitive to drugs than those cut parallel to this axis. The frequency and magnitude of spontaneous movements/

movements and the response to drugs were, however, similar for both types of preparation. Strips taken from different areas of the rumen and of the reticulum were not significantly different in spontaneous movement or in response to drugs.

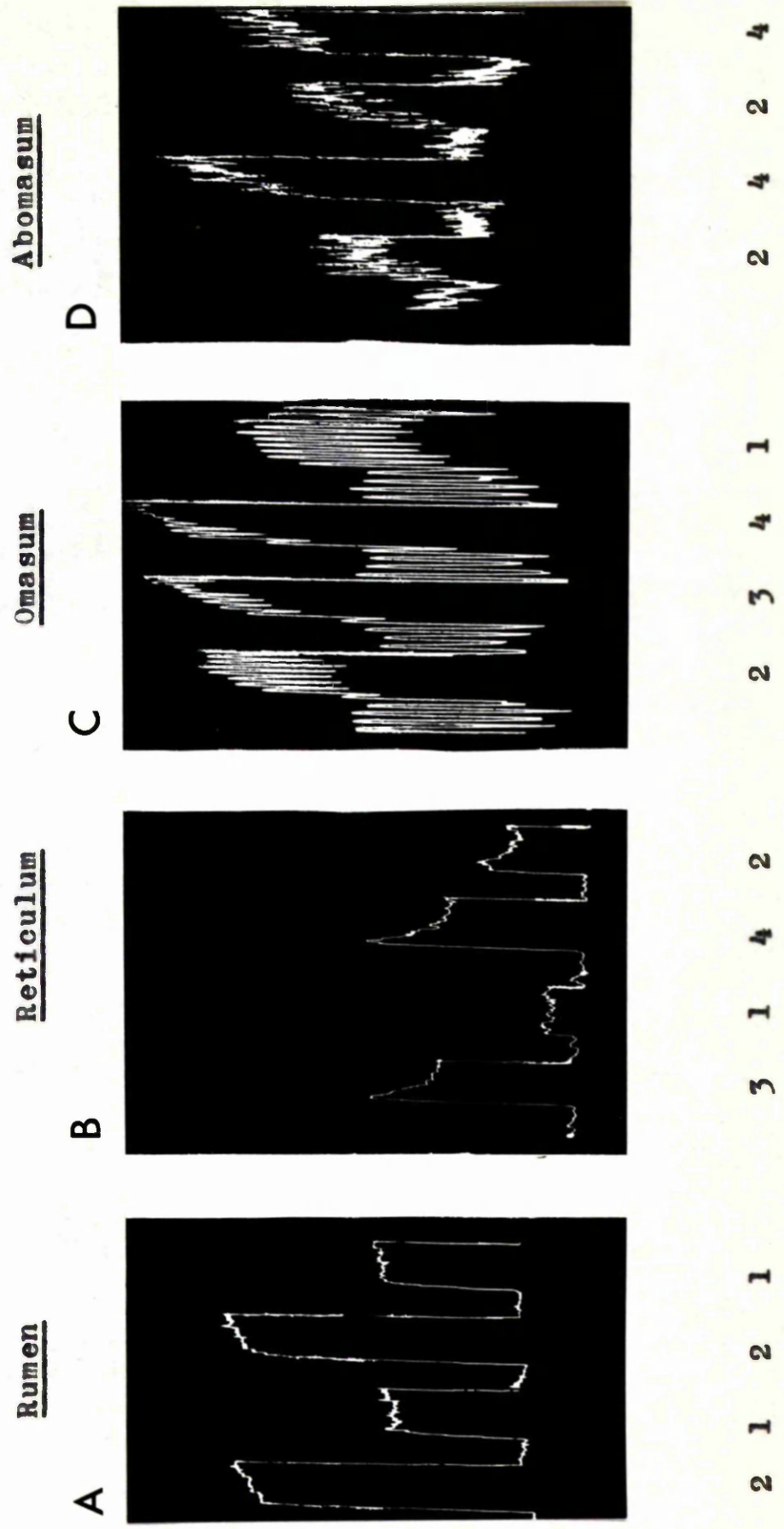
Action of drugs on Isolated Muscle preparations.

Acetylcholine.

In general, isolated strips from all regions of the stomach contracted in response to acetylcholine. Contractions could usually be obtained within 15 minutes of setting up the tissue. On occasions when a preparation did not respond to acetylcholine, it invariably failed to respond also to other drugs. This was used as an initial test for each preparation before studying the response to other drugs. During the course of an experiment, the sensitivity to acetylcholine might be either increased or decreased, but in general the changes observed were small.

Strips from all four compartments showed a consistent response to acetylcholine when it was added at intervals of 6 minutes. In all, the response of strips from 90 sheep stomachs were examined, and in each preparation, there was a direct relation between the height of contraction and/

Fig. 8. Response of isolated strips to acetylcholine.
Concentrations $\times 10^{-7}$ acetylcholine.



and the concentration of acetylcholine. There were some differences in the nature of the response, especially between reticulum and rumen on the one hand, and omasum and abomasum on the other.

When acetylcholine was added to the bath, the strips of reticulum contracted rapidly and reached maximum height within 30 seconds. The effect was transient and the reticulum had usually started to relax before the drug was washed out after two minutes. The response of the rumen was usually similar to that of the reticulum in its rapid onset, but the contraction was maintained for a longer time and the preparation did not always relax before the drug was washed out. See figs. 8A and B. On the other hand, some preparations of rumen did start to relax before the end of two minutes. See fig. 12.

Strips from the omasum and abomasum contracted more slowly in response to acetylcholine and at the end of two minutes, the contraction was still maintained. The persistence of spontaneous movements in the presence of acetylcholine was frequently/

frequently observed in both omasum and abomasum, as can be seen in Figs. 8 C and D. Rhythmical contractions persisted even when the response to acetylcholine had reached its maximum. The concentrations of acetylcholine required to produce these effects did not differ significantly for strips taken from the different compartments. Dose response curves could be obtained using concentrations of acetylcholine between 10^{-8} and 10^{-6} .

Fig. 9.

Effect of atropine on acetylcholine contractions of rumen strip. Contractions in response to 1×10^{-7} acetylcholine.

ATR = 10^{-7} atropine sulphate.

Atropine was allowed to act for 5 minutes, and was added 3 mins. before acetylcholine.

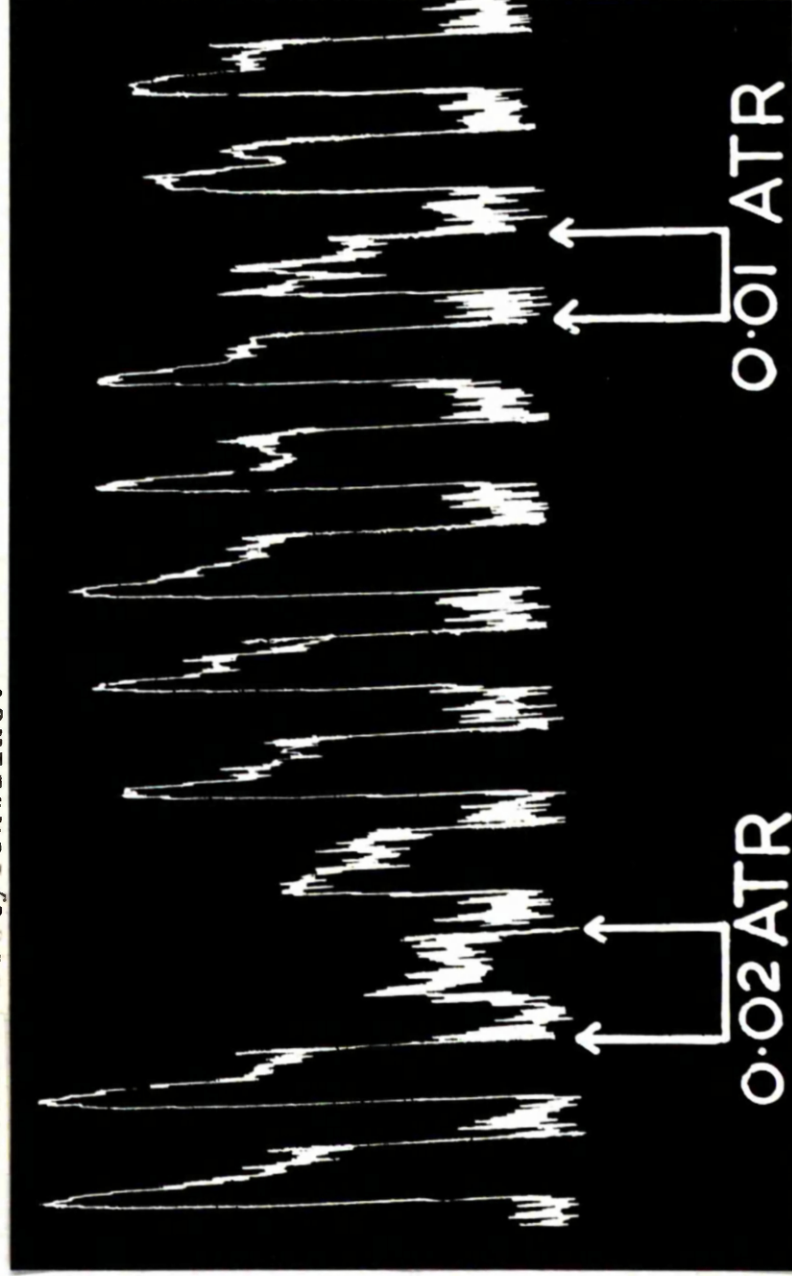
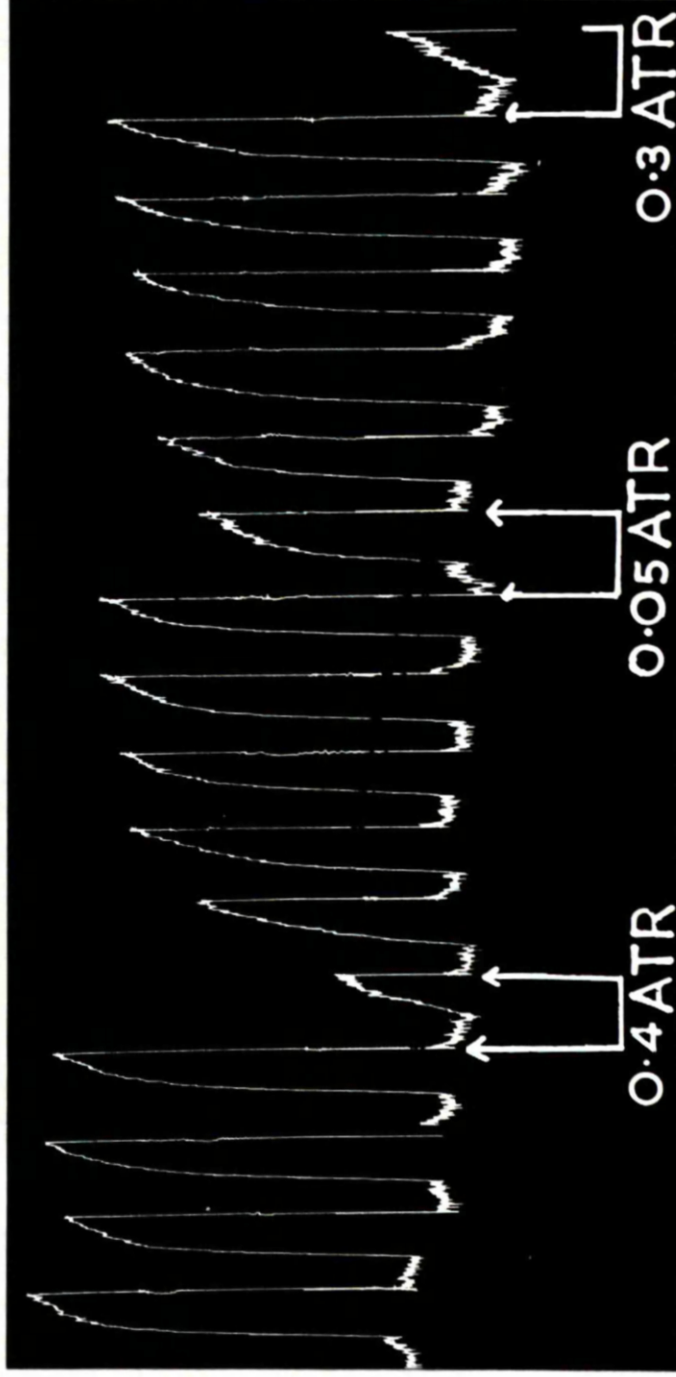


Fig. 10.

Effect of atropine on acetylcholine contractions of abomasum strip. Contractions in response to 1×10^{-6} acetylcholine.

ATR = 10^{-7} atropine sulphate.

Atropine was allowed to act for 5 minutes, and was added 3 mins. before acetylcholine.



Experimental Studies of Isolated Tissue.

Atropine. In concentrations of 10^{-9} - 5×10^{-8} atropine sulphate did not influence the spontaneous contractions. When it was added three minutes before acetylcholine, it antagonised the contractions produced by the latter in proportion to the concentration of atropine used. This effect was observed with preparations from all four compartments and representative tracings are shown for rumen and abomasum in figs. 9 and 10. The strips recovered their normal response to acetylcholine within 12 - 18 minutes of washing out the atropine.

Neostigmine. The effect of neostigmine in potentiating the response to acetylcholine was observed in strips from all compartments. When neostigmine methylsulphate was added to Tyrode's solution in concentrations of 10^{-8} - 5×10^{-8} , a gradual increase in the response to acetylcholine was observed, reaching its maximum after 30 - 50 minutes. Representative tracings obtained from experiments/

Fig. 11. Effect of neostigmine on acetylcholine contractions of reticulum strip. Contractions in response to 1×10^{-7} acetylcholine.

$N = 1 \times 10^{-7}$ neostigmine methylsulphate.

Acetylcholine was allowed to act for 1 min. and neostigmine was present for 30 mins.

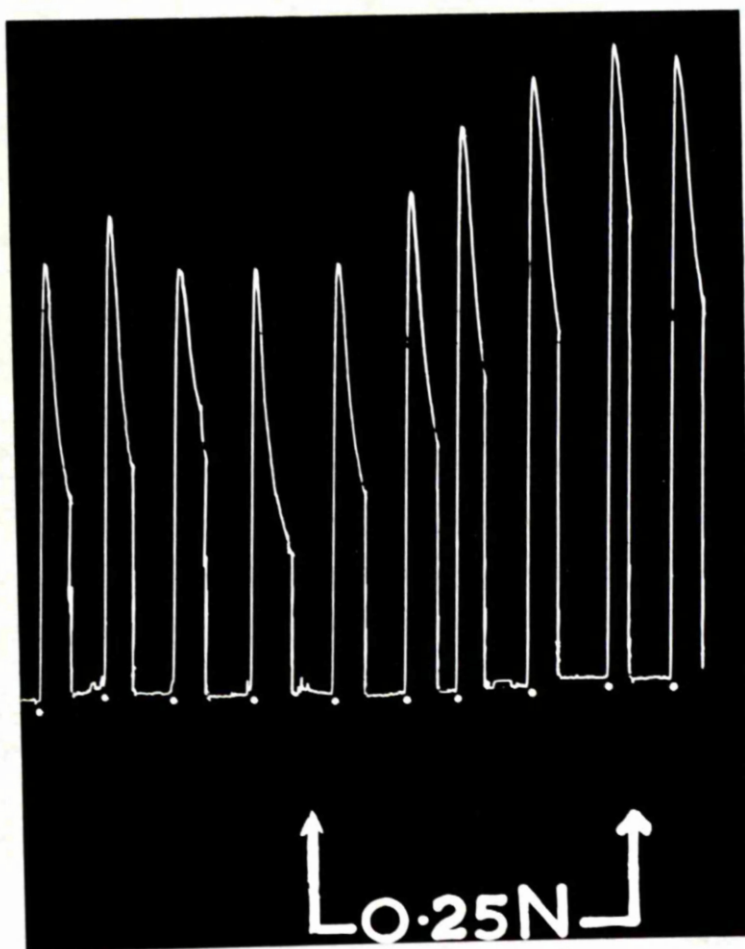


Fig. 12. Effect of neostigmine on acetylcholine contractions of abomasum strip.

Contractions in response to 4×10^{-7} acetylcholine.

$N = 4 \times 10^{-7}$ neostigmine methylsulphate.

Neostigmine was present for 60 minutes.

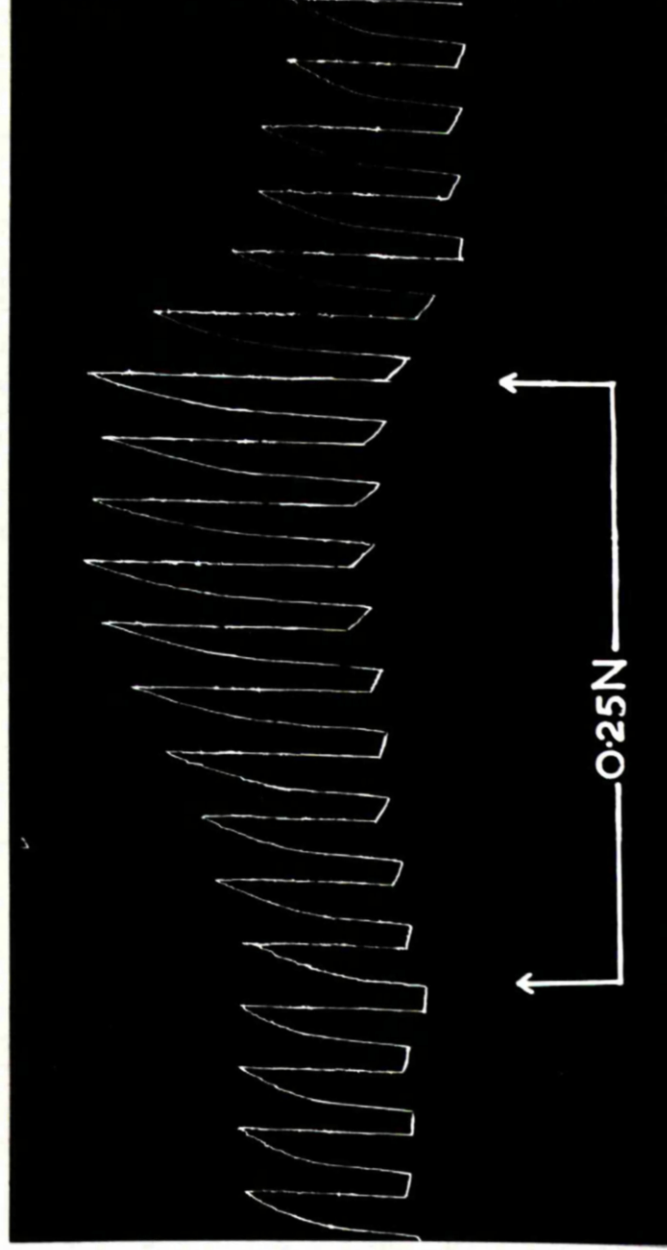
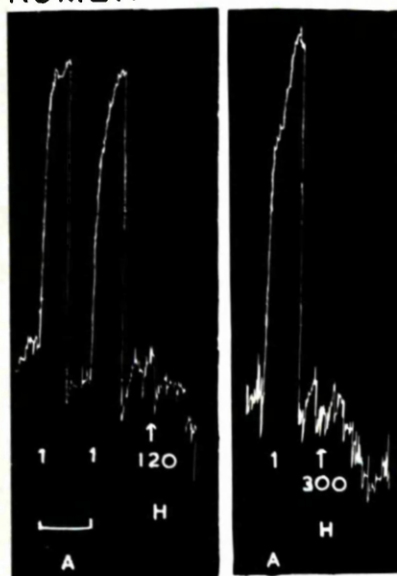


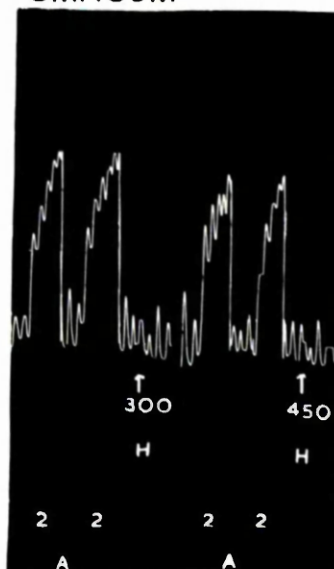
Fig. 13. Responses to histamine and to acetylcholine.

A = $\times 10^{-7}$ acetylcholine. H = $\times 10^{-7}$ histamine.

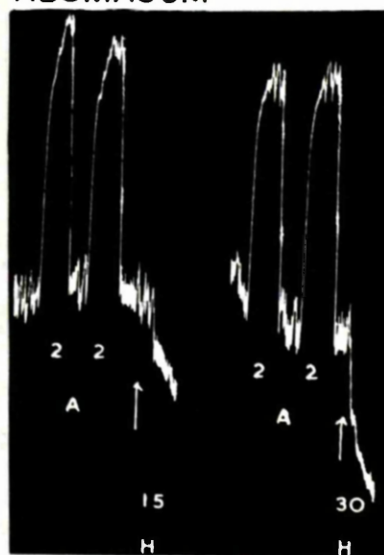
RUMEN



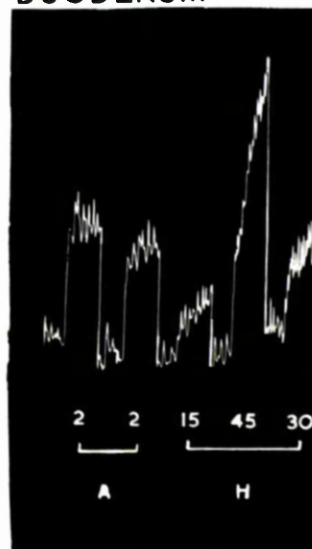
OMASUM



ABOMASUM



DUODENUM



experiments with isolated strips of reticulum and abomasum are shown in figs. 11 and 12. The potentiating effect continued for some time after the addition of neostigmine had been discontinued and even after several hours, slight potentiation of acetylcholine could still be seen.

Histamine. The experiments on the response of strips of muscle to histamine gave quite different results from those obtained with acetylcholine. The addition of histamine to the bath did not produce contractions of any of the muscle strips. This is illustrated in fig. 13 which shows the response produced by the addition of acetylcholine and of histamine to muscle strips obtained from rumen, abomasum, omasum and duodenum. For convenience of illustration, the responses of the reticulum are not included, since they were identical to those of the omasum. It will be seen from the figure that a typical contraction was produced in all strips by the addition of acetylcholine and that the addition of histamine produced a contraction of the duodenum when added in/

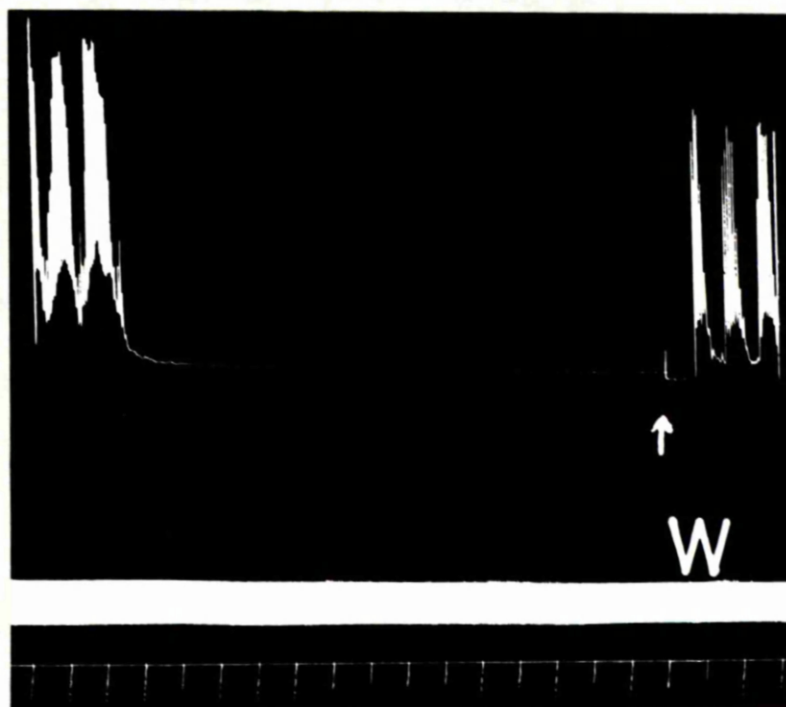
in concentrations of $1.5 - 4.5 \times 10^{-6}$. None of the strips from any of the compartments of the stomach contracted in response to histamine, even when it was added in doses of up to twenty times the amount which produced a contraction of the duodenum. On the other hand, it will be seen that relaxation of the abomasum occurred when histamine was added to the bath in the same concentrations as those which produced contractions of the duodenum.

A more detailed study was made of the response to histamine using strips from all four compartments obtained from twelve sheep stomachs. In general, it can be said that there was a clear distinction between the responses of the rumen and abomasum on the one hand, and those of the reticulum or omasum on the other. No evidence of contraction or relaxation of reticulum or omasum was observed when histamine was added to the bath in concentrations up to 5×10^{-5} .

When histamine was added to the bath in which a strip of abomasum was spontaneously contracting, it/

Fig. 14. Relaxant action of histamine on
abomasum strip. Histamine washed out
at W. Time marker 1 min.

6×10^{-7} histamine.



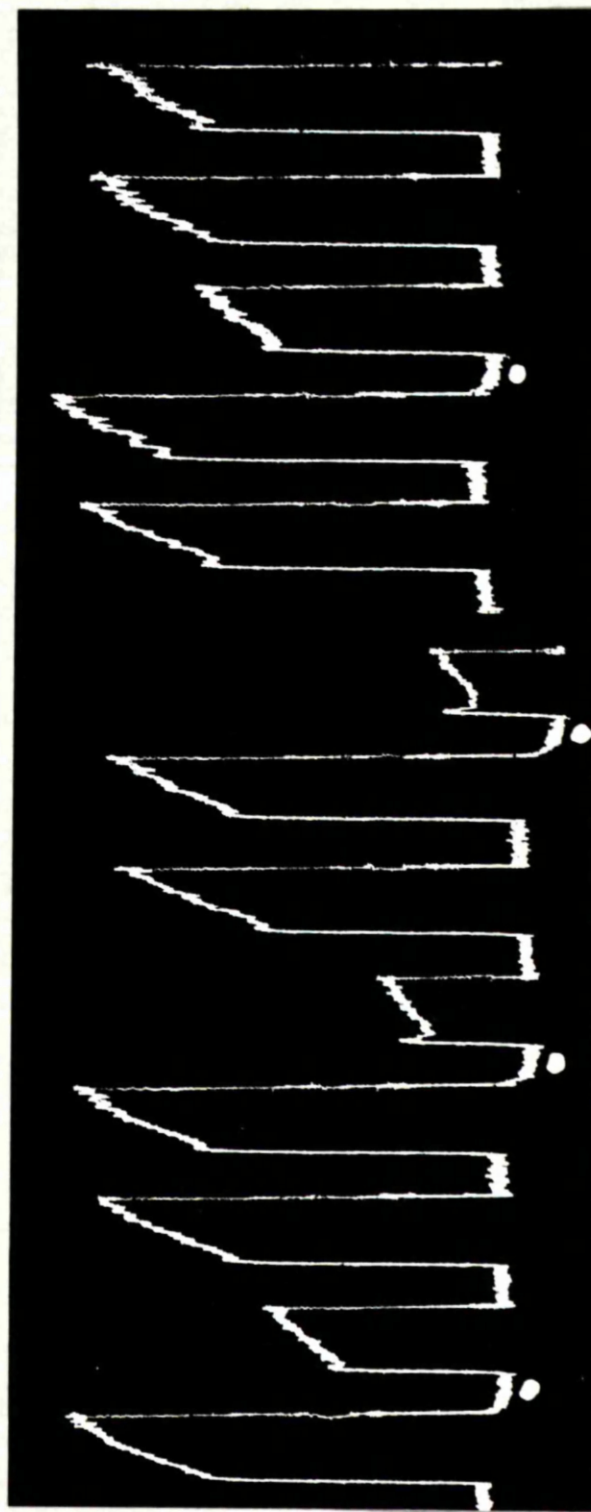
it produced an immediate relaxation which had reached its maximum within two minutes. Graded responses were usually observed with concentrations of histamine ranging from 5×10^{-7} - 10^{-5} . Sometimes, when the spontaneous activity was well marked, this persisted during the relaxation. More usually, however, spontaneous activity did not continue while the histamine was present in the bath. If histamine was allowed to remain in the bath, the return of spontaneous movements was often delayed for as long as fifteen minutes. See figure 14.

Some of the strips of the abomasum did not show spontaneous movements and when histamine was added to the bath, no effect was observed, even though the muscle contracted in response to the usual concentration of acetylcholine.

In addition to causing relaxation, histamine also diminished the contractions produced by acetylcholine. If histamine was added to the tissue bath in a concentration of 5×10^{-7} - 10^{-5} , thirty seconds before a dose of acetylcholine, the resultant/

Fig. 15.

Inhibitory action of histamine on acetylcholine contractions of strip of abomasum. Histamine added 30 secs. before acetylcholine. $H = 4 \times 10^{-7}$ histamine. Contractions in response to 4×10^{-7} acetylcholine.



30
H

60
H

90
H

15
H

Fig. 16. Relaxant and inhibitory action of histamine on acetylcholine contractions of abomasum strip.

Contractions in response to 1×10^{-7} acetylcholine. 10H = 1×10^{-6} histamine.

Histamine added 30 secs. before acetylcholine.

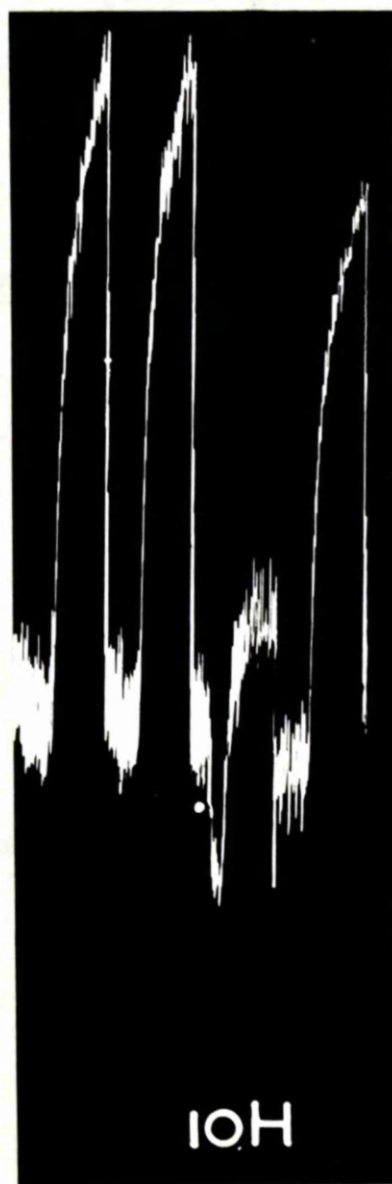


Fig. 17. Relaxant effect of histamine without inhibition of acetylcholine. Abomasum strip. Contractions in response to 2×10^{-8} acetylcholine. $H = \times 10^{-7}$ histamine. Histamine added 30 secs. before acetylcholine.

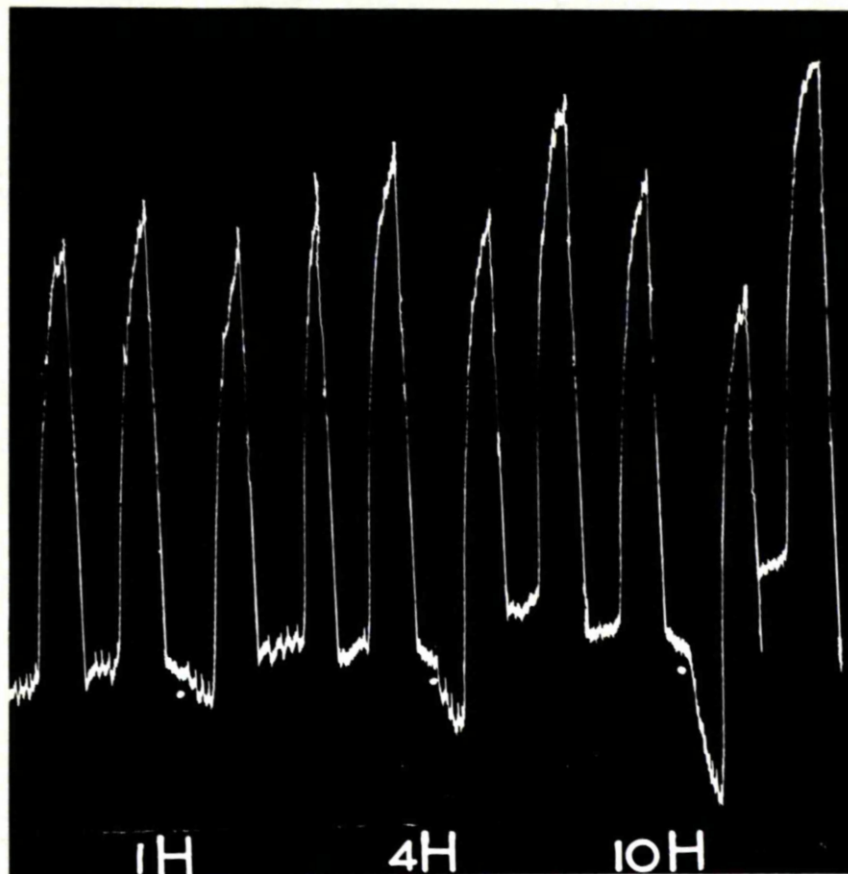


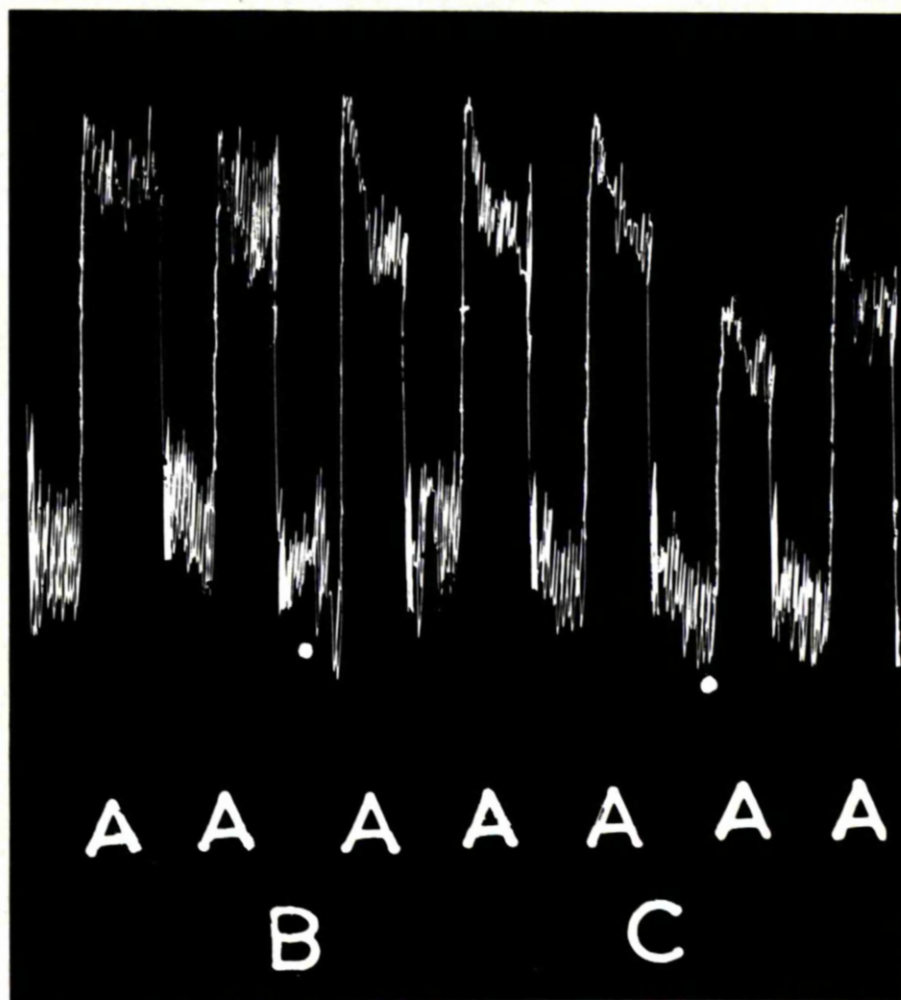
Fig. 18. Effect of histamine on acetylcholine contractions of rumen strip.

denotes addition of histamine.

A = 1×10^{-7} acetylcholine

B = 3×10^{-6} histamine added 30 secs. before acetylcholine.

C = 3×10^{-6} histamine and 1×10^{-7} acetylcholine added simultaneously.



resultant contraction was much less than in the absence of histamine. The antagonism between histamine and acetylcholine which is illustrated in fig. 15 was demonstrated both in preparations which relaxed and in those which did not (see also fig. 16). In some experiments, even though there was marked relaxation in response to histamine, the subsequent contraction produced by acetylcholine was not inhibited. This effect is illustrated in fig. 17. The contractions produced by 5HT or by adrenaline were not antagonised by histamine.

The response of strips of rumen to histamine closely resembled that of the abomasum. The effect was less marked than was seen with strips of abomasum and higher concentrations of histamine were usually needed to produce it. When histamine was added to the bath thirty seconds before acetylcholine, the subsequent contraction was not inhibited. If histamine was added at the same time as acetylcholine, however, the contraction was diminished. This effect is illustrated in fig. 18. Antagonism between histamine and acetylcholine was/

Fig. 19. Effect of histamine on acetylcholine
contractions of rumen strip.

Contractions in response to 1×10^{-7}
acetylcholine.

1×10^{-5} histamine for 27 mins. between arrows.

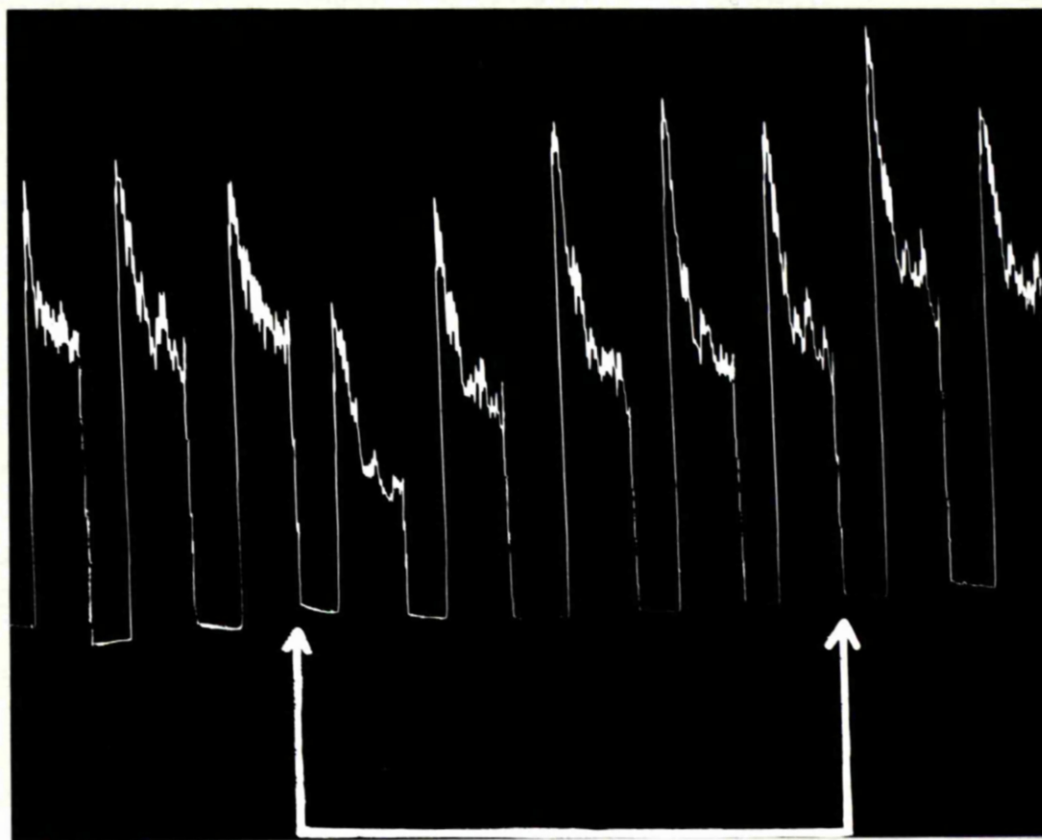
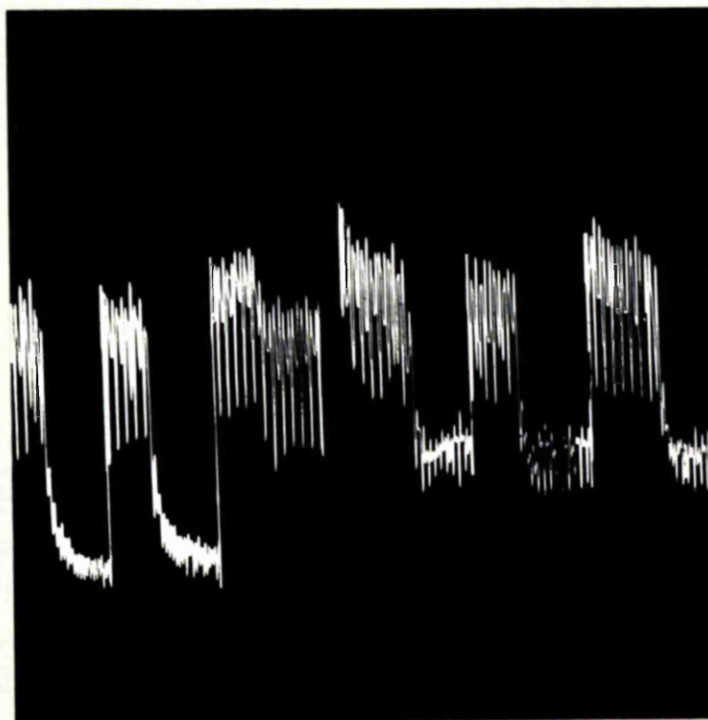


Fig. 20. Effect of mepyramine on response to histamine. Abomasum strip.
Relaxation in response to 2×10^{-6} histamine.
Mepyramine added 3 mins. before histamine.



2×10^{-6} mepyramine.

was thus more difficult to demonstrate on the rumen than on the abomasum. Even when histamine was added to Tyrode's solution and was therefore continuously present in the bath, the contractions produced by acetylcholine were diminished only for a brief period (fig. 19).

Some experiments were performed to see whether the relaxation produced by histamine on the abomasum and rumen could be inhibited by an anti-histamine compound. Mepyramine maleate was used but some difficulty was experienced in finding a concentration of this drug which would antagonise the effects of histamine without causing a progressive rise in the tone of the muscle strip. It was possible, however, to demonstrate that when a dose of the antagonist similar to that of histamine was added three minutes before histamine, subsequent relaxation diminished. This is illustrated in fig. 20.

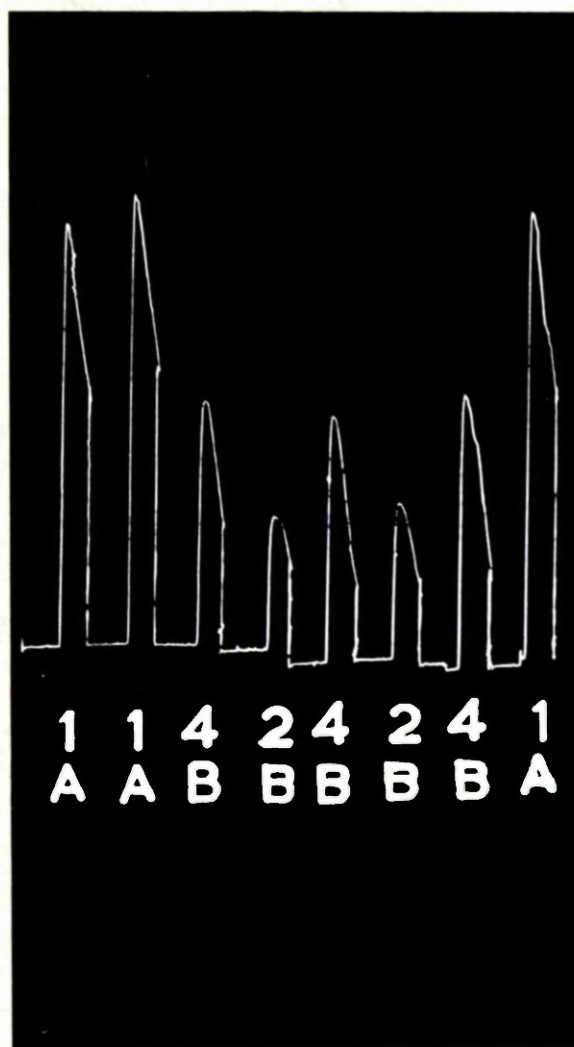
Numerous attempts were made using both strips of abomasum and of rumen to demonstrate that mepyramine would antagonise the inhibition of the/

Fig. 21. Responses to adrenaline and to acetylcholine. Reticulum strip.

A = $\times 10^{-7}$ acetylcholine.

B = $\times 10^{-7}$ L-adrenaline.

Drugs were allowed to act for 1 min.



the response to acetylcholine produced by histamine. In all these experiments, there was no convincing evidence that the presence of mepyramine had any significant effect.

Adrenaline. The response to adrenaline of strips from either the same or from different compartments varied much more than the response to acetylcholine. Sometimes relaxation was observed and sometimes no response was obtained, but contraction usually resulted when the concentration of adrenaline was increased. The response of the tissue was generally slower in onset than with acetylcholine and was more prolonged.

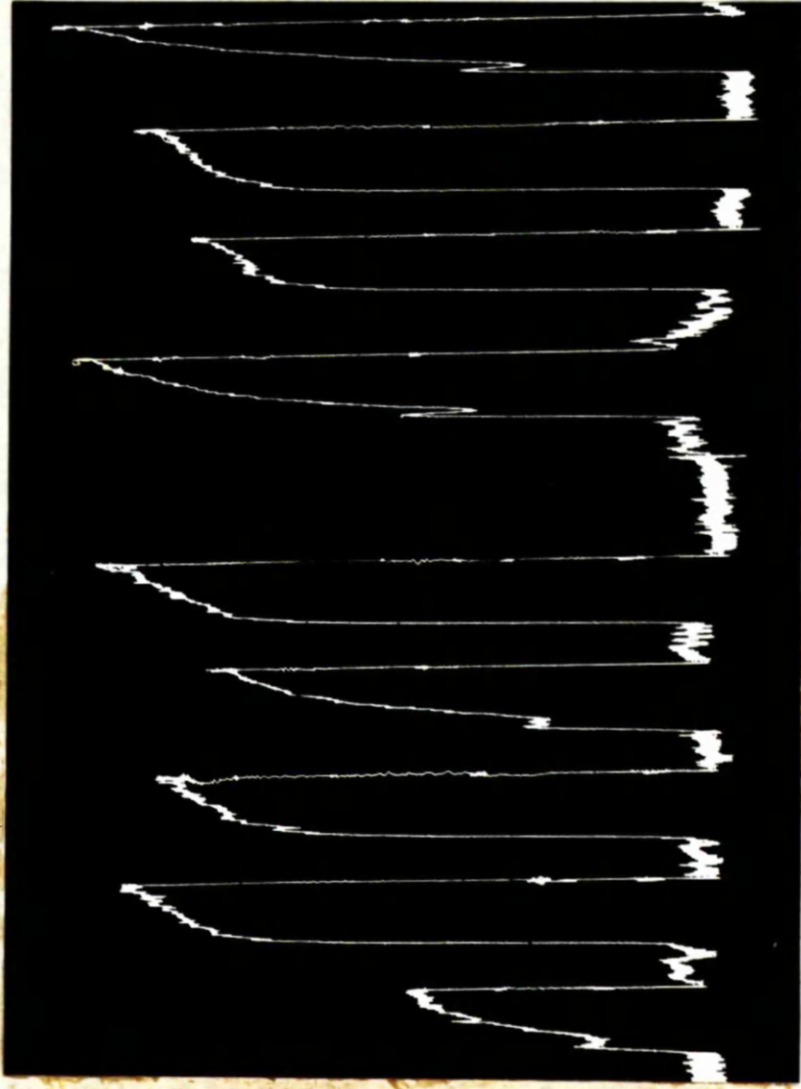
With strips of reticulum, contraction occurred in only two out of six preparations in response to adrenaline in a concentration of 2×10^{-7} (fig. 21). The other four preparations did not respond to adrenaline in concentrations up to 10^{-5} even though all six appeared to be equally sensitive to acetylcholine.

Strips of rumen and of abomasum responded similarly/

Fig. 22a. Response to adrenaline
of abomasum strip. Relaxation
in response to 1×10^{-7}
L-adrenaline.



Fig. 22b. Contractions of abomasum strip in
response to acetylcholine & adrenaline.
A = 1×10^{-6} acetylcholine.
D = 1×10^{-7} L-adrenaline.



5
D

A A

10
D

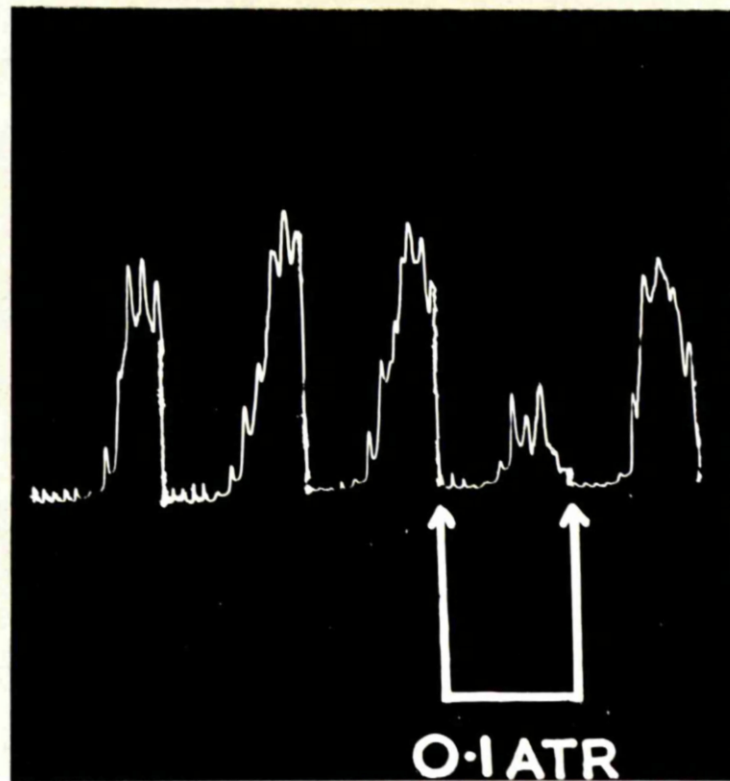
A

15
D

A A

15
D

Fig. 23. Effect of atropine on adrenaline
contractions of abomasum strip. Contractions
in response to 4×10^{-7} L-adrenaline.
ATR = $\times 10^{-7}$ atropine sulphate which was allowed
to act for 5 mins.



similarly to adrenaline. When added in concentrations of less than 5×10^{-7} , relaxation was usually observed, and when concentrations of about 10^{-6} were used, a slow contraction (as shown in fig. 22) was produced. The abomasum contracted in response to adrenaline more slowly than to acetylcholine and the contraction did not reach its maximum until after two minutes. The effect of adrenaline persisted after washing out the bath, and the preparations usually returned to the normal resting state some fifteen minutes later. When a further dose was added before 15 minutes the contraction produced was greater than to the same dose given after the tissue had recovered. Contractions in response to adrenaline could be antagonised by atropine added to the bath before adrenaline, but the concentration of atropine required to produce this antagonism was usually ten times more than was needed to antagonise the effect of acetylcholine (see fig. 23).

Strips of omasum were usually unresponsive to adrenaline but occasionally a contraction was observed/

Fig. 24.

Inhibitory action of adrenaline on acetylcholine contractions of reticulum strip.

Contractions in response to 4×10^{-7} acetylcholine.

D = $\times 10^{-7}$ L-adrenaline which was allowed to act for 5 mins.

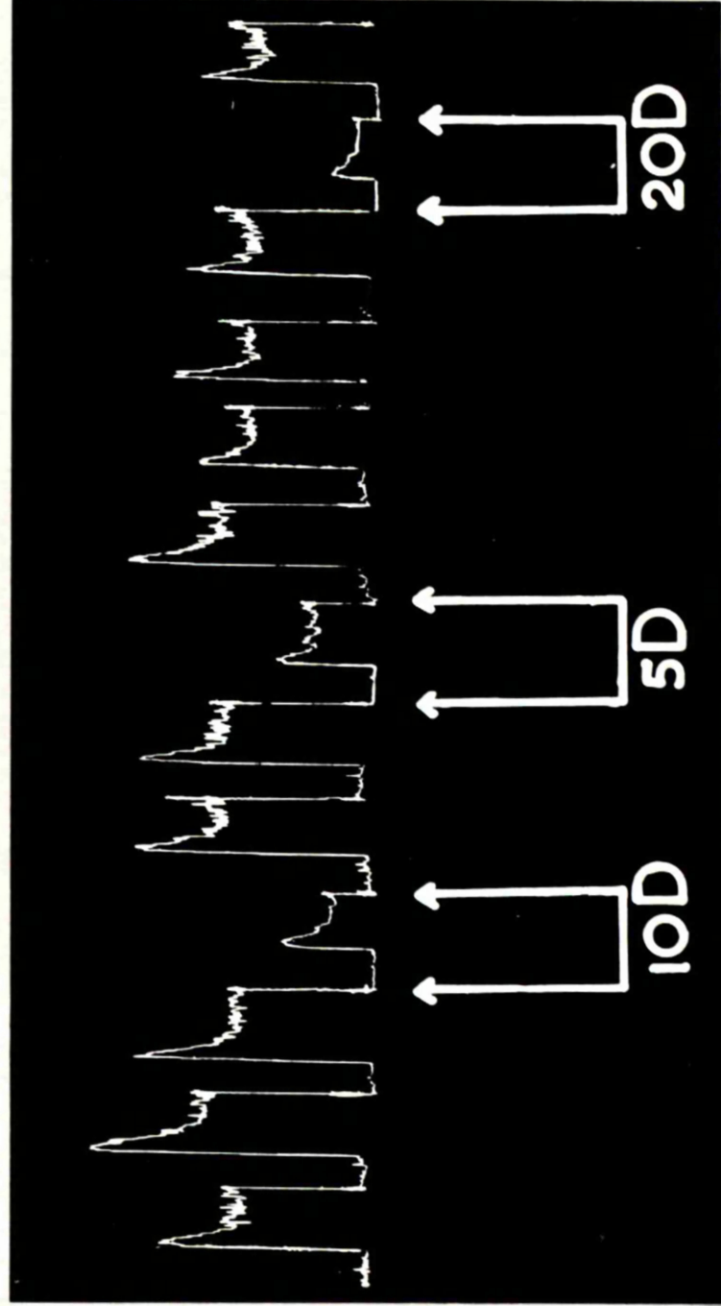


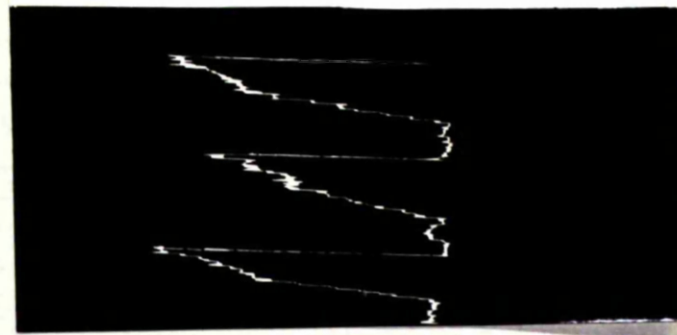
Fig. 25. Response of isolated strips to 5-hydroxytryptamine creatinine phosphate. Concentrations $\times 10^7$.

Rumen

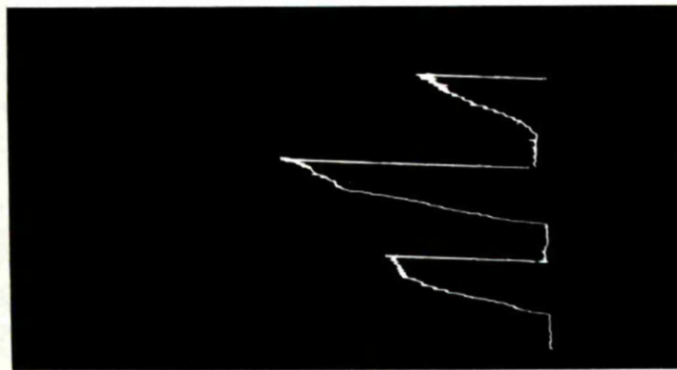
Reticulum

Omasum

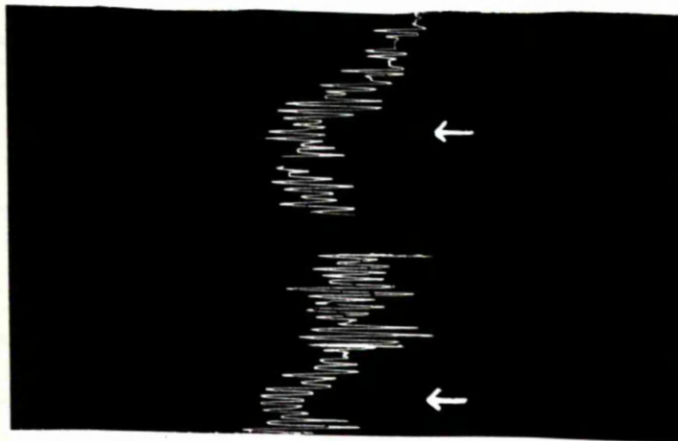
Abomasum



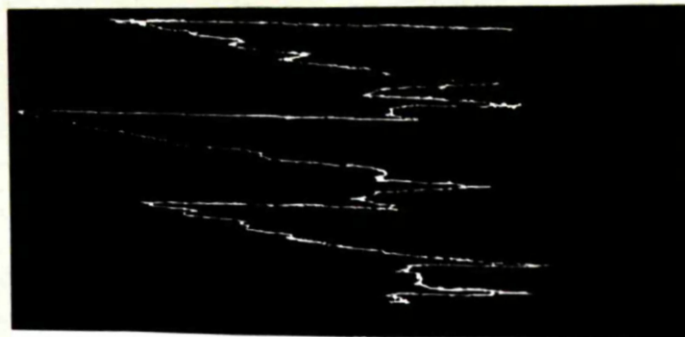
1.5 1.0 1.5



1 2 1



10 50



1 2 1

observed when concentrations of 10^{-6} - 2×10^{-5} were added to the bath. Concentrations of adrenaline, which when added to the bath appeared to produce no effect on rumen, reticulum or omasum often inhibited the response to acetylcholine. An example of this antagonism between adrenaline and acetylcholine is shown in fig. 24, in which contractions produced by acetylcholine in a concentration of 4×10^{-7} were antagonised by the previous addition of adrenaline in a concentration of 5×10^{-7} - 2×10^{-6} .

5-Hydroxytryptamine. 5-Hydroxytryptamine (5HT) in concentrations of 5×10^{-8} - 10^{-5} caused contraction of strips of abomasum, reticulum and rumen, but in some preparations, repeated doses showed tachyphylaxis with a rapidly decreasing response. Contractions produced by 5HT were slower in onset than those produced by acetylcholine, and did not usually reach their maximum until after two minutes. This is illustrated in fig. 25 where it will be seen that there was no significant response of the omasum even when doses were used which were 100 times greater than those which produced contraction of strips from the other compartments. Further investigation of the response/

Fig. 26.

Response of omasum strip to 5-hydroxytryptamine showing tachyphylaxis after repeated doses.

T = $\times 10^{-7}$ 5HT creatinine phosphate.

A = $\times 10^{-7}$ acetylcholine.

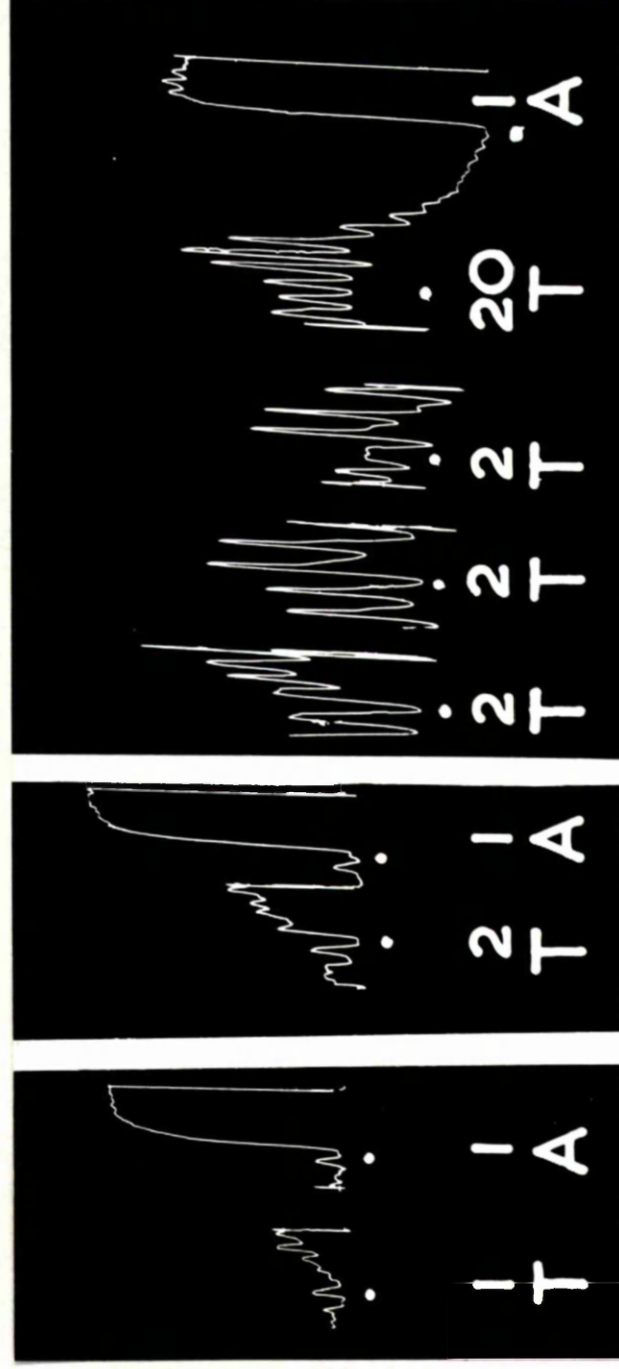
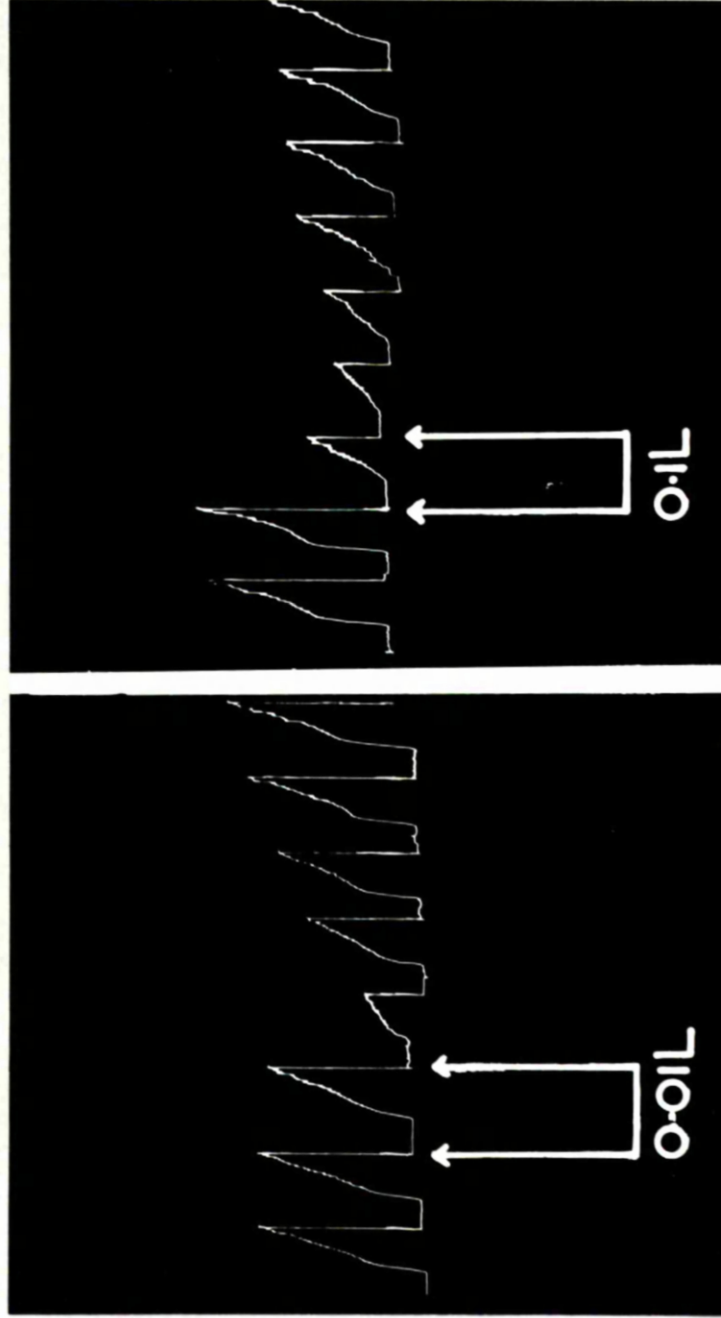


Fig. 27. Effect of lysergic acid diethylamide (LSD) on 5-hydroxytryptamine contractions of abomasum strip. Contractions in response to 4×10^{-7} 5HT creatinine phosphate. L = 10^{-7} LSD tartrate which was allowed to act for 9 minutes.



response of the omasum to 5HT revealed that when low concentrations (10^{-7}) of the drug were employed, a contraction was sometimes obtained. If this dose was repeated, however, a marked tachyphylaxis occurred, and no further contractions were seen. See fig. 26.

Strips of reticulum and rumen contracted more rapidly than those of abomasum, but there were no significant differences in their sensitivity to 5HT.

Antagonism by lysergic acid diethylamide (LSD) was demonstrated in preparations of reticulum, rumen and abomasum. The concentration of LSD employed was 10^{-9} - 4×10^{-9} and the effect was not fully developed until 15 - 18 minutes after it was added to the bath. This delayed effect is shown in fig. 27, where it can be seen that a much greater inhibition of the response to 5HT was obtained nine minutes after the antagonist had been washed out than occurred when it was present in the tissue bath. These concentrations of LSD did not influence the response of the tissue to acetylcholine.

Discussion.

Little difficulty has been experienced in these experiments in obtaining active preparations of isolated strips from each compartment of the sheep stomach. It is possible that the explanation for the difficulties encountered by Duncan (1954) may be associated with a delay in setting up the muscle strips. This is certainly the only factor which, in the present investigation has been associated with a failure of the preparations to give a typical response.

It may be noted also that both Duncan (1954) and Dussardier and Navarro (1953) maintained the temperature of the tissue bath at 38°C , whereas, in the present experiments, it was found that although preparations kept at this temperature were active initially, they failed to respond to drugs after two or three hours. On the other hand, when the temperature was maintained at 37°C , the strips of isolated tissue remained sensitive for several hours.

Spontaneous movement of the isolated strip of tissue did not give a reliable indication of its sensitivity to drugs. Unlike Duncan, who used this as a means of selecting suitable preparations for her experiments, more consistency was obtained in the present experiments when preparations were selected/

selected according to their ability to give a consistent preliminary response.

It is not clear how relevant spontaneous movements in isolated tissue preparations are to their response to drugs. Dussardier and Navarro (1953), however, investigated this aspect in some detail, using isolated strips of ox stomach. They observed that it was possible to demonstrate a change in the rate and size of spontaneous movement in response to drugs. No special study has been made of these movements in the present investigation. Some observations, however, were made on the effects of some drugs on the spontaneous movement of isolated strips of tissue taken from different compartments of the sheep stomach, but no consistent effects were observed when nicotine, hexamethonium, atropine and lysergic acid diethylamide were added in different concentrations to the isolated tissue bath.

Of all the drugs tested in this investigation, only acetylcholine unfailingly caused rapid/

rapid contractions. The differences in the speed of contraction of isolated strips from each compartment are approximately the same as those which have been observed for spontaneous movements in the intact sheep. Thus, strips of reticulum contracted fully within a few seconds, strips of rumen within 30 second, whilst strips of omasum and abomasum contracted much more slowly, taking several minutes to reach a maximum. These findings are consistent with the generally accepted view that acetylcholine is probably the mediator of the vagus nerve in the sheep stomach. Dale and Feldberg (1934) have provided conclusive evidence that there is an increased output of acetylcholine from the dog stomach following stimulation of the vagus nerve. Such evidence has not yet been obtained for the sheep stomach.

The results of the investigation of the action of adrenaline recorded in this thesis do little more than confirm the previous reports of Dussardier and Navarro (1953) and Duncan (1954). A dual action of adrenaline on isolated strips of fundus of rat stomach was reported in 1926 by Brown/

Brown and McSwiney. These authors found that when the tonus of the preparation was high, adrenaline caused a relaxation, but when the tonus was low, contraction was seen. They also noted that when relaxation did occur, it might be followed by what they termed "an indefinite motor effect".

In the ruminant, adrenaline given intravenously inhibits stomach movements. Dussardier (1954) using spinal sheep and Duncan (1954) using vagotomised sheep have each shown, however, that when the normal spontaneous movements of the stomach are absent, intravenous injection of adrenaline sometimes causes contraction of the reticulum.

It seems clear, therefore, that the action of adrenaline on isolated strips of muscle from the sheep stomach is similar to its action on the stomach in vivo.

The actions of 5-hydroxytryptamine (5HT) in isolated strips of sheep stomach are similar/

similar to that observed in many other types of smooth muscle. The tendency of preparations to show tachyphylaxis after repeated doses is well-known, and Gaddum (1953) showed that guinea-pig ileum could be made insensitive to further doses of 5HT by allowing it to remain in contact with a large dose of this drug for a prolonged period. Gaddum and Hameed (1954) have classified receptors of 5HT into two groups:-

- a) those which are not desensitised by repeated doses, but in which the response is readily antagonised by lysergic acid diethylamide.
- b) those which are easily desensitised by repeated doses of 5HT and in which contractions are not so readily antagonised by lysergic acid diethylamide.

The results of the present investigation indicate that receptors in the rumen, reticulum and abomasum should be included in the first group, and those of the omasum in the second.

The/

The consistent relaxation of abomasal strips produced by histamine was rather surprising in view of the conclusion by Duncan (1954) that histamine had little effect on such preparations. Examination of an illustration in her paper, however, shows that a slight relaxation of an isolated strip of abomasum occurred in response to histamine. The apparent failure of Duncan to obtain a consistent response to histamine may have been due, at least in part, to the use of preparations which were less sensitive than those used in the present investigation.

The responses to histamine described were obtained consistently and it is reasonable to conclude that they are characteristic of histamine and not of the phosphate radical. Addition of sodium acid phosphate to the bath in the same concentration as occurred in a solution of histamine acid phosphate produced no effect.

The significance of the apparent antagonism between histamine and acetylcholine is not clear. It/

It is difficult to account for the fact that histamine sometimes failed to inhibit the response of isolated strips of abomasum to acetylcholine, although it produced its typical relaxation. Only by further study of this problem could it be decided whether or not this can be attributed to a dual effect of histamine.

Histamine did not cause contraction of isolated strips from any compartment of the sheep stomach, an observation which has also been noted by Duncan (1954). The inability of muscle from the sheep stomach to contract in response to histamine is rather surprising, in view of the fact that contractions of stomach muscle of other species have been observed. Under the conditions used in the present investigation, it was found that histamine contracted isolated strips of muscle from the guinea-pig stomach. Von Graf, and Delak (1951) found that histamine contracted isolated strips from the ox rumen and Waldor (1953) showed that histamine also contracted isolated muscularis mucosae of the human stomach. On the other hand, Alexander (1951)/

(1951) found that in the horse, histamine given by intravenous injection had little effect on spontaneous contractions of the stomach, although an increase in tonus was observed.

The inability of mepyramine to antagonise the action of histamine on strips of abomasum is rather difficult to explain. Concentrations of mepyramine, which inhibited the response of isolated duodenum to histamine, did not inhibit relaxation of strips of abomasum produced by similar concentrations of histamine. It is well known that anti-histamine drugs do not inhibit the secretion of gastric juice in response to histamine, although they effectively antagonise the action of histamine on smooth muscle. Although the response of both rumen and abomasum strips to histamine could sometimes be antagonised by mepyramine, this was never well marked in the abomasum.

On the basis of this evidence, it is suggested that the relative ineffectiveness of mepyramine may be due to its inability to penetrate abomasal tissues/

tissues in a concentration sufficient to antagonise the action of histamine. This is supported by the experiments which showed that, whilst antagonism occurred fairly quickly in preparations of rumen, it was not observed even after strips of abomasum had been exposed to the same concentrations of mepyramine for 20 - 30 minutes.

In conclusion it would appear from these results that histamine has no direct action on the smooth muscle of reticulum and omasum and has only a slight depressant action on muscle from the rumen. Abomasal muscle, on the other hand, is relaxed by histamine and its normal response to acetylcholine is diminished. It is, therefore, unlikely that paralysis of the reticulum and rumen which follows the intravenous injection of histamine is due to any action on stomach muscle. Paralysis of the abomasum, may, however, result from such a direct action of histamine.

THE ACTION OF HISTAMINE IN THE SHEEP

SECTION II -- THE ACTION OF HISTAMINE IN THE SHEEP

Methods for Recording Stomach Movements in
Conscious Sheep.

A procedure suitable for the continuous recording of stomach movements was described in detail by Schalk & Amadon (1928). Similar methods were employed by Phillipson (1939) and have been used by most other workers interested in this problem. Sheep, cattle or goats are fitted with permanent rumenal and abomasal cannulae and movements of these compartments are recorded as changes in pressure in inflated balloons introduced through the cannulae. By this means movements of the reticulum may also be recorded as this compartment can be reached from the rumenal cannula by directing forward a long tube bearing a balloon. Contractions of the omasum cannot be satisfactorily recorded in this way as it is not possible to direct a balloon into this compartment blindly. Furthermore the presence of an inflated balloon in the omasal canal might tend to hinder the flow of food material and so create an artificial obstruction.

A/

A method for recording actual pressure changes by means of an open-ended tube introduced through a cannula has also been described (Dougherty 1941). This has the disadvantage that it is only satisfactory in regions of the stomach which are normally occupied by gas so that the tube does not become blocked by fluid and semi-solid food material. In spite of this obvious limitation such a method for recording actual gas pressures may be of particular value in experiments involving gaseous distension of the rumen. Miniature pressure transducers connected directly to amplifiers and recording apparatus have also been used by Hill (1960) to study the changes in pressure which occur in the oesophagus during regurgitation.

In most investigations of gastric motility pressure changes in an inflated balloon have been transmitted either to a tambour or to a water manometer. The latter has been fitted with a float bearing a writing point whilst the tambour has been/

been connected to a frontal writing lever. In both cases tracings have been made on a rotating smoked drum. Although such apparatus can function in a perfectly satisfactory manner it is susceptible to all extraneous vibrations and furthermore it is often difficult to prevent the float of the water manometer from sticking. Although, at the start of the present investigation water manometers were used, these were eventually replaced by electronic pressure transducers coupled to ^a multi-channel pen oscillograph.

Experimental Investigation.

Recording Apparatus.

In the first stage of this investigation, pressure changes in the balloons were indicated by water manometers. These were of a conventional pattern (made by C.F. Palmer Ltd.) and bore recording floats with writing points which traced on a smoked paper kymograph. The balloons were inflated to a pressure of 10 - 15 cm. of water which was sufficient to give a sensitive response to movements of the stomach.

Although such a system has the great merit of simplicity it was not easy to align more than one manometer against the kymograph without risk of overlapping. Furthermore, there was sometimes a tendency for floats to stick in the tubes whilst the pointers moved erratically at the height of each contraction.

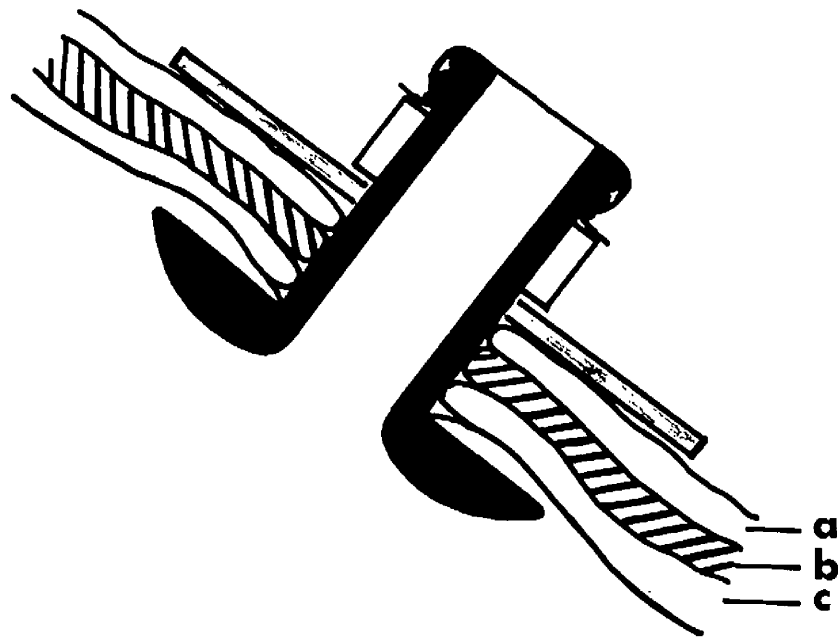
When sufficient apparatus became available to permit the electronic recording of pressure changes, the use of water manometers was abandoned. The electronic apparatus consisted of a pressure sensitive/

sensitive transducer, amplifier and pen recorder and two such channels were available. At the start of each experiment the apparatus was calibrated against a water manometer so that, if required, pressure changes could be expressed in cm. of water. Although this equipment was somewhat cumbersome, the electronic transducers were more sensitive and the traces obtained were free from many of the artefacts seen in the smoked paper records. Brief details of the equipment used are given in Appendix 1.

Fig. 28. Rumen cannula.



Fig. 29. Position of rumen cannula in relation to tissues.



{ a. skin
b. muscle
c. rumen wall

Materials and Methods.

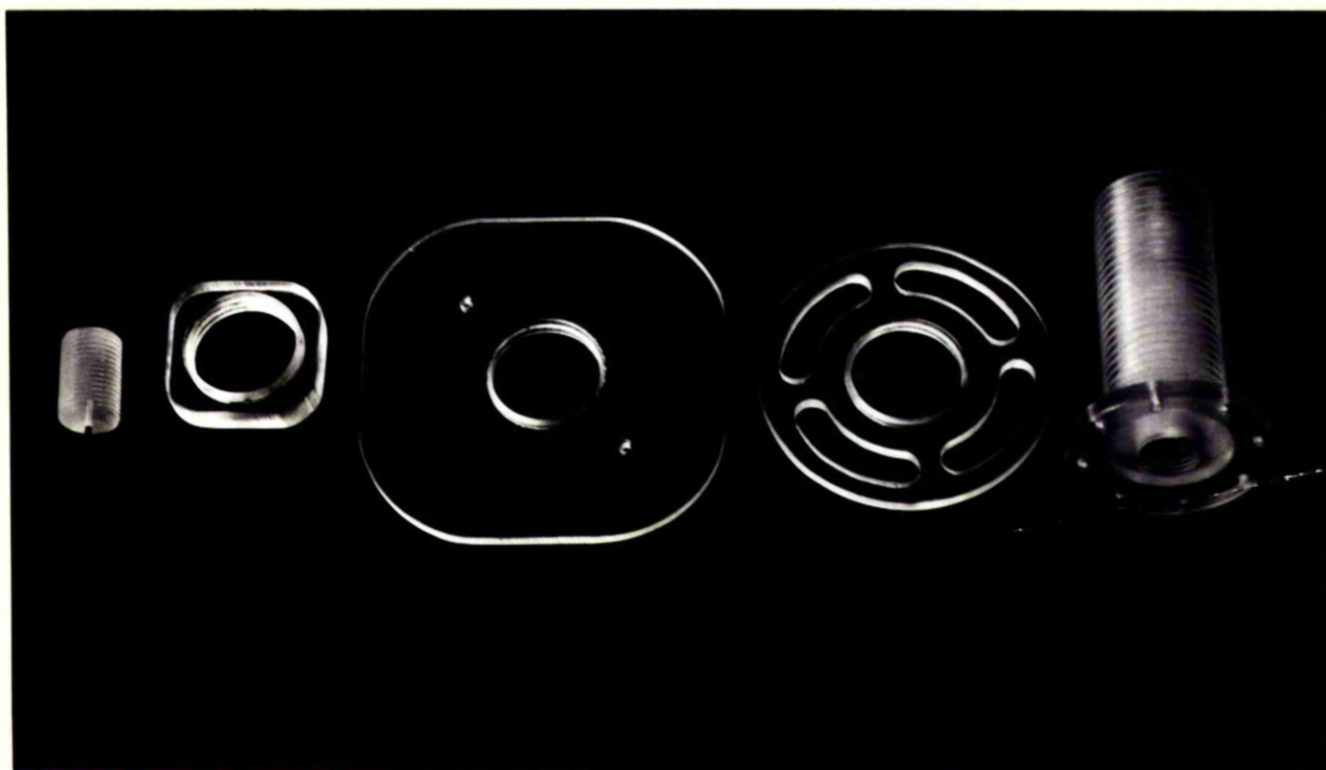
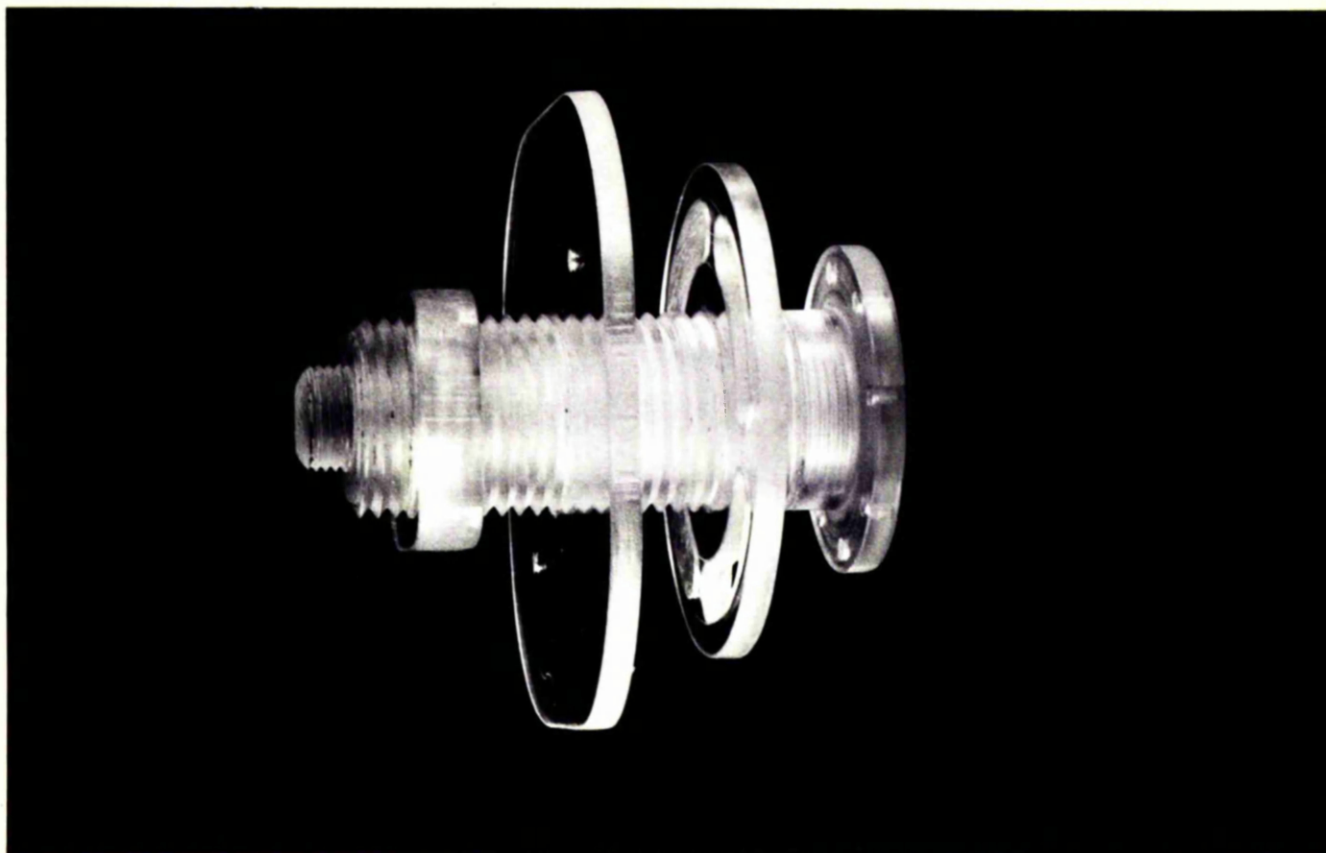
Rumenal cannula. This was of the type first described by Jarrett (1948) and is illustrated in figs. 28 and 29. The cannula was constructed of polyvinyl chloride tubing 1" internal diameter to which PVC sheet was welded to give the requisite shape. The tube was 3" long and bore a double collar at the outer end. The inner end was connected to a wide circular flange 3" diameter so constructed that it was flexible enough to allow the cannula to be tightly fitted to the wall of the rumen.

When fitted, a large perspex washer 3" diameter was passed over the tube externally and this was held in place by a polythene collar as shown in the diagram. The cannula was closed either by means of a rubber bung or more often by a piece of polythene sheeting held in place by a rubber band.

Cannulae retrieved from sheep which had been in place for several months had lost much of their elasticity and were not used again in other/

Fig. 30. Abomasal Cannula.

Above: Assembled. Below: Component parts.



other sheep. (Rumen cannulae were made by Portland Plastics Ltd., Hythe, Kent).

Abomasal cannulae. Rigid perspex 1" diameter cannulae were used in the abomasum as illustrated in fig. 30. The cannula was threaded to take two washers and a stopper to close it when not in use. The internal end of the cannula carried a rim $1\frac{1}{4}$ " diameter. The fenestrated collar was attached to the serosal surface of the abomasum to lie between this organ and the parietal peritoneum whilst the other plain washer was fitted externally to hold the cannula firmly in place. (Abomasal cannulae were constructed by Mr. Turnbull of the Veterinary School.)

Preparation of Sheep with Permanent Stomach Cannulae.

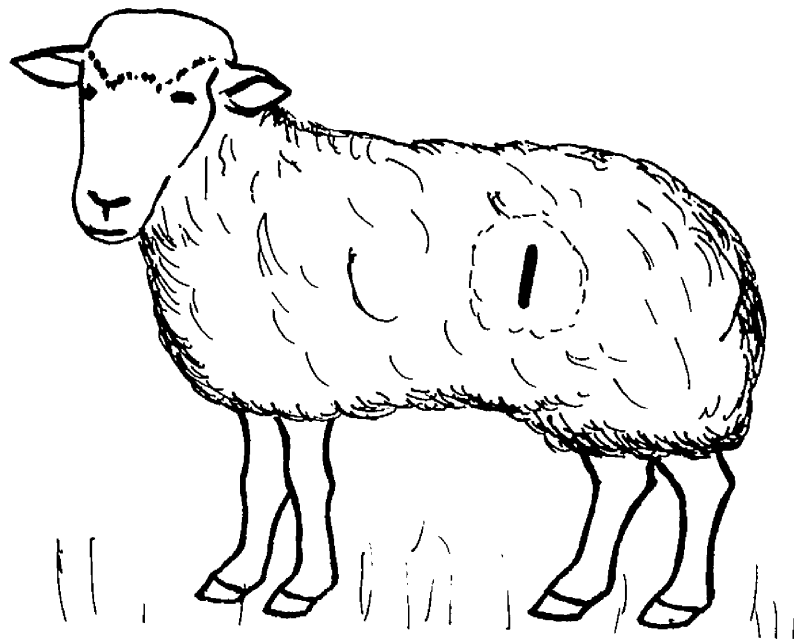
Nine half-bred ewes were fitted with rumen cannulae and in three of these the abomasum was cannulated also. Adult ewes were obtained in small lots at a local market and were kept at pasture until required for experiment when they were housed individually in indoor sheep pens approximately 7' x 4'.

In/

In the course of these experiments two sheep died within 21 days after operation for insertion of the rumen cannula. In neither case was there any obvious cause of death and the operation sites did not appear to be infected or abnormal in any other way. As there was a complete absence of lesions samples were taken for bacteriological examination but were negative for clostridial toxins. In spite of this, however, it was thought that death had possibly resulted from *Clostridium welchii* infection which has been encountered by other workers. (Hill 1951, Singleton 1959 personal communication). For this reason all experimental sheep were subsequently vaccinated using 2 ml. of a polyvalent *Clostridium welchii* vaccine given subcutaneously. ("Ovilin" Glaxo Ltd.)

The operation for cannulation of the rumen was performed in two stages with an interval of 7 - 10 days between each stage. First the rumen was sutured to the abdominal wall in the left flank region so that permanent adhesion occurred. An incision was then made through the adherent area/

Fig. 31. Site of rumen adhesion.



area large enough to allow the insertion of a cannula.

Each sheep was housed on the day before the first stage of the operation and food was withheld for 24 hours so that the rumen was not completely filled. The sheep was weighed and anaesthesia was induced with 6% sodium pentobarbitone* given by rapid intravenous injection. The sheep weighed 60 - 80 Kg. and required 25 - 30 ml. of pentobarbitone solution (1.5 - 1.8 G of sodium pentobarbitone) to produce a medium depth of anaesthesia. An endotracheal tube was then inserted and the animal was connected to a closed-circuit anaesthetic apparatus with circle absorber for the removal of carbon dioxide. Anaesthesia was maintained with a cyclopropane-oxygen mixture.

The sheep was placed on its right side and an area of skin on the left flank was prepared. This area was demarcated by the last rib anteriorly and the hind-limb posteriorly and is indicated in figure 31. The operation site was cleaned with/

* "Nembutal" Abbott's.

Fig. 32. Section of body wall showing position of sutures.

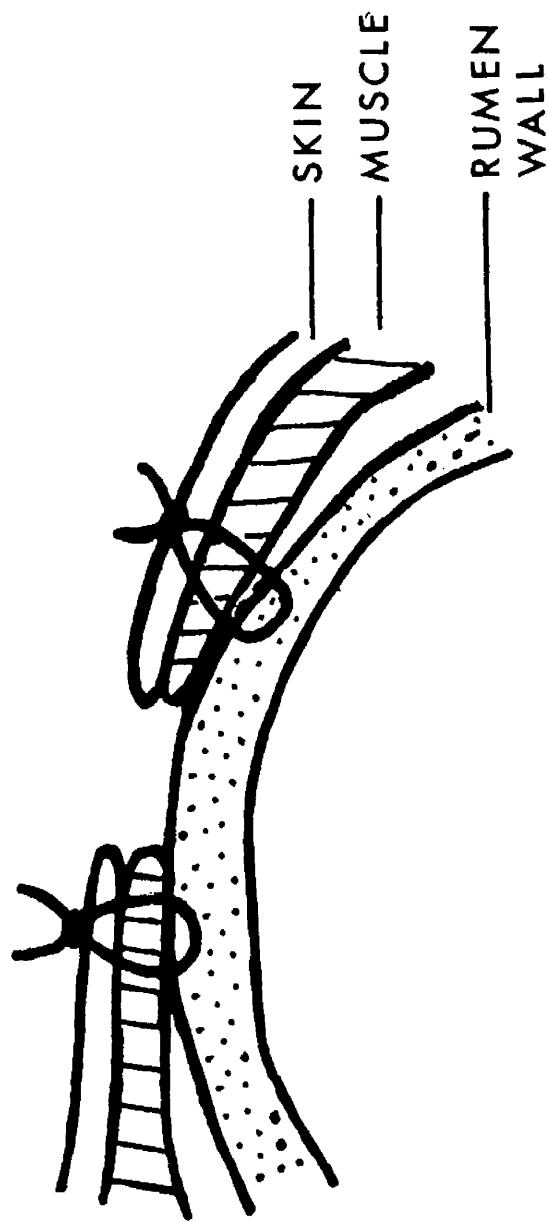
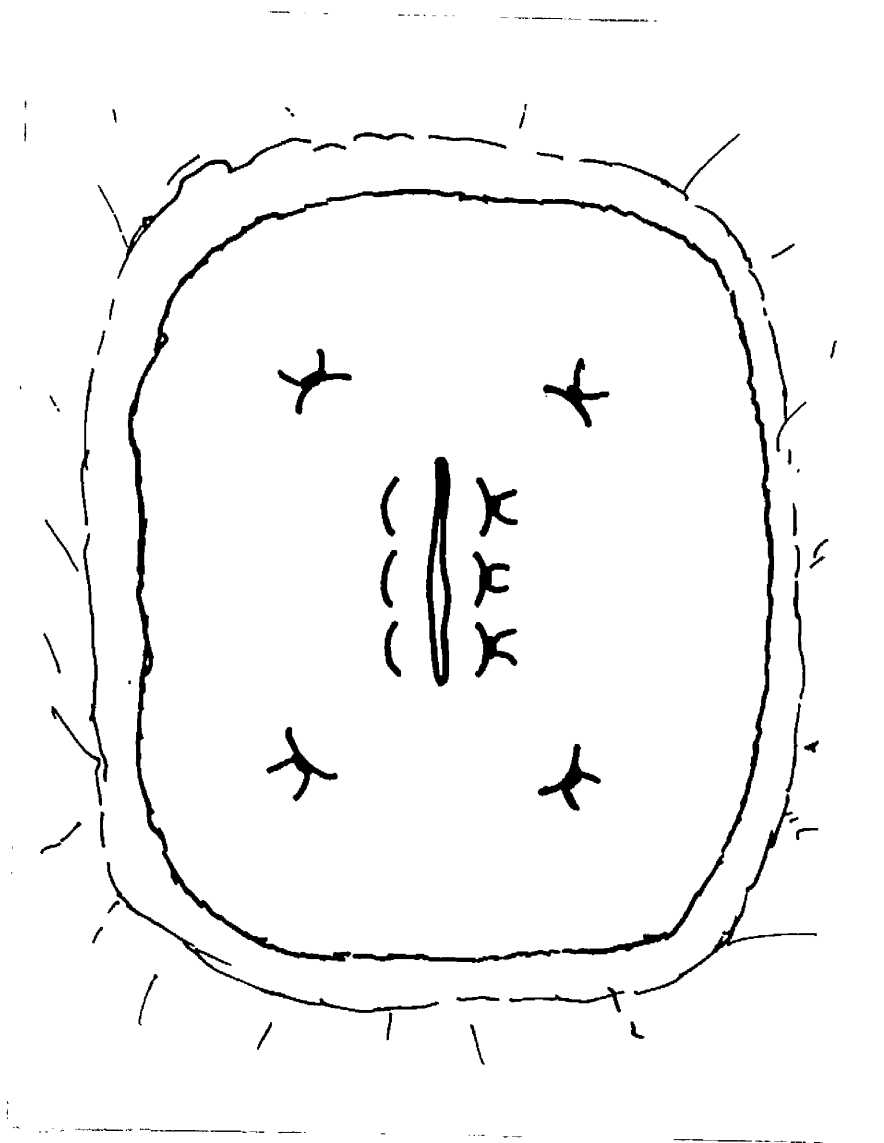


Fig. 33. View of sutures on completion of rumen adhesion.



with cetrimide tincture and sterile drapes were applied. A vertical incision 7 - 8 cm. long was made mid-way between the last rib and the anterior edge of the muscle mass of the left hind limb starting at a point about 10 cm. below the lumbar transverse processes. Having reached the peritoneum this was cut and the rumen was drawn up to the incision and held by two pairs of tissue forceps. Four nylon anchoring sutures were then placed as shown in figure 32 at a distance of about 8 cm. from each other. Each suture was passed through the entire thickness of the abdominal wall and then through the muscular wall of the rumen before returning through the abdominal wall. The portion of rumen wall which projected into the incision was then held by nylon sutures as shown in figure 33. Interrupted mattress sutures were used and three of these were usually sufficient. The sheep was given an intramuscular injection of 1.5 million units of procaine penicillin suspension (Mylipen" Glaxo Ltd) at the end of the operation and this was repeated each day for 4 days.

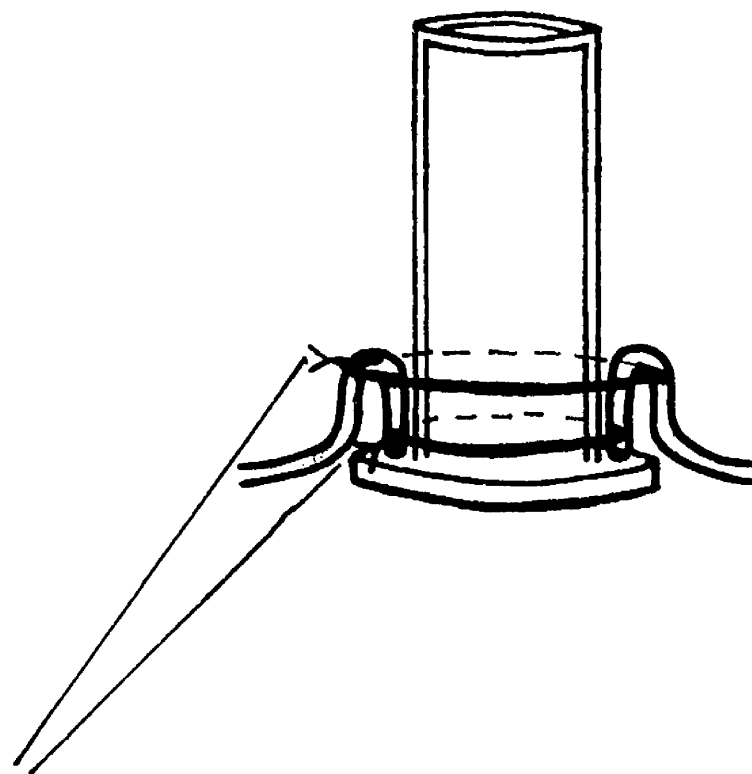
All/

All sheep were able to stand within 3 hours of removal from the surgery and had started to eat within 2 - 3 days. Sutures were removed 4 - 7 days after the operation.

At the second operation the sheep was again anaesthetised and placed on a table on its right side. An incision about 5 cm. long was then made through the adherent tissue at the original incision and an opening was made into the rumen. A rumen cannula was then inserted and held in place by means of the external washer and polythene collar. In one sheep it was found at the second operation that little adhesion had occurred and in this animal the rumen was re-sutured to the abdominal wall and the cannula inserted at one operation. Injections of procaine penicillin suspension were repeated daily for 3 days.

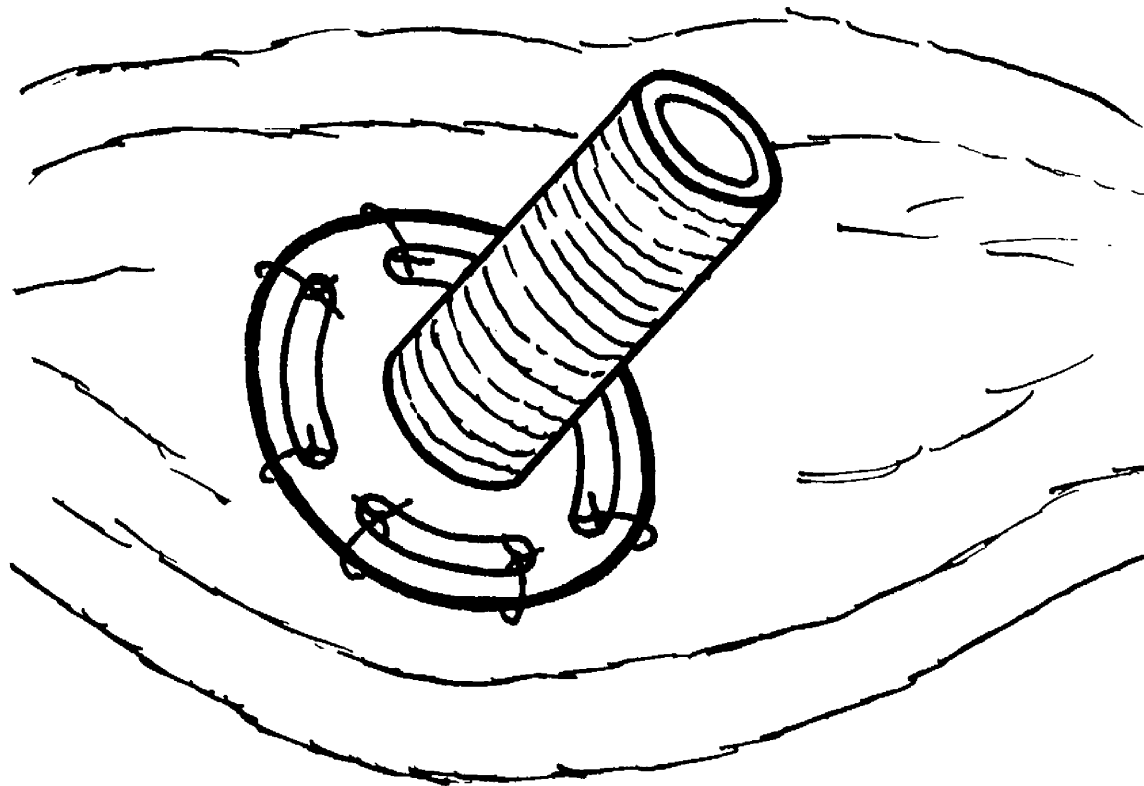
Abomasal cannulae were fitted in three sheep which had been provided with rumen cannulae some weeks previously. The sheep was anaesthetised as described above and placed on the table on its left side. An area of skin over the abdomen to the/

Fig. 34. Abomasal cannula showing method of attachment to wall of abomasum.



Purse string sutures

Fig. 35. View of cannula in position in abomasum.



the right side of the mid-line was clipped, and prepared for operation. A right paramedian incision was then made about 15 - 20 cm. from the mid-line and the abomasum was found lying below this incision.

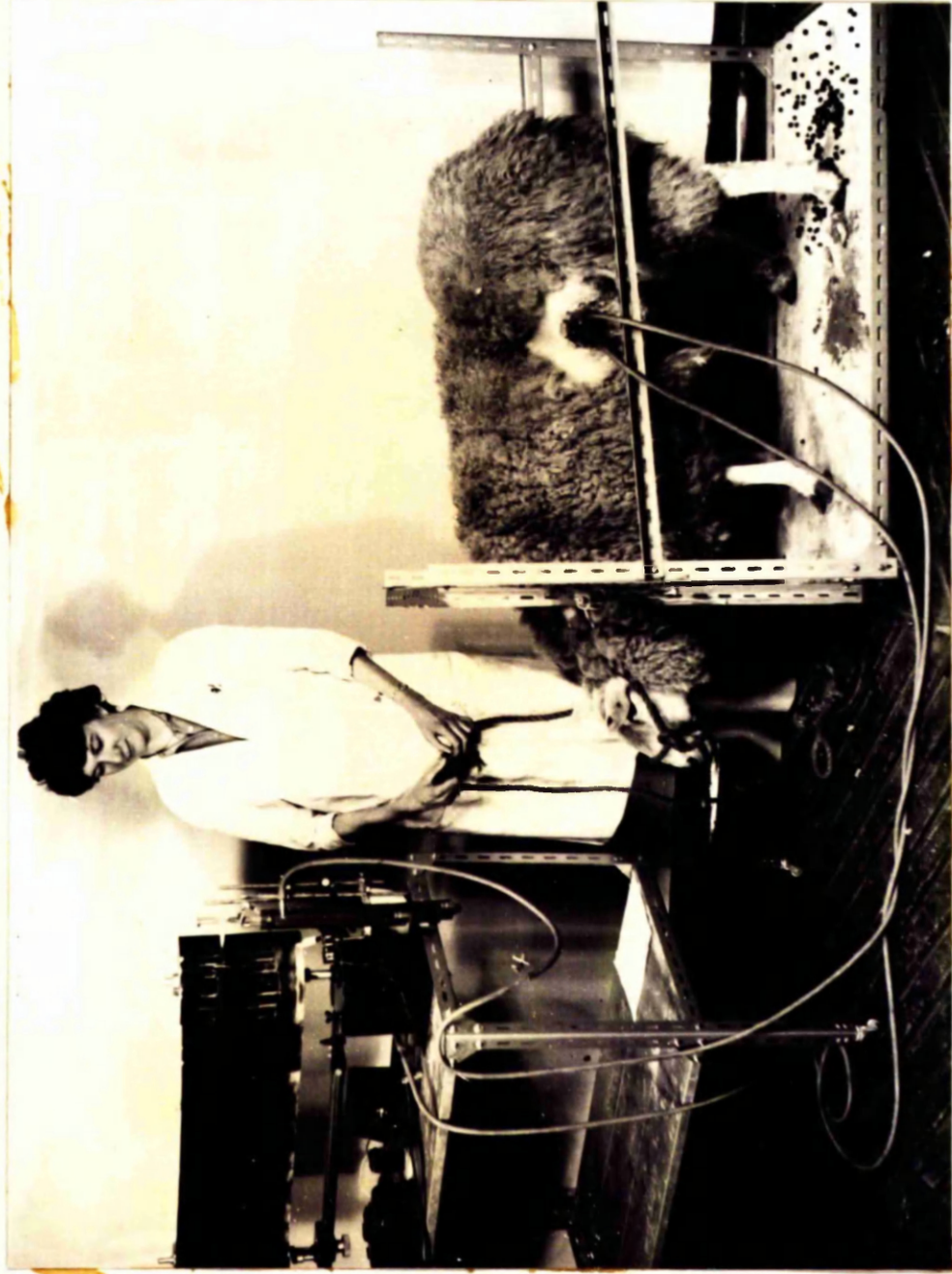
The abomasum was drawn up into the incision by means of tissue forceps and held so that the lateral wall was presented at the junction of the cardiac and pyloric regions. An incision was made in the proximal pyloric region and the cannula was inserted. It was secured by means of a purse string suture after which the sutured edge was invaginated and the fold held in place by a second purse string suture as shown in fig. 34. The fenestrated washer was then screwed on until it was touching the abomasum. It was then attached to the muscular wall of the abomasum by means of sutures in the manner illustrated in fig. 35.

A small stab-wound was made in the abdominal wall between the operation site and the mid-line. The neck of the cannula was directed through this/

this and held in place by means of the second washer. The original incision was then closed by a continuous suture taking in peritoneum and muscle layers whilst the cut edges of skin were drawn together by a row of interrupted mattress sutures. Post-operative treatment was similar to that described for the operation for cannulation of the rumen.

Fig. 36. Recording of stomach movements with water manometers and kymograph.

NOTE Apparatus was moved from sheep hut to facilitate photography.



Method for Recording Stomach Movements.

Cannulated sheep were housed in individual pens and were fed a diet of concentrates and hay with water ad lib. Accurate feeding records were not kept but the sheep were given about 1 Kg of concentrates and unlimited hay daily. A hut was obtained and converted to provide accommodation for two sheep. The fittings in this hut were constructed so that recordings of stomach movements could be made without taking the sheep out of its pen so creating as little disturbance as possible. The general layout of the apparatus and method of restraining a sheep are indicated in fig. 36. This arrangement had the advantage that the sheep did not have to be moved to a strange environment and, as the hut was isolated from the other buildings, experiments could usually be carried on without disturbance.

At the start of an experiment the head of the sheep was drawn through the yoke in the recording stand and its collar was tied to the framework. Sheep stood in the stand for 3 - 5 hours but often/

often became restless towards the end of a long period of recording. They appeared to stand more quietly when attended and were sometimes quite agitated if left alone. The presence of another sheep in the opposite pen also seemed to have a reassuring influence. This peculiarity was noted by Phillipson (1939) who found that sheep often refused to eat normally if kept alone.

The cover was removed from the rumen cannula and tubes bearing the recording balloons were introduced. Surgeons finger cots 6.0 cm. x 1.5 cm. in diameter were used as balloons and were securely tied to the ends of 8 mm. external diameter pressure tubing. In most sheep the reticulum balloon was introduced on a wire director which was bent to facilitate passage over the anterior pillar and reticulo-ruminal fold. The position of this balloon was determined by the nature of the movements which it recorded. When typical rapid reticular movements were not obtained, the/

Fig. 37. Bung in position in rumen cannula. Note tubes for reticulum and rumen balloons.



the balloon was withdrawn and re-introduced. Although it was impossible either to view or to palpate the reticulum balloon in situ, it was usually possible to decide whether the reticulum had been reached by the ease with which the balloon had been introduced. The rumen balloon was carried on a tube 18 cm. long and was directed through the cannula at right angles to the long axis of the sheep.

The tubes carrying the balloon were connected to a bung which was fitted in the cannula (fig. 37). Connections to the recording apparatus were made with lengths of pressure tubing. A similar balloon was used for recording movements of the abomasum and this was fitted to a polythene tube 4 mm. in diameter and 10 cm. long.

Fig. 38. Normal movements of reticulum and rumen
10 sec. time marker.

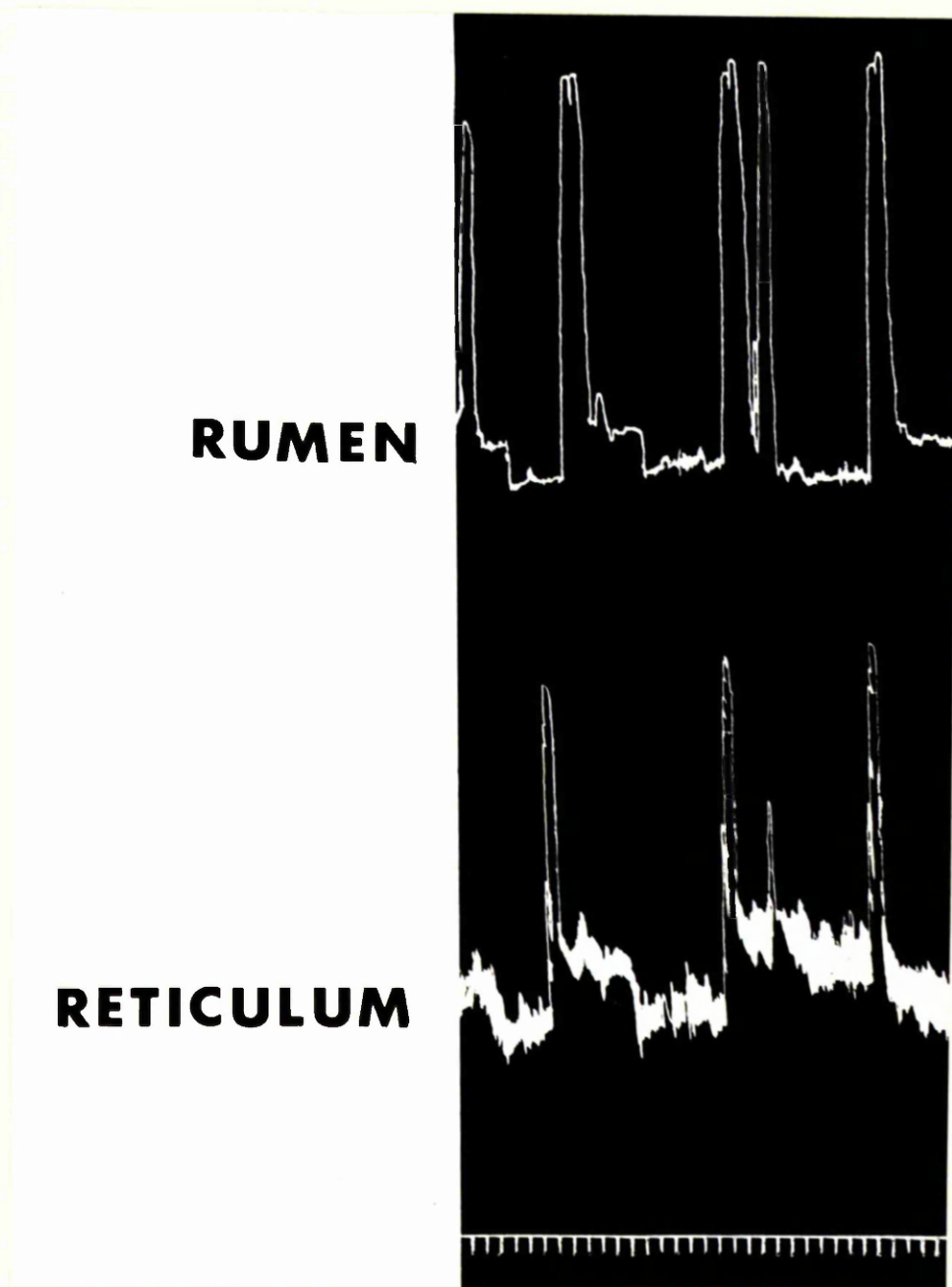


Fig. 39. Normal movements of reticulum and rumen. Note 2 single rumen movements followed by 1 double movement. Respiratory movements are seen on reticulum tracing.

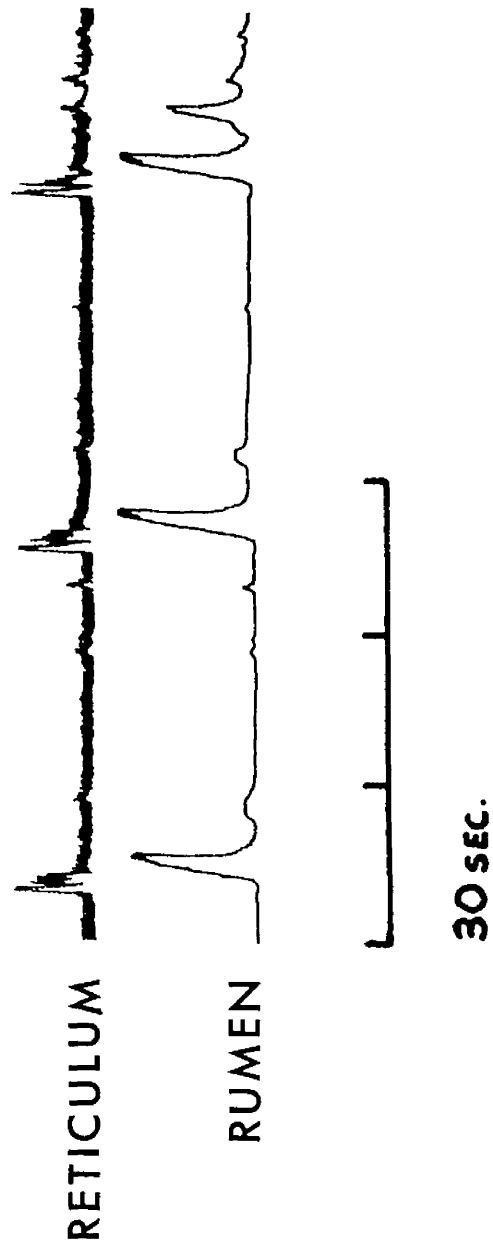
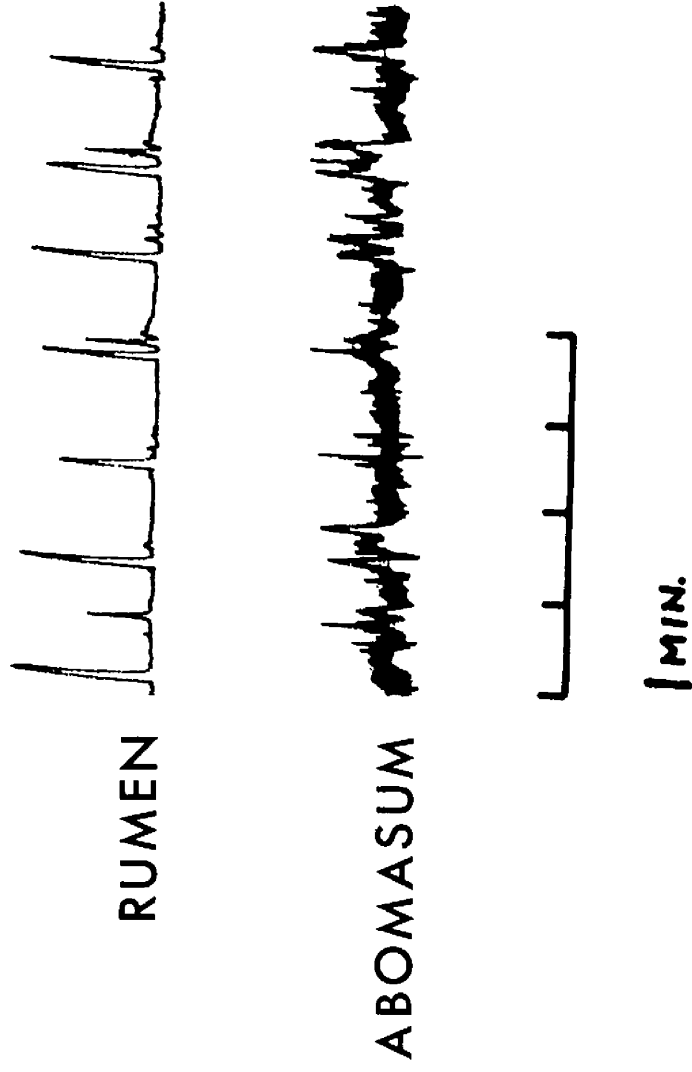


Fig. 40. Normal movements of rumen and abomasum.



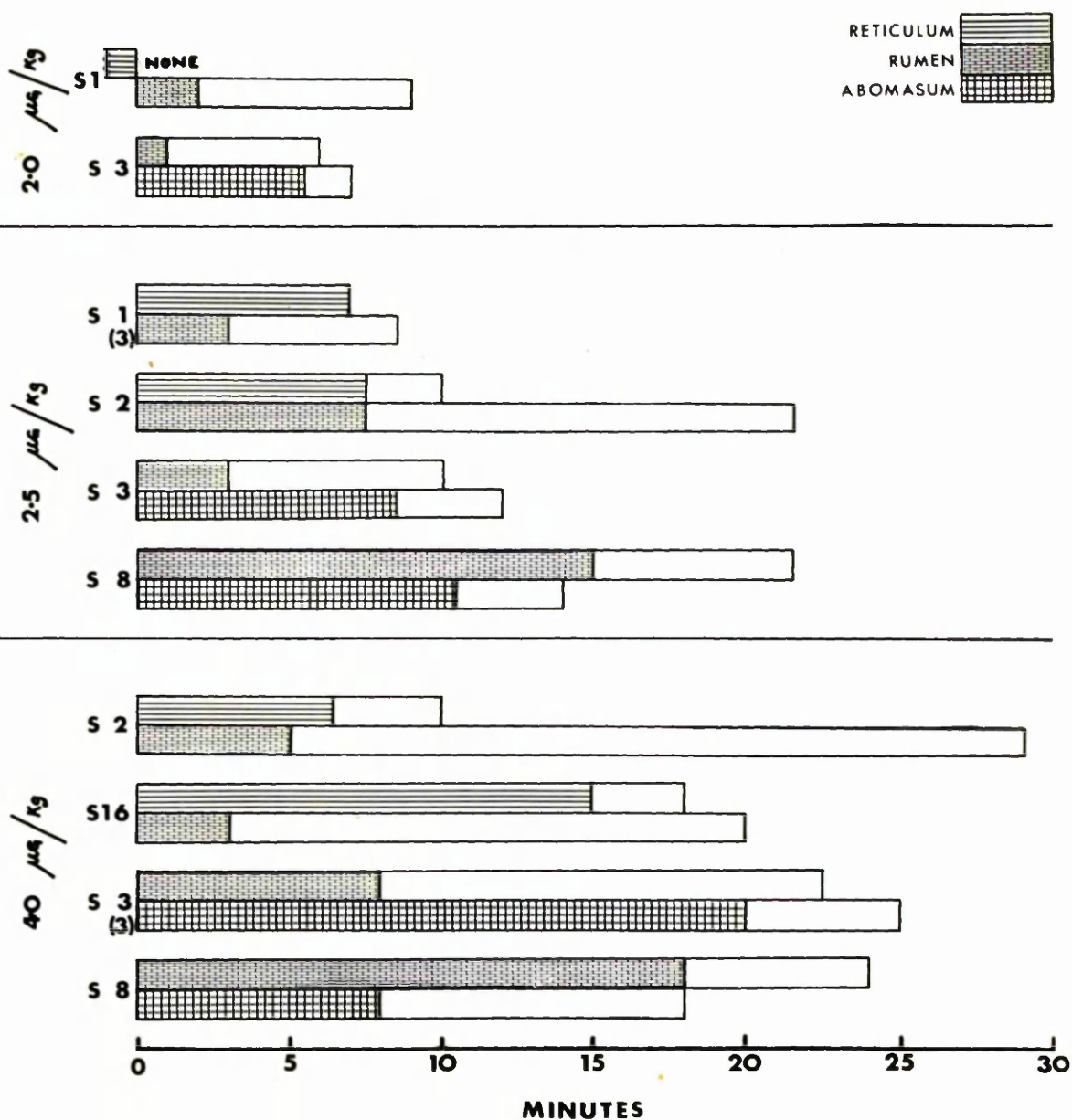
Results.

i) Normal Movements of the Stomach.

Movements of the reticulum and rumen were recorded in five sheep. Records of abomasal movements were also obtained from two of these sheep. Typical records are shown in figs 38 and 39. The reticulum invariably showed a double contraction starting just before the contraction of the dorsal sac of the rumen. The latter usually contracted once in each cycle but double ruminal contractions sometimes alternated with single movements a pattern described by Phillipson (1946). This pattern is illustrated in fig. 38. The sequence was not constant and in some recordings two cycles with single contractions of the rumen might be followed by a double contraction as seen in fig. 39.

Movements of the abomasum are shown in fig. 40. These varied considerably in force and frequency and it was not possible to correlate abomasal contractions with movements of the reticulo-rumen. In some experiments however, large/

Table I. Inhibitory effect of 2.0 - 4.0 $\mu\text{g}/\text{Kg}$ histamine I.V. on movements of reticulum, rumen and abomasum.
Shaded areas = complete inhibition.
Clear areas = recovery period.



large abomasal movements appeared to follow contraction of the rumen in each cycle.

The frequency of stomach movements was increased by feeding whilst, during rumination, an extra contraction of the reticulum preceded each regurgitation. Respiratory movements were frequently recorded by balloons in the reticulum and abomasum but rarely affected the balloon placed in the rumen.

ii) The Effect of Histamine on Stomach Movements.

Histamine was given by intravenous injection in 16 experiments. The results obtained for each sheep are summarised in Table I. Doses of histamine greater than $2.0 \mu\text{g/Kg}$. invariably gave rise to some depression of the movements of all three compartments. In general, movements of the reticulum were inhibited less completely than those of the rumen and also tended to return more rapidly to normal. Inhibition of ruminal contraction was usually followed by a prolonged recovery period in which the movements slowly increased/

Fig. 41.

Inhibitory effect of histamine on movements of reticulum and rumen. 4.0 $\mu\text{g}/\text{kg}$ histamine I.V. given at first arrow. Second arrow marks point at which respiratory rate had returned to normal. (This was not clearly seen on tracing).

M = movements of sheep due to sneezing.



Fig. 42a. Inhibition of movements of rumen and abomasum in response to 4.0 μ g/Kg histamine I.V. given at

Interval of 4 mins. between 1 & 2.
" " 12 " " 2 & 3.

Note relaxation of abomasum.



Fig. 42b. Inhibition of movements of rumen and abomasum in response to 2.5 μ g/Kg histamine I.V. given at

Mj= movement of sheep.

M ↓ M ↓



increased in force to reach the pre-injection level. In the early part of the recovery period ruminal movements tended also to be rather irregular.

The effect of an intravenous injection of histamine is shown in fig. 41. In this experiment movements of the reticulum were inhibited for 6 minutes and those of the rumen for 5 minutes. The recovery period for ruminal movements was 22 minutes. It can be seen from Table I that the duration of depression of stomach movements is proportional to the dose of histamine injected.

Inhibition of abomasal movements was often preceded by a decrease in tonus of this compartment. This was clearly seen in fig. 42. Movements of the abomasum tended to return slowly after an injection of histamine. The abomasum was not, however, noticeably more sensitive to the action of histamine than the fore-stomach compartments.

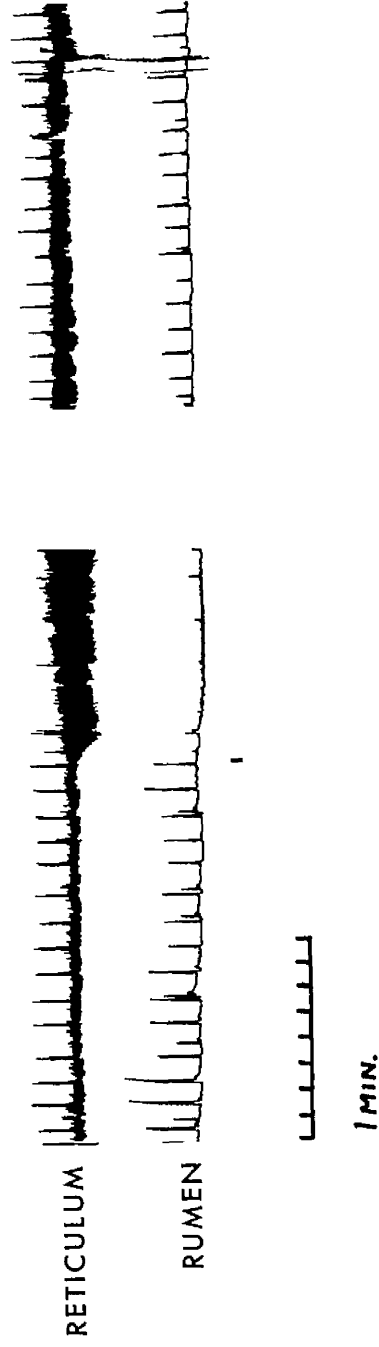
Doses/

Fig. 43.

Effect of infusion of histamine on movements of reticulum and rumen. Infusion $6 \mu\text{g}/\text{kg}/\text{min}$. histamine started at 1 and continued for 30 mins. Second tracing starts 10 mins. after end of infusion.

NOTE.

Large increase in respirations.



Doses of histamine greater than $4.0 \mu\text{g/Kg}$ gave rise to restlessness with frequent defaecation and sneezing. Respirations were also accelerated and became more vigorous.

Having established a threshold dose for the suppression of stomach movements attention was turned to the production of prolonged inhibition by the intravenous infusion of histamine. An infusion rate of $2.5 \mu\text{g/Kg}$ per minute caused inhibition of movements of the reticulum and rumen during the period of infusion and for some time afterwards. Ruminal movements tended to reappear gradually as in the experiments in which a single dose of histamine was given. The effect of an infusion on movements of the reticulum and rumen is shown in fig. 43. Infusions were continued for 30 minutes to one hour and sheep defaecated frequently during this period. Repeated sneezing was also commonly observed. Infusion at a rate of $8.0 \mu\text{g/Kg}$ per minute in one sheep caused great respiratory distress with marked hyperpnoea. The distress continued for approximately/

approximately 24 minutes after the infusion had been stopped and contractions of the reticulum and rumen re-appeared at about the same time as the breathing returned to normal.

Sheep were still able to eructate even when movements of the reticulum and rumen were absent, although this appeared to require more effort than normal.

iii) Antagonism of the Action of Histamine by Mepyramine Maleate.

Intramuscular injections of 0.5 mg/Kg. mepyramine maleate partially antagonised the effect of histamine but did not completely abolish the depression of movements. Doses of 1 mg/Kg. completely antagonised the effect of 4 μ g/Kg histamine.

Actions of Large Doses of Histamine in Conscious Sheep.

In the experiments described in the foregoing section the observations were confined mostly to the effects on stomach movements. Other effects were noted in addition, however, but these were usually seen only when doses of histamine were used which were larger than those required merely to inhibit movements of the stomach. The effects observed included repeated defaecation, impaired eructation and respiratory embarrassment. These effects were produced by doses of 5 - 10 μ g/Kg.

The actions of much larger doses of histamine were examined in four conscious sheep to provide some indication of the symptoms of severe histamine intoxication in this species. Histamine was given by rapid intravenous injection and the sheep were restrained only during the period of injection. Observations of respiratory rate, and ruminal movements were made at $2\frac{1}{2}$ minute intervals until the sheep had returned to normal.

A/

A brief description of the experiments is given below.

1. A half bred ewe weighing 82 Kg. was given 0.2 mg/Kg. histamine intravenously into the jugular vein. Within one minute the animal showed severe hyperpnoea but without any marked increase in respiratory rate. During the next 20 minutes the sheep defaecated several times and eructated with effort although the rumen did not become tympanitic. A small amount of saliva appeared round the lips and there was slight facial oedema which involved the eyelids particularly. The sheep did not collapse but was unwilling to move. These effects gradually became less marked and at 30 minutes after the injection respirations were almost normal. Ruminal movements could not be palpated until one hour after injection.
2. A blackface ewe weighing 60 Kg. was given 0.2 mg/Kg. histamine intravenously. After one minute slight facial oedema and considerable nasal discharge was apparent. In the second minute the sheep defaecated and after 4 minutes it became recumbent. The respiratory rate rose during the first minute after dosing and remained raised for 8 minutes. After this the sheep breathed more slowly but irregularly for a further 10 minutes when respirations became more normal. The nasal discharge and oedema were greatly reduced at 20 minutes after the injection and 40 minutes later the sheep was apparently normal. There was no evidence of impaired eructation although ruminal movements could not be palpated during this period.
3. A blackface ewe weighing 50 Kg. was given 0.4 mg/Kg. histamine intravenously. Nasal discharge and saliva appeared after one minute and the sheep stood with its head lowered. The respiratory rate was markedly reduced/

reduced and gurgling sounds were heard at each respiration. By the end of the second minute there was severe oedema of the eyelids and muzzle and the respiratory rate had risen considerably. At 6 minutes after injection the sheep became recumbent and lay with its head outstretched breathing through its mouth. There was a copious frothy discharge from the mouth and nostrils. During the next 25 minutes the sheep appeared to be extremely uncomfortable and stood up and lay down several times. Respirations were rapid and exaggerated and repeated sneezing started 18 minutes after dosing. Throughout this period the sheep defaecated several times. There was slight tympanitis of the rumen but this was never marked. At 40 minutes after the injection, the condition was becoming less severe and at 90 minutes the sheep was apparently normal.

4. A half bred ewe weighing 84 Kg. was given 0.8 mg/Kg. histamine intravenously over a period of one minute. Thirty seconds later the sheep collapsed, and urinated and defaecated whilst lying down. Respirations became increasingly rapid and laboured and the sheep grunted repeatedly. During the next 5 minutes the eyelids and muzzle became grossly oedematous, but there was no swelling of the limbs. There was a copious nasal and lachrymal discharge and excessive salivation. Ruminal movements ceased and the rumen became tympanitic although the animal appeared to eructate with considerable effort. For 15 minutes after the injection these symptoms became increasingly severe and gurgling sounds were heard at each respiration. At this time it appeared that the condition might prove fatal and to spare the sheep from further distress 100 mg. of mepyramine maleate was given by intramuscular injection. Fifteen minutes later the facial oedema seemed less severe and the sheep made several strained eructations. At 17 minutes after the injection of mepyramine the sheep coughed, stood up and defaecated several times. Thereafter the condition became progressively less severe until at 30 minutes after the mepyramine respirations were only slightly increased/

increased in force and the oedema had almost disappeared. Ruminal movements did not return, however, until 30 minutes later.

From these experiments it may be inferred that the toxic dose for histamine given intravenously to an adult sheep lies in the range of 0.4 - 0.8 mg/Kg. The rapid recovery of the first three sheep suggested also that, as in other species, histamine must be cleared quickly, more especially as the effects were not much more severe than those produced in previous experiments by the continuous infusion of $8 \mu\text{g/Kg/minute}$.

The Action of Histamine on Blood Pressure and
Respirations of the Anaesthetised Sheep.

With the exception of gastric secretion the most characteristic effects produced by small systemic injections of histamine in other species involve the circulatory and respiratory systems. In the dog and cat, for instance, intravenous injections of histamine produce severe hypotension whilst in the guinea-pig asphyxia results from intense bronchoconstriction.

As it appeared likely that similar actions might occur in the sheep a series of experiments were performed in anaesthetised sheep using those doses of histamine which had been shown to inhibit contractions of the stomach.

Method.

Each sheep was anaesthetised by an intravenous injection of 6% sodium pentobarbitone solution (25 - 30 mg/Kg). It was then intubated using a cuffed endotracheal tube and connected to a closed circuit anaesthetic apparatus. Anaesthesia was/

was maintained with a mixture of cyclopropane and oxygen. The sheep was placed on a table in dorsal recumbency and a polythene cannula was inserted in the tarsal vein to permit intravenous injections or infusions. A carotid artery was exposed in the neck region and a glass cannula filled with heparinised saline was inserted. The arterial cannula was connected by a column of saline to a mercury manometer with writing float tracing on a smoked paper kymograph. A T-piece was inserted in the endotracheal tube and the side arm was connected to a recording tambour which also traced on the kymograph. The latter gave an indication both of respiratory rate and of tidal volume. Having completed the surgical preparation, the sheep was disconnected from the anaesthetic circuit and anaesthesia was maintained by the intravenous injection of 50 mg/Kg chloralose in a 1% solution. A more detailed description of the experimental procedure is given in Appendix II.

In one experiment the vagus nerves were divided/

divided in the cervical region and electrical stimulation was applied to the peripheral ends from a square wave stimulator. Movements of the reticulum and abomasum were recorded by means of balloons placed in these compartments and connected to water manometers as already described for recording stomach movements in conscious sheep.

Table II. Effects of intravenous injection of histamine in sheep anaesthetised with 50 mg/Kg chloralose. Blood pressure was measured from a carotid cannula and respirations were recorded from a T-piece in the endotracheal tube.

Dose of histamine. μg/Kg.	No. of Sheep.	Effect on Blood pressure.	Effect on Respirations.
0.20	1	None	None
0.25 - 1.0	3	Depressed for 30 - 50 secs.	None - transient decrease in depth.
2.0 - 10.0	5	Triphasic response. Total period of depression 100 to 300 secs.	Depression in depth and rate sometimes followed by brief hyperventilation.
11.0-20.0	4	Triphasic response. Total period of depression 210 to 400 secs.	Depression and sometimes apnoea up to 40 secs. duration. Followed by hyperventilation.

Fig. 44. Effect of histamine on carotid B.P. and respirations 40 Kg. sheep. Chloralose anaesthesia.

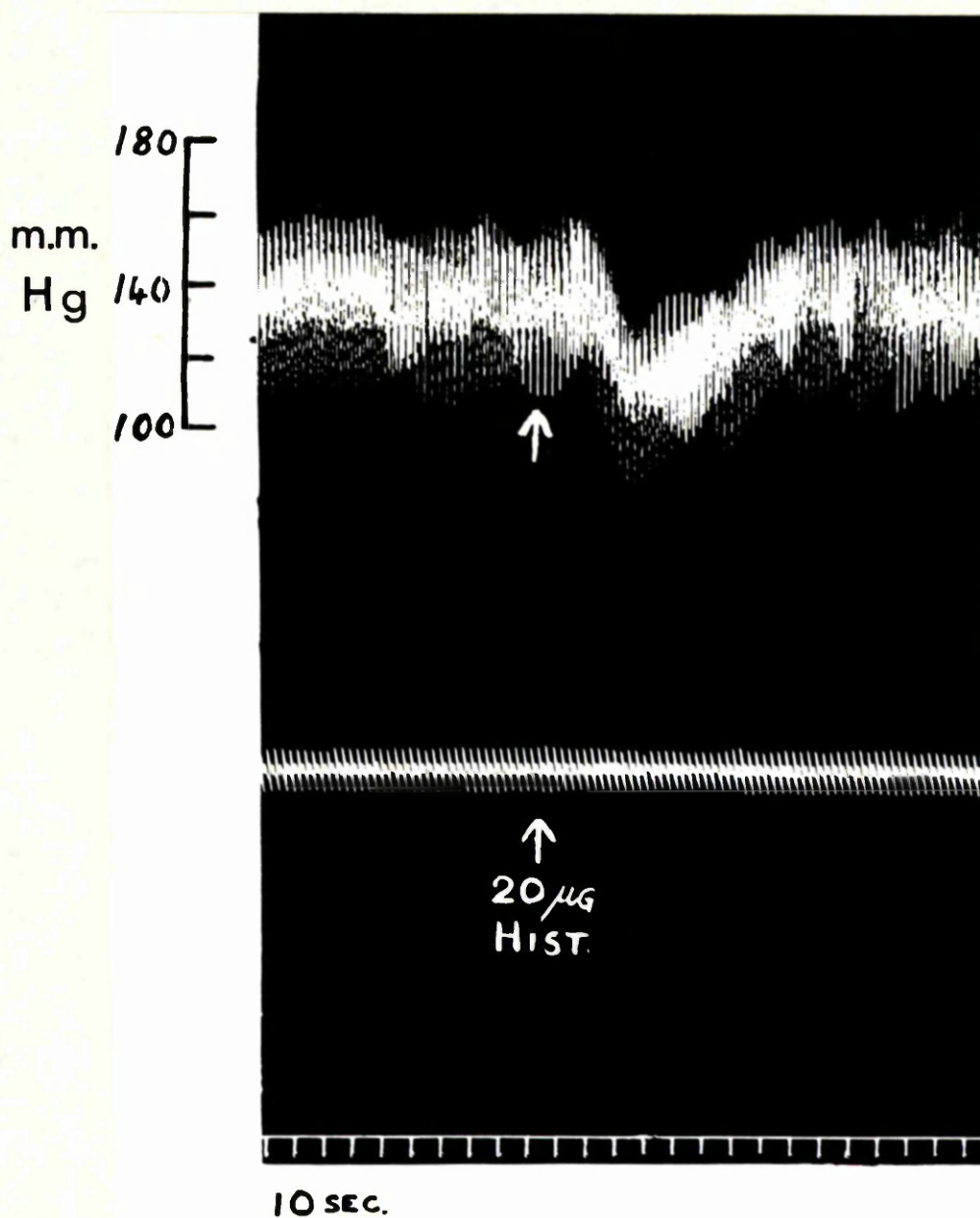


Fig. 45. Effect of histamine on carotid B.P. and respirations 40 Kg. sheep. Chloralose anaesthesia.

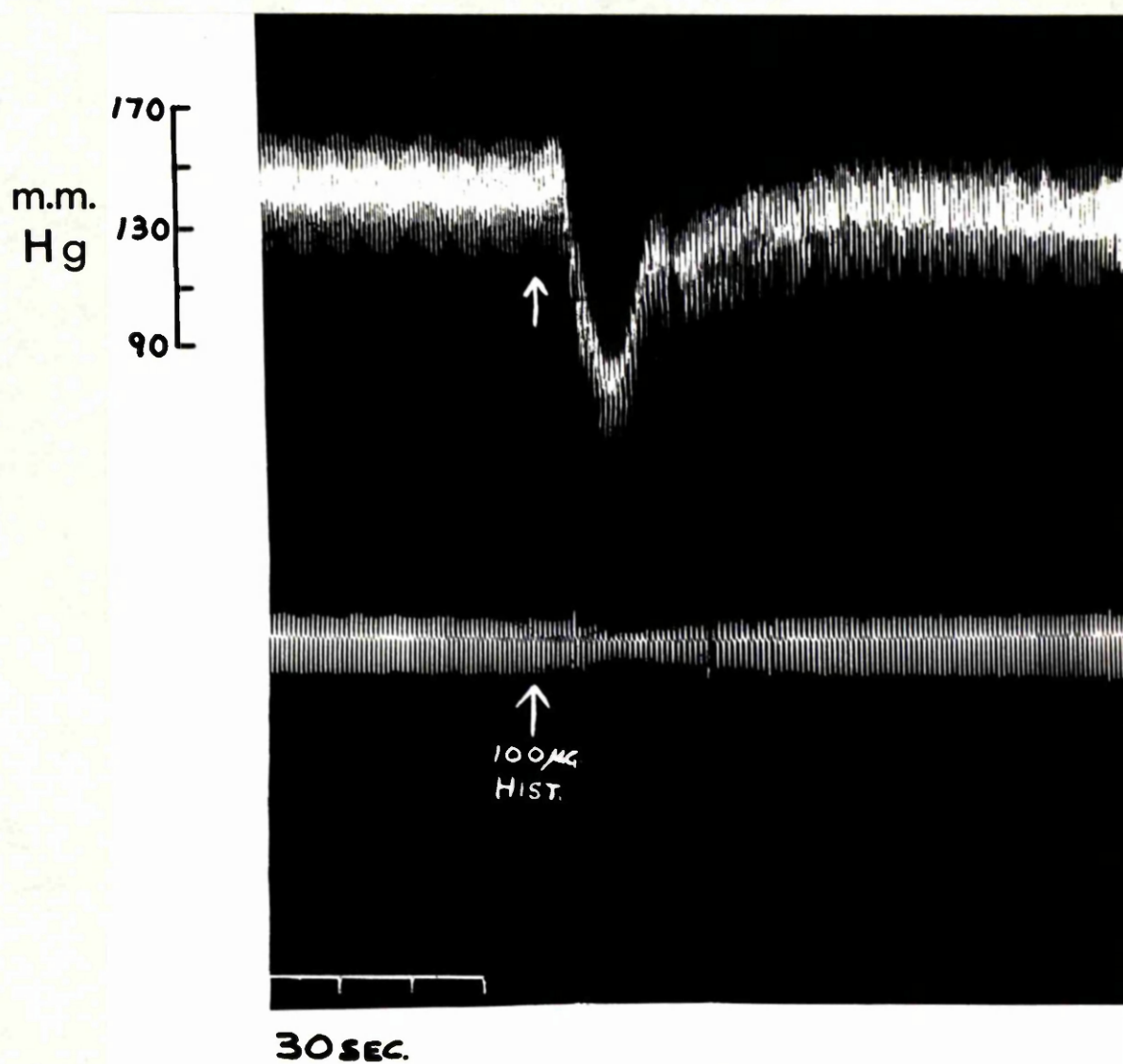
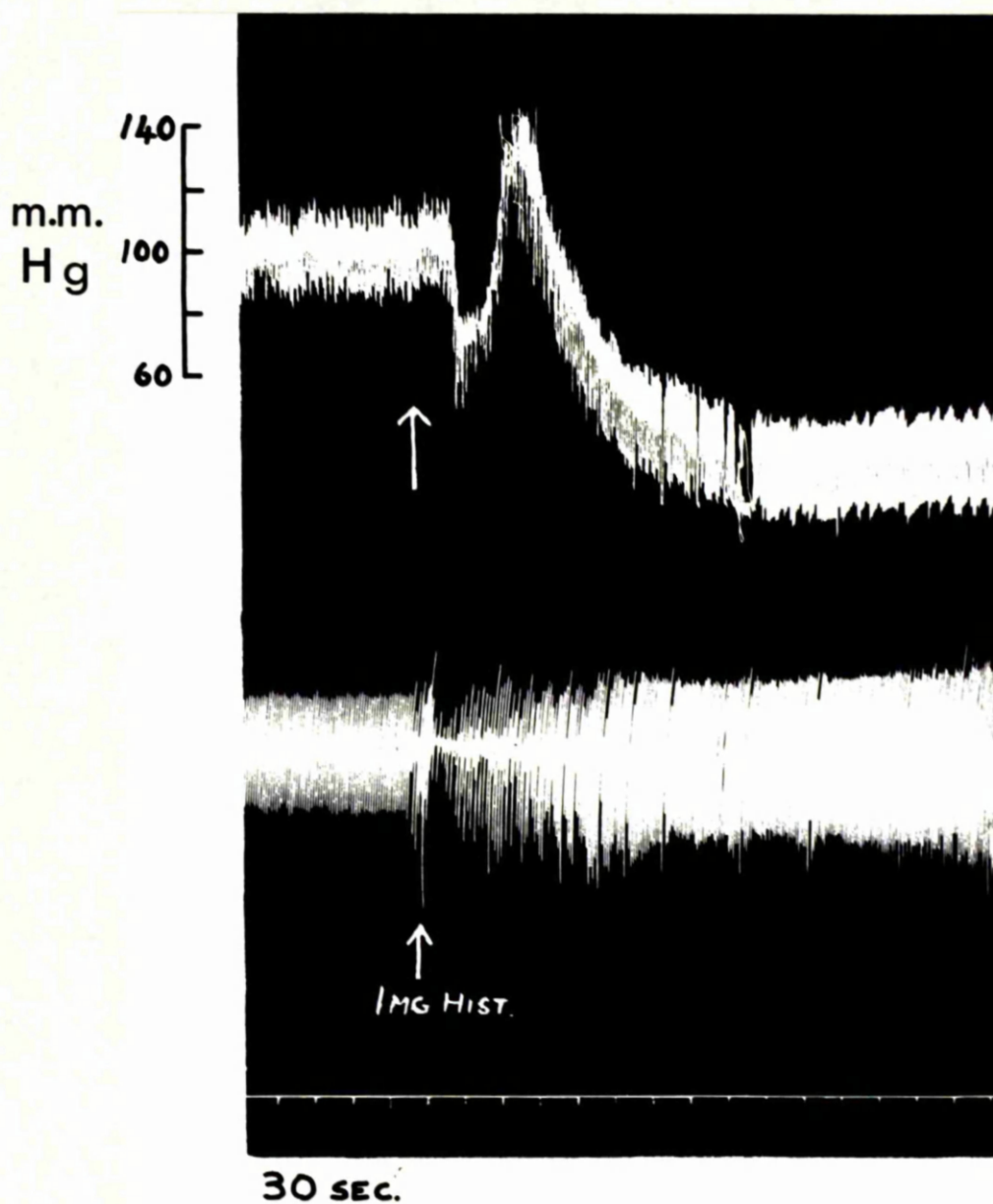


Fig. 46. Effect of histamine on carotid B.P. and
respirations 42 Kg. sheep. Chloralose anaesthesia.



Results.

In 9 acute experiments, doses of histamine ranging from 0.2 to 20.0 $\mu\text{g/Kg.}$ were given by intravenous injections. The results are summarised in Table II and typical tracings are shown in figs. 44 - 46. The threshold dose required to produce an effect on blood pressure was found to be 0.25 $\mu\text{g/Kg.}$ approximately. This dose had no effect on respirations but the mean arterial pressure fell by 10 - 20 mm. of mercury. Doses up to 1.0 $\mu\text{g/Kg.}$ had a similar action but sometimes also produced some decrease in the depth of respirations without affecting the respiratory rate.

Doses of histamine greater than 2.0 $\mu\text{g/Kg.}$ usually gave rise to a triphasic response of the arterial pressure. This is clearly illustrated in fig. 46. An initial rapid fall in blood pressure was followed by a sharp rise to a point which often exceeded the pre-injection level. This was followed by a slower and more prolonged secondary fall which lasted from one to several minutes according to the size of the dose.

Respirations/

Respirations were depressed both in depth and also in rate by doses of $2.0 - 10.0 \mu\text{g/Kg}$. histamine. This period of depression was sometimes followed by a brief period of hyperventilation. Depression of respirations was still more marked with larger doses of histamine and short periods of apnoea occurred. The depression was followed by a period of hyperventilation corresponding to the phase of secondary depression of arterial blood pressure. The effect on respirations was most marked when histamine was injected rapidly and if the same dose was given slowly over 1 or 2 minutes little change in respirations was seen. It appears from these results that respirations were less sensitive to histamine than the circulatory system.

Infusions of histamine at rates of $7.0 - 10.0 \mu\text{g/Kg/min}$. resulted in a steady fall in blood pressure which recovered rapidly when the infusion was stopped. No triphasic response was seen and respirations were only slightly depressed at these rates of infusion.

Response of the Reticulum to Vagal Stimulation.

This experiment was performed in one sheep only.

The peripheral end of the cut left cervical vagus nerve was attached to a bipolar electrode. Repeated sub-maximal stimuli of 20 volts for 5 m. secs.

were given at a frequency of 10 per sec. for 3 second periods. Each period of stimulation gave rise to a contraction of the reticulum but contractions of the abomasum did not occur.

Stimulation of the peripheral end of the right vagus also failed to cause regular abomasal contractions but occasional movements were seen.

Periods of stimulation were repeated at intervals of 1 minute and regular single contractions of the reticulum were obtained. The characteristic double contraction seen in the conscious sheep did not occur at any stage of the experiment. Stimulation caused a slowing of the heart and a temporary fall in blood pressure followed by a rise lasting for approximately 10 seconds.

Intravenous/

Intravenous injections of $8 \mu\text{g/Kg}$. histamine gave rise to a marked fall in blood pressure but the response of the reticulum to electrical stimulation was not affected. Subsequently $50 \mu\text{g/Kg}$. atropine sulphate was given intravenously. This considerably reduced the response of the reticulum to vagal stimulation but did not inhibit it altogether. It was concluded that histamine had no direct action on the reticulum.

Discussion.

The results described in the previous section demonstrated clearly that histamine has no stimulatory action on smooth muscle of the sheep stomach. This finding has been confirmed by experiments in conscious sheep and is consistent with the observations of Clark (1950) and Duncan (1954).

The recordings of stomach movements from conscious sheep indicate that contractions of the reticulum, rumen and abomasum are all inhibited by intravenous injections of histamine. The "in vitro" experiments, however, showed that the abomasum and, to a lesser extent, the rumen were directly affected by histamine. It must be inferred that the action of histamine in suppressing movements is indirect, at least in the reticulum and possibly also in the other compartments. Clark (1950) considered that this was likely as he found that contractions of the reticulum and rumen elicited by electrical stimulation of the vagus were not affected by injections of/

of histamine. This experiment was repeated in one sheep during the course of this study and its results were confirmed.

Since strips of abomasum were more sensitive to histamine than those from other compartments it was expected that, in the conscious sheep, the abomasum might be more sensitive to histamine than the other compartments. The only sign of any direct action observed, however, was the relaxation which preceded the inhibition of abomasal movements. The reticulum recovered more rapidly from the effects of an injection of histamine but it was not possible to say whether or not this difference was due to the lack of a direct response of the smooth muscle of this compartment.

The ability of mepyramine to antagonise the depressant effect of histamine on stomach movements contrasted markedly with its relatively poor anti-histaminic action on isolated tissue. This gives further support to the possibility of an indirect action of histamine of a kind which could be readily antagonised by a specific antihistaminic/

antihistaminic agent.

Indirect suppression of the contractions of the sheep stomach might be a result either of changes in blood flow or of an inhibitory reflex. The former mechanism has been examined by Alexander (1952), in the horse, who considered that it was an important factor, causing changes in intestinal motility. There is as yet no evidence to show whether the doses of histamine which inhibit ruminal movements act by producing changes in blood flow through the stomach and further investigation would be needed to establish this point.

Several inhibitory reflex mechanisms have been described (Phillipson 1939, Titchen 1958, Ash 1959) and it is possible that some of these may be affected by histamine. The inhibition of movements of the reticulo-rumen following dilatation of the abomasum might, for instance, be stimulated by the relaxant action of histamine on abomasal muscle. Histamine might also modify the permeability of the ruminal wall to substances affecting/

affecting the acid receptors postulated by Ash (1959). On the other hand the stimulation, by histamine, of acid abomasal secretions (Hill, 1960, unpublished observations) would tend to stimulate contraction of the reticulum (Titchen 1958). Although these possibilities are at present entirely speculative, they might usefully be investigated in a further series of experiments in decerebrate sheep.

The prolonged inhibition which resulted from intravenous infusions of histamine demonstrated that persistent stasis might result from the continuous absorption or release of histamine. During prolonged intravenous infusion some signs of respiratory embarrassment occurred suggesting that this might also be expected in histamine intoxication. There was no evidence of laminitis, noted by Scarisbrick (1954) as a symptom of acid indigestion and thought by Fraser (1959), to be due to the action of histamine. Injections of histamine made the sheep restless but this might be related to abdominal discomfort.

The/

The general features of the response of sheep to histamine are similar in many respects to those seen in other species.

Small intravenous doses of histamine caused a temporary fall in blood pressure whilst larger doses produced a triphasic response. This response was very similar to that first described in the cat by Dale and Laidlaw (1919). These authors considered that, in the cat, the initial fall in pressure was due to constriction of the pulmonary arterioles and that the following rise was due to systemic arteriolar constriction. The secondary and more prolonged fall was believed to be due to dilatation and increased permeability of the capillaries. Later Burn and Dale (1926) showed that the secondary rise in pressure was greatly reduced after adrenalectomy indicating that the release of adrenaline was also involved in this response.

The failure of continuous intravenous infusion to produce a similar response was also to be expected. Dale and Laidlaw (1919) found that/

that an infusion of histamine to a cat at a rate of 0.1 mg/min. (approx. 50 μ g/Kg/min.) caused a prompt fall in blood pressure which thereafter remained steady at a lower level for several minutes. Weiss, Robb and Ellis (1932) found that, in man, the systolic blood pressure remained unaltered during infusion. Wakin, Peters, Terrier & Herton (1949) obtained similar results although they noted that the diastolic pressure tended to fall slightly. The lack of any marked hypotension was believed to be due to compensatory vasoconstriction aided by the release of adrenaline. If it can be postulated that similar conditions obtain during the infusion of histamine in conscious sheep, the persistent stasis of the stomach may be a result of the release of adrenaline.

Anaesthesia appeared to modify the action of histamine on the respirations of sheep. In conscious sheep the usual response was an increase in both the rate and depth of respirations. This response was also noted by Duncan (1954).. In anaesthetised sheep, however, there was an initial decrease/

decrease in the depth of respiration and with larger doses of histamine the respiratory rate was also decreased.

There was no evidence of the intense bronchoconstriction characteristic of the response to histamine of the guinea-pig, (Dale & Laidlaw 1910). Bronchoconstriction is not, however, seen to the same extent in the cat or rabbit (McDougal & West 1953) and there appears to be considerable species variation in this response. In both conscious and anaesthetised sheep, injections of histamine appeared to increase the secretions of the respiratory tract. This effect would tend to decrease respiratory efficiency and so stimulate an increase in respiratory rate.

In general the experimental results indicated that doses of histamine sufficient to inhibit contractions of the stomach might also be expected to have actions elsewhere in the body. Other effects seen included defaecation, increased secretions in the respiratory tract and an increase in the respiratory rate. In anaesthetised sheep similar doses of histamine caused a fall in blood pressure and had a primary depressant effect on respirations.

PART III

FORMATION AND ABSORPTION OF HISTAMINE IN THE RUMEN
OF THE SHEEP

FORMATION OF HISTAMINE IN THE ALIMENTARY TRACT.

The ability of certain enteric micro-organisms to form histamine by the decarboxylation of histidine has been recognised for many years. (Mellanby and Twort 1912, Kendall and Gebauer 1930). In a series of papers Hanke and Koessler (1924 a,b, and c) have assessed the conditions under which organisms were able to decarboxylate amino-acids. They found that histamine and tyramine were produced "in vitro" only when the culture medium contained a source of carbohydrate such as glucose or glycerol and when an acid reaction developed during the course of incubation. A medium containing milk was found to be most suitable for producing these conditions. With faecal micro-organisms, Hanke and Koessler observed that deamination occurred unless culture took place in the presence of a suitable nitrogen-containing compound such as potassium nitrate or ammonium chloride.

In their experiments, Hanke and Koessler used organisms recovered from human faeces but more/

more recently Irvine, Duthie and Waton (1959) have shown that intestinal contents removed from the jejunum and ileum of an anaesthetised dog were also able to form histamine, when incubated for 3 hours in tyrode solution containing histidine and at a pH value similar to that found in the intestine. These authors also produced evidence to show that histamine formation was depressed if succinylsulphathiazole was added to the incubation mixture.

Gale (1940) re-examined the conditions suitable for decarboxylation of amino-acids by coliform bacteria. He found that organisms which had been cultured for 14 - 16 hours in a broth containing 2% glucose developed potent decarboxylase activity. Such organisms attacked arginine, lysine, histidine, glutamic acid and ornithine although some cultures were not able to decarboxylate all of these acids. The organisms were cultured anaerobically and, as incubation proceeded, fermentation of glucose made the medium more acid. Decarboxylation was estimated manometrically and the amines produced were identified by chemical analysis. In the case of/

of histamine, results were also checked by biological assay. The optimum acidity for histidine decarboxylase activity was at pH 4.0 and experiments were carried out at 30°C as the enzymic activity was rapidly lost at higher temperatures. Under these conditions decarboxylation was not enhanced by the addition of glucose nor did the presence of oxygen have any effect.

Further evidence for the formation of histamine by intestinal micro-organisms has been reported more recently by Wilson (1954) and Irvine et al. (1959). Wilson measured the total urinary output of histamine in rats over 24 hour periods. The histamine content of the rats' urine was decreased when the animals were given chlortetracycline, chloramphenicol, penicillin or phthalylsulphathiazole. All four compounds produced a similar effect which was assumed to be due to their antibacterial action reducing the number of enteric organisms available for histamine formation. Irvine and his colleagues also measured urinary histamine. They noted that, in both human subjects and in dogs, the free histamine content of urine increased after a meal/

meat meal or after feeding L-histidine in milk. The increased excretion of histamine which occurred during the five hours following such a meal was significantly reduced when an anti-bacterial agent had been given previously. Human subjects were treated with succinylsulphathiazole for 5 days whilst the dogs were given this drug for 4 days and also received chlortetracycline for the last 36 hours of this period. In both man and dog similar results were obtained.

The evidence described indicates clearly that certain enteric-micro-organisms can decarboxylate histidine and that such organisms are found in many species of animals. The work of Wilson (1954) and Irvine et al. (1959) suggested also that some at least of this histamine is absorbed and ultimately excreted in the urine and this aspect of the subject will later be considered in detail. Before proceeding to this, however, it is pertinent to examine the evidence for the decarboxylation of histidine by bacteria in ruminants.

Studies/

Studies of protein metabolism in ruminants have made it clear that much of the plant protein ingested is broken down by ruminal micro-organisms to yield ammonia. Attention has been drawn to this loss of protein-forming nitrogen by McDonald (1948), Chalmers Cuthbertson & Synge (1954) and Lewis, Hill and Annison (1957). The latter authors have also shown that ammonia formed in the rumen is readily absorbed but is mostly removed from the blood during its passage through the liver (Lewis et al. 1957). McDonald (1952) has observed that ammonia constitutes the main component of non-protein nitrogen in ruminal fluid and this is utilised by the micro-organisms present. Several authors have noted the occurrence of free amino-acids in ruminal fluid, however, (Duncan, Agrawala, Huffman & Luecke 1953, Lewis 1955, and Annison 1956) and there is evidence to show that the concentration of these acids fluctuates with feeding. Annison (1956) found that the amino-acid content fell rapidly 2 - 3 hours after feeding and presumed that this was due either to the uptake or to the de-amination of these acids by micro-organisms. Lewis (1955) found/

found that amino-nitrogen in the ruminal fluid of sheep was less in animals on a diet of hay than in those having additional protein-containing food. He observed also that, when individual amino-acids were placed in the rumen of cannulated sheep, deamination occurred with the production of ammonia. The optimum conditions of acidity for this reaction were at pH 6.5, a normal value for ruminal fluid of healthy sheep. Histidine was among the amino-acids tested by Lewis and he showed that this acid was also deaminated when incubated with ruminal contents.

Apart from the ability of ruminal micro-organisms to undertake protein breakdown there is also evidence to show that amino-acids may be synthesised in the rumen from simpler nitrogen-containing compounds. Duncan et al. (1953) found that calves fed urea as their only source of nitrogen synthesised amino-acids in the rumen. In general the proportions of individual acids formed were similar to those found in calves on a normal ration but in this respect histidine was exceptional, being much less in the urea-fed calves/

calves. There was clear evidence, however, that histidine was formed in the rumen of these calves and the concentration regularly increased after feeding. Agrawala, Duncan and Huffman (1953) reported further that ruminal organisms in urea-fed calves were able to synthesise protein from urea within 6 hours after feeding.

The amino-acid content of ruminal organisms has been examined by Weller (1957) who studied hydrolysates of microbial preparations. He separated bacteria and protozoa from ruminal fluids of sheep fed on four different types of ration. Amino-acids were separated from the extracts by ion-exchange column chromatography and analyses were carried out to estimate the concentration of individual acids. Histidine was present in both bacterial and protozoal fractions and there did not appear to be any marked difference in the histidine content of each.

Although the presence in ruminal fluids of amino-acids including histidine is now well established; the way in which these are metabolised is less certain. Sirotnak, Doetsch, Brown and Shaw/

Shaw (1953) have investigated the metabolism of individual amino-acids by "in vitro" incubation with ruminal contents. Incubation was carried out at 39°C and each substrate was buffered by a phosphate solution to pH 6.9. Sodium sulphide was also added to stabilise the redox potential and incubation was continued for 72 hours. Of 22 amino-acids tested evidence of decarboxylation or de-amination was obtained with only 6; aspartic acid, glutamic acid, serine, arginine, cystine and cysteine. Histidine was apparently not affected under these conditions.

Formation of Histamine in the Rumen. The studies of ruminal metabolism which have been discussed do not suggest that any appreciable quantity of histamine is likely to be found in ruminal contents. There is evidence, however, that histamine and tyramine are sometimes detectable in ruminal fluid of cattle. The circumstances in which Dain et al. (1955) discovered abnormal amounts of histamine in the ruminal fluid of sheep have already been described.* It is necessary only to recapitulate the conditions under which this occurred. Hungate et al. (1952) had previously noted that the feeding of excess grain or glucose to sheep led to a change in/

in the ruminal flora. Gram +ve organisms showed a relative increase in number whilst the concentration of non-volatile acids, and in particular lactic acid, increased markedly. This increase resulted in a fall in pH to 4.1 - 4.6. Under similar conditions produced by over-feeding experimental sheep Dain et al. (1955) showed that the formation of histamine increased as the acidity of the ruminal fluid increased. At pH values of < pH 4.5 the histamine content rose markedly and amounts exceeding 90 μ g/ml. were obtained. Histamine was extracted chromatographically and the presence of tyramine was also recorded in this way. In general, these workers found that the severity of the experimentally induced disease was proportional to the histamine content of the ruminal fluid. Recent experiments by Van der Horst (1961) have supported the previous findings. He extracted keto-acids and amines from the ruminal fluid of cannulated cattle. If glucose was added to the ruminal contents pyruvic acid formation increased rapidly and the pH fell. The concentration of amino-acids and of other basic compounds also increased. Chromatographic/

Chromatographic separation revealed the presence of large quantities of cadaverine, putrescine and colamine and smaller amounts of histamine, tyramine and tryptamine. No quantitative estimation of these amines was reported, however.

Although these reports provide evidence from which it might be inferred that excessive production of histamine in the rumen contributes to the syndrome of "over-eating" such an inference depends on the assumption that free histamine can be absorbed from the alimentary tract. The evidence in support of this assumption will next be discussed.

Absorption of Histamine from the Alimentary Tract

There appears to be no published evidence for the absorption of histamine in the rumen apart from a brief report by Dougherty and Cello (1949). These authors stated that histamine solution sprayed through a cannula onto the dorsal wall of the rumen of a cow caused cessation of ruminal motility and marked hyperpnoea. On the other hand Bourlé (1959) reported that 10 grams of histamine placed in the rumen of a cow produced no effect although he attributed this to the presence of histamine-destroying enzymes.

It is well-known that absorption of some metabolites does take place in the rumen. Absorption of short-chain fatty acids via water-filled pores and through the lipoid membranes of epithelial cells has been described by Danielli, Hitchcock, Marshall and Phillipson (1946). There is evidence also that lactate is absorbed in the alimentary tract of ruminants but it is doubtful whether this takes place in the rumen/

rumen (Broberg 1960, Dobson 1961).

The situation in regard to nitrogenous compounds has received much less investigation. The absorption of ammonia from the reticulo-rumen has been demonstrated by McDonald (1948). Annison (1956) stated, however, that amino-acids did not appear to be absorbed from the rumen, but were normally de-aminated.

Although no reports have been found describing the intestinal absorption of histamine in ruminants, there is considerable evidence for the occurrence of this process in several other species. Meakins and Harington (1923) found that when histamine was present in doses of 20-50 mg/Kg in the alimentary tract of anaesthetised cats, there was sufficient absorption to produce a fall in carotid blood pressure. The effect was most marked when histamine was present in the ileum and was less in the duodenum and much less in the stomach and caecum. Absorption did not appear to be related to any abnormality of the intestine under these conditions as when the gut was made anoxic by occluding the blood supply for/

for 5 - 15 minutes, the passage of histamine was not facilitated. Mammoser and Boyd (1929) found that 5 mg/Kg doses of histamine placed in the duodenum did not affect the blood pressure. When histamine was present with another compound, however, a profound fall in blood pressure occurred. The substances which allowed histamine to exert this action were 0.4% HCl, carbon tetrachloride and 15 - 30% ethyl alcohol; each being given in amounts of 20 - 40 ml.

Results of a series of experiments by Hanke and Koessler (1924) led these authors to conclude that free histamine was not absorbed from the gut either in the dog or in the guinea pig. They observed, however, that histamine disappeared from the stomach and intestines and suggested that it might be inactivated in the intestinal wall.

Mitchell & Code (1954) measured changes in the 24 hour urinary output of histamine to indicate variations in the rate at which it was absorbed. These authors found that fasting was accompanied by reduction in output whilst a meat diet increased a urinary concentration of both free and conjugated histamine. When histamine/

histamine itself was taken by mouth, however, there was no change in the urinary free histamine although an increase in the conjugated form was observed. If histamine was taken by mouth with a meal of milk, bread and butter a large increase in free urinary histamine resulted.

Irvine, Duthie, Ritchie and Waton (1959) found that, in dogs with denervated gastric pouches, acid secretion increased after doses of 10 - 1000 mg. histamine in saline had been introduced into the jejunum through a cannula. The hourly urinary output of free histamine was also measured and this increased to reach a maximum in the third hour after dosing. After portocaval venous anastomosis in some dogs, the acid response to small doses of histamine placed in the jejunum was greatly augmented. These authors considered that this effect was due to lack of the metabolism which normally occurred in the liver as a similar response was obtained when dogs were given aminoguanidine which suppressed tissue histaminase activity.

The/

The problem of the conjugation of histamine either in the gut contents or in the intestinal wall will not be considered in any detail as this investigation is concerned primarily with evidence for the absorption of free, physiologically active histamine. Adam (1950a) observed that following the oral administration of histamine in man, about 1% of the dose appears in the urine in a conjugated form, whilst there is only a very small increase in free histamine. He further found (Roberts & Adam 1950) that there was a wide variation in the daily output of conjugated histamine in man whereas free histamine remained relatively constant. These observations led him to conclude that histamine absorbed from the alimentary tract was mostly, if not entirely, in a conjugated form. Wilson (1954) obtained evidence however, to suggest that in rats both free and conjugated histamine might be absorbed through the intestinal wall.

Experimental Methods for Investigating Histidine
decarboxylase Activity of Ruminal Contents.

Most methods for examination of the histidine decarboxylase activity of micro-organisms have involved incubation of cultures with solutions of L-histidine. In early experimental work long incubation periods of 2 - 3 days were employed (Mellanby & Twort 1912, Koessler & Hanke 1924) but in more recent investigations incubation for 2 or 3 hours was found to be sufficient (Gale 1940, Irvine et al. 1959).

The optimum conditions for demonstration of amino-acid decarboxylase activity of *Bacterium coli* have been investigated in considerable detail by Gale (1940). He found that anaerobic culture of organisms for 16 hours in a medium containing 2% glucose greatly increased decarboxylase activity. Cultures were then incubated for 1 - 2 hours with L-histidine at pH 4.0. Although enzymic activity was initially greater at 37°C it declined rapidly at that temperature and most of Gale's experiments were performed at 30°C. Decarboxylation was assessed by the production of CO₂ in a Warburg manometer but the histamine/

histamine formed was both identified chemically and assayed biologically.

Van der Horst (1961) employed a similar approach to the study of amine formation by ruminal contents. He incubated ruminal liquid with glucose for 24 hours adding either fodder cake, casein or individual amino-acids. Amines were extracted with butanol and separated by paper electrophoresis and subsequent chromatography. In the present investigation incubations have been carried out under a variety of experimental conditions. Activity has been assessed by biological assay of the histamine produced and no measurements of CO_2 production have been made.

Experimental InvestigationMaterials & Methods.

Ruminal contents have been obtained from healthy sheep fitted with rumen cannulae. These sheep were kept indoors and fed a diet of hay and concentrates. Samples were usually taken in the morning 15 hours after the previous feed of concentrates. Each sample was centrifuged for 15 minutes at 2,000 revs/min. to remove coarse material and the supernatant was decanted and immediately prepared for incubation. The pH value of the samples was checked to avoid grossly abnormal material, but invariably lay within the range 5.6 - 6.8.

Samples were made up in 25 ml. conical flasks and were incubated in a metabolic shaking incubator. Except where otherwise stated samples were incubated for 3 hours with shaking at a rate of approximately 50/min. At the end of the incubation period each sample was brought to pH 4.0 by the addition of 1 N.HCl and was then boiled for one minute to prevent further fermentation (Watson/

(Watson 1956). Samples were diluted in Tyrode solution and assayed against standard solutions of histamine on the guinea-pig ileum preparation suspended in atropinised Tyrode solution at 37°C. (See Appendix IV). The nature of the contracting substance was verified by antagonising with mepyramine maleate. Accurate measurements of pH were made electrometrically, but where it was sufficient to make only a rough determination B.D.H. indicator papers were employed.

Solutions. Solutions of salts were made up from analytical grade reagents. Solutions of L-histidine were prepared from L-histidine monohydrochloride (L.Light & Co.), and pyridoxal 5-phosphate and aminoguanidine bicarbonate were also obtained from L.Light and Co.

Results.

i. Incubation of ruminal contents with L-histidine and other agents.

Ruminal fluid was incubated under a variety of conditions in a series of preliminary experiments suggested by previous reports which have already been discussed. Variations in the temperature of incubation indicated that there was no evidence of histidine decarboxylation at 30°C as suggested by Gale (1940) and all subsequent experiments were performed at 37°C. Tests of incubation time showed that one hour was insufficient whilst periods greater than 3 hours gave no greater yield of histamine. Accordingly incubation for 3 hours at 37°C was employed as a standard procedure in all subsequent experiments.

The effects of adding various accessory compounds to the L-histidine substrate were next examined. In each case the incubation mixture consisted of 2 ml. ruminal fluid + 1 ml. of L-histidine solution (15 mg/ml) + 6 ml. Tyrode solution and 1 ml. of a solution of the test compound/

TABLE III

The effect of various compounds on the formation of histamine by ruminal fluid incubated for 3 hours at 37°C.

Concentrations shown in brackets are for total incubation mixture.

L-histidine was present in a concentration of 1.5 mg/ml.

<u>Compound added.</u>	<u>Hist. content μg/ml of ruminal fluid.</u>	
	<u>Test sample.</u>	<u>Sample containing boiled ruminal fluid.</u>
Ruminal fluid alone. L-histidine	0.5	0.20
" + pyridoxal 5 phosphate (0.1 mg/ml).	0.45	0.23
" + ammonium chloride (0.1 mg/ml.)	0.32	0.40
" + benzene (1 drop)	0.10	0.60
" + aminoguanidine bicarbonate (0.015 mg/ml)	0.65	0.75
" + lactic acid (to pH 4.0)	0.25	0.22
" + 1% glucose	0.78	0.67

compound. In all experiments duplicate samples were prepared and concentrations of histamine obtained were compared with those given by similar samples containing boiled ruminal fluid. The results of a series of preliminary tests are shown in Table III.

Pyridoxal 5-phosphate was included as it had been suggested by Blaschko (1956) that this was an essential co-enzyme for mammalian histidine decarboxylase. At a concentration of 0.1 mg/ml. there was only a slight increase in histamine formation. Greater concentrations could not be used as they stimulated contractions of the guinea-pig ileum preparation. Ammonium chloride was employed by Koessler and Hanke (1924) to increase decarboxylase activity. In the concentration recommended by these authors however, it had no marked action. Benzene was found by Waton (1956) to greatly increase the activity of mammalian tissue decarboxylase but in the present experiments it appeared to exert a depressant effect. The addition of aminoguanidine, which inhibits the action of histaminase (Waton 1956) also failed/

failed to increase the histamine content of the incubated samples.

Since the report by Dain et al. (1956) associating histamine formation in the rumen with an increasing concentration of lactic acid, this compound was added to incubation mixtures in amounts sufficient to reduce the pH to 4.0. The result shown in Table III is typical in that under these conditions the histamine concentration was increased only slightly. Hanke and Koessler (1924) emphasised the need for a supply of fermentable carbohydrate in cultures of organisms showing decarboxylase activity. In the present experiments the addition of glucose had little effect on samples incubated for 3 hours, but in subsequent experiments more prolonged incubation with glucose was found to have a marked effect. The results of these tests will be described later.

It is apparent from these results that little histidine decarboxylase activity can be demonstrated by the incubation of ruminal contents with L-histidine in the manner used successfully for intestinal/

TABLE IV

Changes in acidity of ruminal contents following incubation at 37°C with 2% glucose.

- indicates NOT DONE.

<u>Sample No.</u>	<u>pH of Sample</u>				
	0 hrs.	18 hrs.	24 hrs.	48 hrs.	72 hrs.
S 8/1	6.50	4.80	4.70	-	-
S 8/2	5.90	-	5.30	4.00	4.00
S 16/1	6.50	-	4.55	4.40	-
S 16/2	6.10	-	4.75	4.15	4.05
S 16/3	6.20	-	4.95	4.20	4.00
S 5/1	6.40	-	4.35	4.10	-
S 5/2	5.75	-	4.45	4.00	4.00
S 5/3	5.60	-	4.55	4.00	4.00

intestinal contents by other workers. The normal rate of production of histamine in the rumen must consequently be very small, although the situation may be different for sheep on other diets.

ii. Prolonged Incubation of Ruminal Fluid with Glucose.

Glucose was added to a series of samples of the supernatant from centrifuged ruminal contents in amounts sufficient to give a concentration of 2% W/V of the fluid. These samples were then incubated for long periods with periodic examination at 18 or 24 hour intervals. It soon became clear that, in samples treated in this way, the acidity increased steadily as incubation proceeded. This is shown in Table IV in which results of pH determinations are recorded for 8 samples taken on different occasions from 3 sheep. During the first 24 hours there was a rapid fall in pH value to 4.1 - 4.7. At 48 hours the pH had fallen to 4.0 - 4.3. Incubation for longer than 48 hours produced little further change. The rate of fall of the pH value for each sample appeared to be related/

related to its initial acidity and this was lowest in S5 although all three sheep were receiving identical rations.

The acidity never increased beyond pH 4.0 even when samples in subsequent experiments were incubated for 72 hours.

Samples incubated in this way were assayed for histamine content at 24 hour intervals. In a preliminary experiment a sample from sheep S5 increased its histamine content from 0.45 to 2.4 $\mu\text{g/ml}$ in 48 hours. Over a similar period, however, a sample from sheep S16 increased from <0.04 to only 0.2 $\mu\text{g/ml}$. After 24 hours there was no clear evidence of histamine formation. Although the differences were of a similar order in both samples the actual concentrations of histamine were very different. This individual variation was further emphasised in a series of experiments with samples of ruminal fluid taken from S5, S8 and S16. The pH and histamine content of each sample was estimated after 24, 48 and 72 hours/

TABLE V.

The formation of histamine by incubation of ruminal contents with glucose. Samples were taken from two sheep S5 and S16. Histamine content is expressed as $\mu\text{g/ml}$. of ruminal fluid. Each figure represents the average of 2 samples.

	Period of incubation hours.			
	0	24	48	72
<u>S5</u> with 2% glucose	0.58	2.70	2.25	3.75
after incubation with histidine	-	-	2.87	3.19
pH of fluid	5.70	4.40	4.00	4.00
<u>S5</u> with 4% glucose	0.58	-	1.18	3.45
after incubation with histidine	-	-	2.86	3.32
pH of fluid	5.80	4.50	4.00	4.00
<u>S16</u> with 2% glucose	<0.04	0.07	0.18	1.15
after incubation with histidine	-	-	0.47	2.07
pH of fluid	6.00	4.75	4.10	4.00.
<u>S16</u> with 4% glucose	<0.04	-	0.10	1.08
after incubation with histidine	-	-	0.38	2.48
pH of fluid	6.10	4.75	4.20	4.10
<u>Boiled fluid</u>				
<u>S5</u> with 2% glucose	0.58	-	0.08	-
after incubation with histidine	-	-	<1.00	-
pH of fluid	5.90	6.00	4.10	-
<u>S16</u> with 2% glucose	<0.04	-	<0.08	-
after incubation with histidine	-	-	<0.08	-
pH of fluid	6.10	5.00	4.10	-

TABLE VI

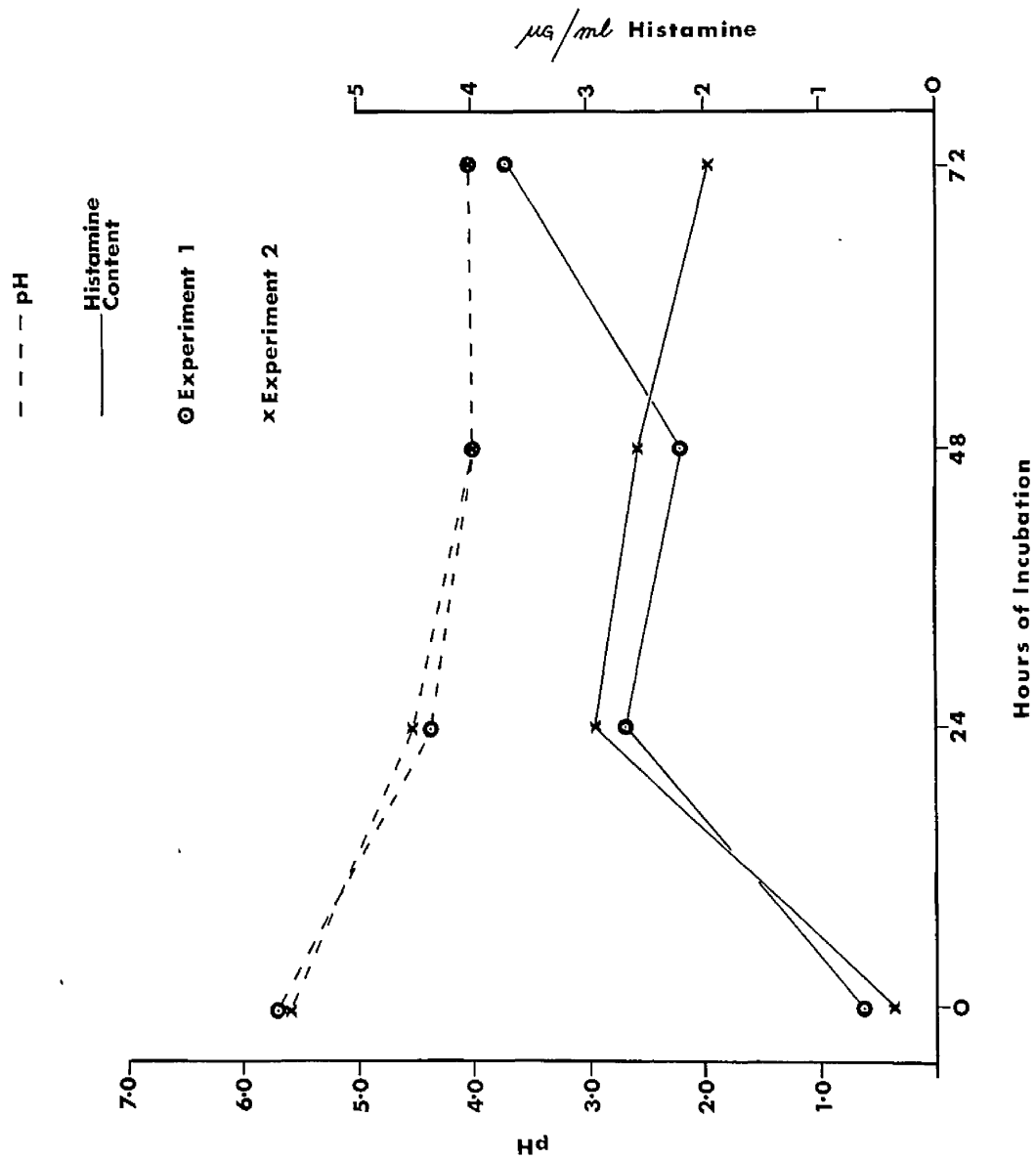
The formation of histamine by incubation of ruminal contents in a solution containing 2% glucose. Samples were taken from 3 sheep S5, S8 and S16. Histamine content is expressed as μ g/ml of ruminal fluid. Each figure represents the average of 2 samples.

	Period of incubation hours.			
	0	24	48	72
<u>S5</u> Histamine content	0.37	2.94	2.60	1.96
after incubation with histidine	-	-	3.16	2.23
pH of fluid	5.6	4.55	4.0	4.0
<u>S8</u> Histamine content	0.1	0.32	0.54	0.13
after incubation with histidine	-	-	0.66	0.37
pH of fluid	5.9	5.3	4.0	4.0
<u>S16</u> Histamine content	<0.04	0.28	0.43	0.40
after incubation with histidine	-	-	0.40	0.84
pH of fluid	6.2	4.95	4.2	4.0

hours of incubation. In addition at 48 and 72 hours 2 ml. of fluid was removed from the sample and was incubated for 3 hours with 1 ml. of L-histidine solution (15 mg/ml) and 7 ml. of Tyrode solution to estimate its potential histidine-decarboxylase activity. Results of these experiments are shown in Table V and VI. Results of incubation with 4% W/V glucose are also included in Table V. It is clear that additional glucose appeared to reduce the rate of histamine formation. For instance sample S5 incubated with 2% glucose reached a histamine concentration of $2.25 \mu\text{g/ml}$ after 48 hours whereas with 4% glucose the concentration had reached only $1.18 \mu\text{g/ml}$. The total amount of histamine present after incubation with histidine was not different being $2.87 \mu\text{g/ml}$ with 2% glucose and $2.86 \mu\text{g/ml}$ with 4% glucose respectively. Results of sample S16 were essentially similar although with this fluid the histamine content did not exceed $1.0 \mu\text{g/ml}$ until it had been incubated for 3 days.

Results from samples taken from sheep S5, S8 and S16 are recorded in Table VI. For S5 and S16 the/

Fig. 47. The effect of incubating ruminal contents containing 2% added glucose. Each point represents the average of 2 samples.



the amounts of histamine formed were of a similar order to those shown in Table IV. The greater histamine-forming potential of S5 is clearly shown in these results whilst it is apparent from Table VI that S8 resembles S16 in this respect. Changes in pH and histamine content are shown plotted against time for 2 experiments with S5 in fig. 47.

It may be concluded from these results that the formation of histamine in ruminal fluid containing 2% glucose incubated at 37°C, was increased as the sample became more acid. There was no direct correlation however, between pH value and histamine content. The acidity increased fairly consistently in every sample whereas the concentration of histamine showed no such consistent pattern. The lack of direct correlation is further emphasised by the results for boiled samples in Table V. These were boiled for 15 minutes, before incubation. Such a procedure effectively inhibited subsequent histamine formation but did not prevent an increase in acidity.

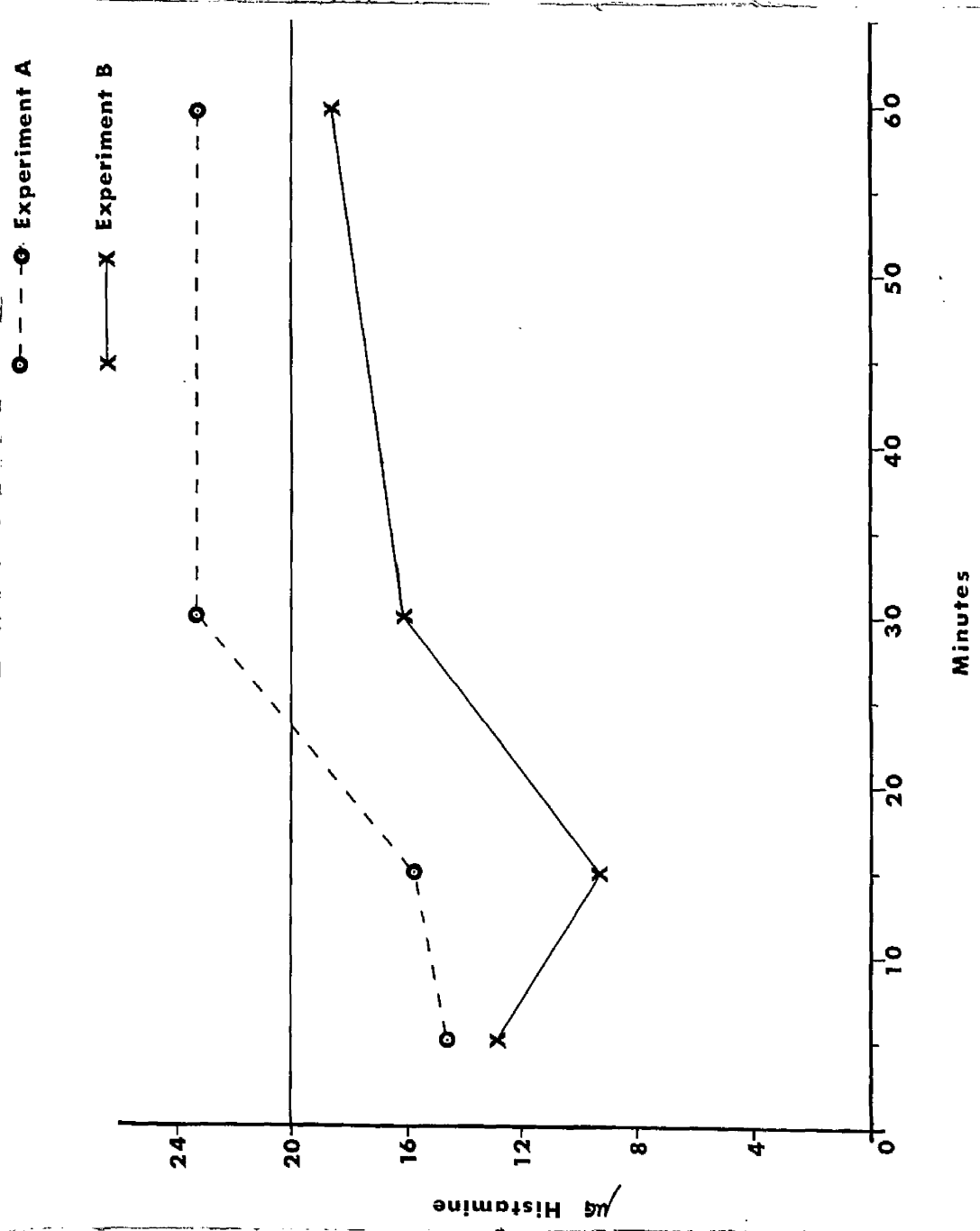
Decarboxylation/

Decarboxylation under these conditions was seen only in samples incubated for 24 - 48 hours. Incubation with L-histidine at the end of this period gave rise to very little more histamine showing that there was no shortage of the amino-acid substrate. Incubation for 72 hours tended in some cases to reduce the histamine content compared to 48 hours samples. Seventy-two hours incubation gave a marked increase in only one sample, S16 in Table V. In general, samples having a low histamine content formed more on incubation with L-histidine than those in which histamine was already present, but there was no consistent pattern in these results.

iii. Incubation of Ruminal Fluid with Histamine.

The amount of histamine formed by the incubation of ruminal fluid with glucose was less than might have been expected from the report of Dain et al. (1955). These authors found quantities of histamine greater than 90 μ g/ml in the ruminal contents of sheep which had been fed on excess of carbohydrate/

Fig. 48. The effect of incubating ruminal fluid with 20 μg histamine. Each point represents the average of 2 samples.



carbohydrate. It was possible, therefore, that some inactivation or breakdown of histamine had occurred during incubation. This possibility was examined by incubating histamine with ruminal contents in two series of experiments.

In the first, 1 ml. of a solution of histamine acid phosphate in saline containing 20 μ g/ml. of histamine base was incubated with 4 ml. saline and 1 ml. of ruminal fluid. Eight such samples were prepared and incubated at 37°C. Pairs of samples were removed at 5, 15, 30 and 60 minutes and were acidified, boiled, neutralised and assayed for histamine. The results obtained from other similar samples without histamine were subtracted in each case. Results of two such experiments are shown in fig. 48. In both these experiments there was an apparent loss of histamine after 5 - 15 minutes incubation but at one hour there was an approximately complete recovery of histamine.

In other experiments 20 μ g histamine base was incubated/

incubated for 3 hours at 37°C with 2 ml. ruminal fluid and 7 ml. Tyrode solution, and samples were set up in triplicate. In two such experiments average amounts of histamine present at the end of 3 hours were 22.4 μ g and 19.1 μ g respectively. In none of these experiments was there any appreciable loss of histamine and it was inferred that ruminal contents did not destroy or inactivate histamine under these conditions. The initial fall in histamine content did suggest, however, that some temporary change may occur.

Experimental Methods for Investigation of the
Absorption of Histamine from the Alimentary Tract.

The absorption of histamine may be assessed either directly, by measuring the histamine content of plasma or urine, or indirectly, by noting characteristic changes in blood pressure and respirations. The latter method does not show that histamine is specifically involved but this may be assumed if the effects are antagonised by an anti-histaminic drug.

Direct estimation of histamine has the advantage that absorption can be expressed quantitatively and free histamine can be distinguished from conjugated forms. In most of the previous studies, which have already been discussed, changes in urinary histamine have been used to indicate absorption. The publication by Adam Hardwick and Spencer (1957) of a simple method for the extraction of plasma histamine has made it possible to measure changes in the histamine content of circulating blood directly. The method employs ion exchange chromatography using a cationic/

cationic exchange resin on which histamine is bound and subsequent eluted with H Cl. As the conditions required for the extraction of free histamine differ from those at which conjugated forms are removed, separation of active and inactive compounds can be achieved. This method is much simpler than the earlier method of Barsoum and Gaddum (1935) modified by Code (1937) in which histamine is extracted by boiling with strong H Cl which is then evaporated by boiling with ethyl alcohol under reduced pressure. This method does not differentiate satisfactorily between free and conjugated compounds as free histamine may be released by boiling with strong H Cl (Adam et al. 1957).

Following extraction, histamine is estimated by bio-assay using the guinea pig ileum preparation. The nature of the contracting substance is confirmed by antagonism with the specific anti-histaminic mepyramine maleate.

In the present investigation measurements of plasma histamine were made in both portal and peripheral/

peripheral venous blood of anaesthetised sheep. Levels in the resting state were compared with those obtained after a solution of histamine in saline had been introduced into the previously emptied rumen. During some experiments attempts were also made to demonstrate changes in respirations. In other experiments with conscious sheep unsuccessful attempts have been made to show some effect on stomach movements following the introduction of histamine through a rumen cannula. Finally experiments were performed in anaesthetised sheep to correlate changes in the histamine content of peripheral venous blood with effects on blood pressure and respirations.

Absorption of Histamine from the Alimentary Tract.

Materials and Methods.

- i. Experiments with Cannulated Sheep. Sheep fitted with rumen cannulae as described in a previous section were used in some experiments. Each sheep was fasted for 24 hours and on the morning of the experiment the contents of the rumen were removed as completely as possible and the interior washed with 5 litres of saline at 37°C. Although it was never possible to remove the contents entirely, inspection with a peritoneoscope inserted through the cannula was used to show how much remained.

After emptying and washing the rumen, recording balloons were inserted and the sheep was placed in a yoke and connected to the apparatus for recording stomach movements. After 30 minutes - 1 hour of recording a solution of histamine in saline was introduced through the cannula and the recording was continued.

Samples of ruminal fluid were withdrawn at intervals. These were centrifuged and the supernatant/

supernatant was boiled with HCl for 1 minute. The samples were assayed against standard solutions of histamine on a guinea pig ileum preparation.

- ii. Experiments with Anaesthetised Sheep. Anaesthesia was induced by the intravenous injection of 6% sodium pentobarbitone solution (25 - 30 mg/Kg). The sheep was placed in lateral recumbency, intubated and connected to a closed circuit system with circle absorber and rebreathing bag.

Anaesthesia was maintained with a cyclopropane oxygen mixture. In experiments in which recordings of blood pressure and respirations were to be made the sheep was subsequently given 50 mg/Kg chloralose intravenously and use of the anaesthetic apparatus was discontinued.

A polythene cannulae 3 mm. ext. dia. was introduced into the tarsal vein for a distance of some 30 cm. sufficient to reach the main trunk of the femoral vein. This cannula was used for taking samples of peripheral venous blood.

In some experiments samples were also taken from/

from the portal vein. A mid-line incision was made and a loop of intestine withdrawn. A similar polythene cannula was introduced into a branch of the mesenteric vein and was passed up into the main portal vein. The position of the tip of this cannula was checked at post mortem examination and in each animal it lay in the portal vein not more than 10 cm. from the liver.

Before taking blood samples each sheep was given 250 i.u./Kg. heparin intravenously to prevent thrombus formation in the cannulae. In spite of this precaution some difficulty was experienced in taking samples of portal blood. In the experiments in which records of arterial blood pressure were made a nylon cannula was inserted in the right carotid artery and connected to a Statham strain gauge transducer and recording apparatus as described in Appendix I.

A polythene tube 1 cm. dia. was introduced into the ventral sac of the rumen, and was secured by a purse string suture. The rumen was first emptied manually through a large ventral incision. The/

The contents were then replaced by 1 litre of saline at 37°C.

- iii. Extraction of histamine from Blood Samples. The method used was that developed by Adam et al. (1957) and a description with details of preliminary tests is given in appendix III. 10 - 15 ml. samples of blood were taken into nylon centrifuge tubes containing 100 i.u. of heparin to prevent clotting. After centrifuging for 10 minutes at 2,000 revs./min. the plasma was removed and subjected to further centrifugation at 3100 revs./min. for 60 minutes to remove platelets. Samples of plasma were then withdrawn and mixed with an equal volume of 6% trichloroacetic acid to precipitate protein. After standing for one hour the samples were centrifuged for 30 minutes at 3100 revs./min. and the supernatant was removed.

Histamine was extracted from the supernatant by passage through columns containing a carboxylic ion-exchange resin (IRC 50) which had been previously prepared by treatment with sodium hydroxide. Samples were run through the columns at pH 7.92 using/

using phosphate buffer to attain the correct degree of alkalinity.

At this pH free histamine is completely adsorbed by the resin whereas acetylhistamine is not retained. The composite columns are also very sensitive to the concentration of sodium ions so care was taken to ensure that this did not exceed the limit of 0.2 N (Adam et al. 1957).

The adsorbed histamine was eluted with a fixed volume of 0.1 N HCl and the eluate was collected and subsequently neutralised and assayed biologically. The rate of flow of solutions through the column was adjusted to 0.2 - 0.3 ml/min. Recovery of histamine both from pure solution and from sheep plasma was also determined and the results of these tests will be described as a separate appendix (See Appendix III).

When the histamine content of a sample was too low for assay at a dilution of 1 in 5 it was used in a more concentrated form and salts were added to produce a composition similar to that of Tyrode/

Tyrode solution (Adam et al. 1957). The salts added were sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium acid phosphate and sodium bicarbonate and details of the solutions are given in Appendix III.

Results.

i. Histamine in Rumen of Conscious Sheep.

Solutions of histamine acid phosphate in saline were put into the partially emptied rumen of sheep in five experiments using three sheep. Concentrations of histamine base ranged from 0.04 to 10 mg/ml. and the dose of histamine given by this route varied from 0.5 - 30.0 mg/Kg. The sheep were observed and continuous records of rumen movements were made for 3 - 4 hours after giving histamine.

This procedure had no effect on ruminal movements nor were there any other signs, such as tachypnoea or repeated defaecation, which might indicate that histamine had been absorbed. On the days following each experiment the sheep fed normally and appeared to be completely unaffected. It was concluded, from the complete lack of response, that free histamine was not absorbed in any significant quantity from the rumen.

During the course of some experiments samples
of/

Table VII.

Histamine content of ruminal fluid after dosing
with 1 gram histamine in 250 ml. saline per
cannula.

<u>Time after dosing.</u> <u>mins.</u>	<u>Histamine content.</u> <u>µg/ml.</u>
0 (before dosing)	< 0.08
30	32
90	600
150	112

of ruminal fluid were withdrawn for determination of pH and histamine content. Results for one such experiment are shown in Table VII. It can be seen that the concentration of histamine was 0.6 mg/ml. after 150 minutes. The low value obtained at 30 minutes might be due to poor mixing in a rumen which had not been emptied completely. On the other hand it was possible that some temporary absorption had occurred and this point will be further considered in the discussion. (See P.171).

In another similar experiment the concentration of histamine in the ruminal fluid was greater than 1.0 mg/ml. for at least 150 minutes after dosing. These results show clearly that histamine is not inactivated by ruminal contents nor is there any evidence to indicate that it is absorbed in the rumen.

Table VIII.

Variations in plasma histamine content of anaesthetised sheep following the administration of a histamine solution into the emptied rumen. Histamine added at 0 minutes.

Details of Experiment.	Time mins.	Plasma Histamine $\mu\text{g/ml.}$	
		Portal	Peripheral
1. 3 grams histamine in 500 ml. saline	0	0.318	0.097
	10	-	0.133
	30	0.192	0.133
	45	0.141	0.101
	60	0.213	0.109
	90	0.118	0.093
2. 5 grams histamine in 500 ml. citric acid phosphate buffer at pH 5.5.	-15	0.102	0.064
	0	0.122	0.065
	10	-	0.068
	20	0.336	0.172
	30	0.099	0.078
	45	0.078	0.086
	60	0.059	0.106
	90	-	0.081
3. 5 grams histamine in 500 ml. acetate buffer at pH 5.5. Histamine solution was removed and replaced by acetate buffer at 60 mins.	-20	0.139	0.140
	- 5	0.214	0.213
	15	0.063	0.078
	30	0.183	0.173
	45	0.181	0.183
	60	0.060	0.108
	75	-	0.129
	90	0.050	0.156

ii. The Effect on Plasma Histamine Content of Histamine in the Rumen of Anaesthetised Sheep.

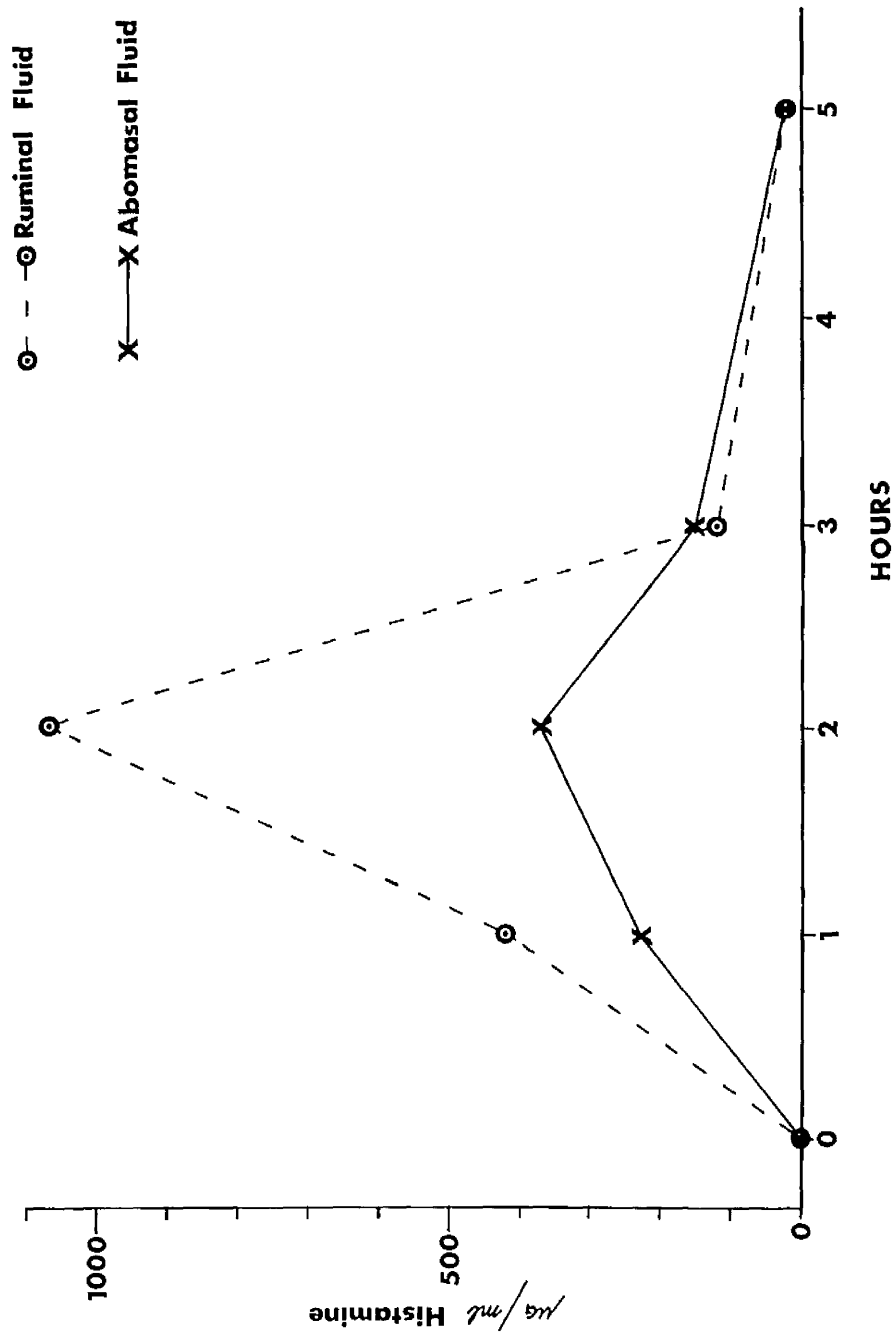
This experiment was performed in 3 half-bred sheep. Histamine was placed in the previously emptied rumen and samples of portal and peripheral venous blood were taken at intervals for the extraction and estimation of histamine. In the first experiment records of arterial blood pressure and of respirations were also made but these were not affected by the presence of histamine in the rumen. In the second and third experiments histamine was dissolved in a buffered acid solution at pH 5.5 using a citric acid-phosphate buffer in one experiment and an acetate buffer in the other. No marked variation in the pattern of the results was seen as a result of this procedure.

Plasma histamine values during each of the three experiments are shown in Table VIII. It is apparent that there is no evidence of a consistent rise in plasma free histamine following the placing of a solution of histamine containing 10 mg/ml.

in/

in the rumen. The histamine content of portal plasma did, however, show a marked temporary increase in experiment 2. In general it can be seen that portal vein plasma tended to contain more free histamine except in experiment 3 where the values were similar for both portal and peripheral blood. A correlation between changes in the histamine content of portal and peripheral plasma was clearly seen in all three experiments with the exception of the final samples in experiments 2 and 3. This well-marked tendency for the histamine content of simultaneous samples of portal and peripheral plasma to show a similar variation, gave considerable support to the validity of these results. In conclusion it can be seen that these experiments give little evidence for the absorption of free histamine through the ruminal wall.

Fig. 49. Passage of histamine from rumen to abomasum. 1 gram of histamine put into rumen through cannula at 0 hours.



iii. Passage of Histamine from Rumen to Abomasum.

It has now been clearly shown that a dose of histamine put into the rumen through a cannula increased the histamine content of ruminal fluid for several hours. During this period movements of the reticulum and rumen continued unchanged so it seemed probable that fluid containing histamine had passed to the abomasum.

This supposition was tested in a sheep fitted with both ruminal and abomasal cannulae as described in part 2 of this thesis. On two occasions separated by an interval of a fortnight a dose of 1 gram of histamine in saline at 37°C was put into the rumen. Samples of ruminal and of abomasal fluid were withdrawn at intervals and their histamine content was estimated in the manner already described. Similar results were obtained in both experiments and details of the second are shown in fig.49. Histamine appeared in considerable concentration in abomasal fluid within one hour of dosing per cannula. The concentration of histamine in subsequent samples from the abomasum showed a marked/

marked tendency to rise and fall with that of the ruminal fluid. In both experiments the histamine content of both stomach compartments had fallen to its normal low level within 18 hours.

From these results it was concluded that histamine present in the rumen passes rapidly to the abomasum and may subsequently reach the small intestine without inactivation. It was not possible to assess the passage of histamine in a quantitative manner as no measurements were made either of fluid volumes or of the rate of flow from rumen to abomasum. The highest concentration of histamine recorded in abomasal fluid was 1.28 mg/ml. This occurred 6 hours after dosing in the first experiment.

Discussion.

Previous reports have indicated that an abnormal concentration of carbohydrate is necessary for histamine formation to occur in ruminal fluid. (Dain et al. 1955, Van der Horst 1961). In the incubation experiments here described a solution containing 2% glucose appeared to provide the most suitable conditions. Although the increase in histamine content was not directly proportional to the fall in pH value there appeared to be some correlation between these two parameters. Increased acidity of ruminal contents was found by Hungate et al. (1952) to be due to the formation of non-volatile acids, particularly pyruvic and lactic acid. Such an increase seems to favour the decarboxylation of histidine which could not be demonstrated in normal ruminal fluid even after incubation for 72 hours (Sirotnak et al. 1953).

Histidine-decarboxylating activity appeared to develop slowly and was probably associated with the multiplication of one particular group of organisms/

organisms. Incubation of fresh ruminal contents for 3 hours with lactic acid or with glucose did not reveal any marked decarboxylase activity. The addition of either pyridoxal 5-phosphate or of benzene to the incubation mixture also failed to enhance this activity which was presumably due to a different histidine decarboxylating enzyme from that found in mammalian tissues (Waton 1956). Although no attempt at bacteriological examination was made it is interesting to note that decarboxylation occurred under similar conditions to those specified for human faecal organisms by Hanke & Koessler (1924) and Gale (1940).

In attempting to assess the results of the incubation experiments two important points arise. These are i) the possible contamination of the samples and ii) the identity of the active substance formed. Contamination was unlikely to be involved as boiled samples incubated under identical conditions showed no histidine-decarboxylase activity. The second point is more difficult to answer satisfactorily. Samples were boiled with 1N HCl for 1 min. (Waton 1956) and assayed in atropinised/

atropinised Tyrode solution. Results were checked in many cases by antagonism of contractions with the specific antihistaminic agent mepyramine maleate. Nevertheless the results might be more reliable quantitatively if a method had been developed for the isolation of histamine from the incubation mixture.

The persistence of histamine in the ruminal fluid, both 'in vitro' and during incubation experiments, does not support the suggestion that ruminal fluid normally contains a histamine destroying enzyme (Bourlé 1959). In view of this, it seems likely that the concentrations of histamine obtained when ruminal contents are incubated with glucose represent the total amounts formed under these conditions. This point is of some importance in assessing the significance of histamine formation as the concentrations of histamine involved ($< 5.0 \mu\text{g/ml}$) were very much less than those recovered by Dain et al. (1955) from the ruminal fluid of an animal which had been fed an excessive amount of carbohydrates (i.e. $> 90 \mu\text{g/ml}$ histamine).
It/

It seems probable that the ability of ruminal micro-organisms to decarboxylate histidine varies considerably according to conditions of feeding and management. It may be concluded that histamine formation in the rumen does not occur to any significant extent under normal conditions of indoor feeding. Histamine formation might be of more significance, however, if the carbohydrate content of ruminal fluid were increased.

When sheep were dosed with histamine per cannula it was found that the histamine content of ruminal fluid increased rapidly to a maximum $1\frac{1}{2}$ - 3 hours later. This rise was entirely unexpected and appeared, at first sight, to indicate inadequate mixing of the dose within the rumen. Similar results were obtained, however, in experiments in which the contents of the rumen were replaced by a solution of histamine in saline. Furthermore, inadequate mixing would be likely to give a haphazard variation in histamine content but not a steady increase. (See Table VII and fig. 4^a). It was also found that histamine had started/

started to pass to the abomasum within one hour of dosing which would tend to produce a fall rather than a rise in the histamine content of ruminal fluid.

There might be several explanations of this situation:

1. Ruminal fluid might be concentrated due to increased absorption of water through the wall of the rumen.
2. Histamine might be rapidly bound or conjugated and later released.
3. The presence of histamine in the rumen might stimulate the formation or release of more histamine.
4. Histamine might become converted to a form having greater biological activity.

The first factor is unlikely and could only be responsible for the results seen if histamine itself modified the normal pattern of gain and loss of water in the rumen. Temporary binding by ruminal fluid was suspected in the experiments in which histamine was incubated with the supernatant from the centrifuged/

centrifuged ruminal contents. The histamine content of the incubated mixture fell during the first 15 - 30 minutes but later rose to reach its starting point in 30 - 60 minutes. If histamine were conjugated it might be converted to acetylhistamine which has no pharmacologic activity. (Anrep, Ayadi, Barsoum, Smith & Tallaat 1944). This form of conjugation is believed to occur extensively in the intestinal tract of many species including man (Adam 1950).

Although acetylhistamine cannot be assayed biologically it can be converted to free histamine by acid hydrolysis and then assayed (Adam et al. 1957). This was carried out with ruminal contents obtained in one experiment by boiling one half of each sample under reflux for one hour with concentrated HCl but there was no evidence that acetylhistamine had been present in the samples.

The possibility of further histamine formation or release has not been explored but no reports have been found which would support this suggestion. The/

The final possibility is that a more active compound was formed. The most likely compound to be involved is N-methylhistamine since Vartiainen (1935) has shown that this compound is about twice as potent as histamine when assayed on the guinea-pig ileum. Schild (1947) found also that N-methylhistamine was antagonised by antihistaminic drugs so that it would not be possible to distinguish it from histamine by the methods used in the present investigation. The possible production of N-methylhistamine is of some importance in assessing the metabolic reactions of the rumen and might profitably be further investigated.

The absence of any evidence for the absorption of histamine through the wall of the rumen was not in agreement with the observation made in a cow by Dougherty & Cello (1949). In the present experiments doses of histamine up to 2.5 grams were completely without effect when given by cannula. The concentrations of histamine attained in the rumen during these experiments (>1 mg/ml) far exceeded those considered potentially toxic by Dain/

Dain et al. (1955). It may be that failure to demonstrate absorption of histamine from the rumen was due to the fact that sheep were used rather than cows. Bourlé (1959) quoted evidence of similar experiments in cows, however, which were completely in agreement with the present findings. It is, however, possible that some histamine is absorbed but is then conjugated in the wall of the rumen or in the liver. This aspect of the problem was not examined as the investigation was concerned only with the absorption of physiologically active histamine.

PART IV

DISTRIBUTION OF HISTAMINE AND MAST CELLS IN THE
STOMACH AND OTHER TISSUES OF THE SHEEP

The initial investigation of the effects of histamine on the motility of the sheep stomach has led, on the one hand to a study of the formation and absorption of histamine and, on the other, to a study of the distribution of endogenous histamine in the alimentary tract. The occurrence and distribution of histamine in animal tissues has been extensively investigated since its discovery was first reported in 1910 by Barger & Dale. It will be relevant, therefore, to review briefly the literature relating to the action of histamine on the stomach. Later, the distribution of histamine in the stomach and its relation to tissue mast cells will be discussed.

Actions of Histamine in the Stomach.

Popielski (1919) and Keeton, Koch and Luckhardt (1920) showed that when histamine was injected subcutaneously it produced a marked increase in gastric secretion, but this effect was not seen when the histamine was given intravenously. An explanation for this difference was/

was provided by the experiments of Ivy & Javois (1925) who showed that the speed of intravenous injection was important. They gave histamine intravenously to dogs by continuous slow infusion and found that a dose of 0.0027 mg/Kg/min. was sufficient to stimulate gastric secretion. This aspect of histamine administration has received further careful investigation by Obrink (1948), who found that there was, in fact, no threshold value for the dose of histamine required to stimulate gastric secretion. He pointed out, however, that although histamine was always present in the blood of the normal animal, the concentration was not sufficient to produce a significant effect on gastric secretion.

Gastric juice produced in response to histamine has a high acid and low pepsin content. The action appears to be direct, as it has been reported that when gastric mucosa is transplanted to a subcutaneous site, it continues to secrete in response to injections of histamine (Ivy & Farrell 1925).

In/

In a study of the function of the stomach of ruminants, Hill (1951) has suggested that histamine probably stimulates acid secretion in the abomasum. He has since verified this suggestion by giving intravenous infusions of histamine to stimulate secretion in a sheep having an abomasal pouch. Code (1956) concluded that histamine is a "universal gastric stimulant". He also stated that "the predominant, if not the entire, secretory effect of histamine on the gastric mucosa is one of stimulation of the production of acid".

Although it is well known that in general histamine contracts smooth muscle, a search of the literature has not revealed any detailed studies of its action on stomach muscle. Dale & Laidlaw (1910) found that when injected intravenously, it caused vomiting and purging in the unanaesthetised cat, but these authors did not comment on the possible mode of action in this case.

According to Salter (1952), histamine does not/

not increase peristalsis of the rat's stomach, whilst Walder (1953) reported that it caused only slight contraction of isolated muscularis mucosa of the human stomach. The actions described by Walder were obtained with concentrations of histamine of 1 in 30,000. The contractions were much smaller than those produced by 1/100th of this concentration of acetylcholine, and no differences were observed in different regions of the stomach.

Distribution of Histamine in the Stomach.

The presence of histamine in the gastrointestinal tract was first reported by Barger & Dale (1910). They extracted it from the intestinal mucosa of the ox, and established its identity by chemical and pharmacological tests. Twenty-two years later, the isolation of histamine from acid extracts was reported by Sacks, Ivy, Burgess and Vandolah (1932). They succeeded in preparing crystals both of the sulphate and of the picrate. No other substance was obtained from the extracts which stimulated gastric secretion, and as the activity of/

of the extracts was destroyed by histaminase, they concluded that histamine was the principal, if not the only, substance in gastric mucosa capable of stimulating secretion.

The distribution of histamine differs in the various tissues and regions of the stomach, and is found in greater concentration in the gastric mucosa than in the other tissues. Gavin, McHenry & Wilson (1933) and Emmelin & Kahlson (1944) found that histamine was present in large amounts in the mucosa of the stomach of the dog, and that the concentration was higher in the body of the stomach than at the pylorus. The former estimated that 80% of the total histamine content of the stomach occurred in the fundic mucosa.

In a detailed study of the histamine content of the gastro-intestinal tract of the dog, Douglas, Feldberg, Paton & Schachter (1951) reported that the wall of the oesophagus contained little histamine. In the stomach, however, large amounts (60 - 127 $\mu\text{g/g}$) could be extracted from the gastric mucosa, but much smaller concentrations were found in/

in the muscularis externa (14 - 32 μ g/G). The wall of the duodenum, like that of the stomach, contained high concentrations of histamine, but the more distal portions of the small intestine contained progressively less histamine.

In a further study of the distribution of histamine in the stomach of the dog, Feldberg & Harris (1953) estimated the amount of histamine which could be extracted from small areas of stomach tissue out on a freezing microtome. In this way, they were able to relate the histamine content to the histological structure of the tissues. They constructed "histamine profiles" for the wall of the stomach which showed how histamine content varied from the lumen to the serous surface. In the body of the stomach, the profile showed two peaks of histamine concentration. One of these was associated with the region of maximum concentration of parietal cells in the mucosa. In the pyloric region, the profile had only one peak which corresponded to the region of the mucosa containing the pyloric glands. Although specimens taken from the body of the stomach/

stomach generally yielded higher histamine values than those from the pylorus, specimens of pyloric mucosa sometimes had peak concentrations of histamine which were as high as those of the body.

These authors reported that although there was considerable individual variation in the histamine content of the tissues, the regional differences were similar in each animal. In reviewing the species differences in the histamine content of gastric mucosa, Feldberg (1956) pointed out that large amounts of histamine occurred in the dog, whilst in the cat there was much less, and very little in the rat and mouse. Comparable figures for other species were not available. Feldberg thought it desirable, therefore, to emphasise the fallacy of describing any organ as having a high content of histamine without reference to the particular species concerned.

Histamine and Mast Cells in the Alimentary Tract.

Mast cells were first recognised and so named by Ehrlich in 1877. He showed that these cells occurred in loose connective tissue and contained granules which stained metachromatically with basic dyes. Later he also reported that the granules were very soluble in water, a fact which makes their recognition in tissue difficult unless suitable fixatives are used. Hardy & Westbrook (1895) found that the basophil granules could be preserved if tissue was fixed in alcohol and stained by alcoholic stains. In the course of their survey, they drew attention to the large number of basophils situated in the sub-mucosa of the intestine of the dog which were not present in the other laboratory animals examined.

The methods used for identifying mast cells and studying their distribution have been extensively reviewed by Riley (1959). Maximow (1924) discussed the origin and embryological development of these cells and stated that whilst in lower vertebrates/

vertebrates they may originate from basophil blood leukocytes, in mammals, connective tissue mast cells and basophil leukocytes are two distinct cell types.

The occurrence of mast cells throughout the digestive tract of the dog was studied by Arvy & Quivy (1955). They reported that they found few cells in the oesophagus and colon, but large numbers in the stomach. In discussing the distribution of mast cells in the stomach, they stated that they found more cells in the mucosa of the lesser curvature and fundus than in the pre-cardia and greater curvature. Mast cells were also found in abundance in the muscularis mucosae, in the parietal cell region of the mucosa and around the necks of the fundic glands.

The distribution of mast cells in the digestive tract of the rat, guinea-pig, cat and dog has been described by Mota, Ferri & Yoneda (1956). In contrast to other species, only small numbers of mast cells were seen in the fore-stomach of the rat, and they were also scarce in the sub-mucosa of the fundic and pyloric regions. They found/

found, however, a rich supply of eosinophils in the gastric mucosa of the rat, to which reference will later be made. In the stomach of the guinea-pig, dog and cat, mast cells were particularly concentrated in the muscularis mucosae and between the necks of the gastric glands, but the site of greatest density occurred in the fundic mucosa. These authors also found that many mast cells were also present in the submucosa and in the muscularis externa in these species.

Although the distribution of mast cells in the ruminant stomach does not appear to have been studied in detail, Riley (1959) has examined various tissues from ruminants. He found that, in cattle, mast cells were numerous in serous membranes and in the connective tissue of the capsules surrounding the viscera. He considered that these cells were associated with a special type of loose connective tissue rather than with the particular organ which they surrounded. Riley also stated that "enormous numbers of mast cells could be demonstrated in any section of the complex gastric organ of cattle", but provided no detailed evidence in support of this statement.

The/

The relation between the mast cell content of an organ and the amount of histamine which can be extracted from it was first demonstrated by Riley & West (1952 and 1953). Jorpes (1946) had previously related the heparin content of several tissues to the number of mast cells which they contained. Riley & West made a study of similar tissues, showing that there was also a relation between the number of mast cells and the tissue content of histamine. In organs which contained few mast cells, such as the rat liver and pig aorta, very small amounts of histamine (less than $1 \mu\text{g/G}$) were found. On the other hand, where mast cells were extremely plentiful, a high yield of histamine ($40 \mu\text{g/G}$) was obtained. They obtained further evidence for the relation between histamine and mast cells by demonstrating that tissues from adult animals contained more histamine and more mast cells than the corresponding tissues from young animals. Furthermore, by the use of a histamine liberator, they were able to show that the subsequent loss of extractable histamine was accompanied by the disappearance of mast cells from the treated tissues. Riley (1959) attempted to determine the quantity of histamine/

histamine associated with each mast cell in the liver capsule of the ox. He calculated this to be approximately 25 μ g. histamine base per single mast cell.

The findings of Riley & West have since been confirmed by several workers. Graham, Lowry, Wahl & Priebat (1953), for example, found a positive correlation between the number of mast cells and the histamine content of the dog skin. These authors calculated that each mast cell had an average histamine content of 6 μ g. This figure is not unlike that obtained by Riley (see above) for mast cells from the ox. Mota, Beraldo & Junquiera (1953) stated that two histamine-releasing agents, compound 48/80 and stilbamidine, caused disruption of mast cells which could be followed microscopically. Fawcett (1954) gave the histamine liberator, compound 48/80 by intra-peritoneal injection. He found that histamine was released into the peritoneal fluid, and that there was also a release of mast cell granules in the peritoneum. He further showed that compound 48/80 failed to release histamine from/

from connective tissue which had previously been depleted of mast cells. Further evidence in support of the relation between histamine and mast cells has been provided by Cass, Riley, West, Head & Stroud (1954), who demonstrated that very large quantities of histamine could be extracted from mast cell tumours of dogs. They reported in one case the extraction of as much as $1,290 \mu\text{g}$. histamine per gram of tissue.

The actual site of histamine in the mast cell has not yet been established with certainty. From the evidence so far discussed, it seems likely that the histamine is contained in granules of the mast cells, as disruption of these is accompanied by a release of histamine. Jorpes (1946) however, confidently asserted that the granular material consisted of heparin. He based this conclusion on evidence that this substance, like the mast cell granules, was stained metachromatically by toluidine blue. He also found that heparin was precipitated by very dilute solutions of this dye, and felt convinced on the evidence/

evidence available at that time that the granules were composed of heparin. Since the discovery that histamine is also present in mast cells, however, it seems probable that the granules contain histamine as well as heparin.

Grossberg and Garcia-Arocha (1954) studied the effects of various substances which release histamine from a suspension of the large granule fraction of the dog liver. They found that substances which caused lysis of cells also produced a rapid and complete release of histamine. They concluded that histamine was probably held in the granules in diffusible form, rather than by a primary chemical bond. These authors tentatively suggested that mast cell granules contain histamine within a membrane which could be lysed by physical or chemical agents. On the other hand, Fawcett (1954) considered that the graded response of mast cells to increasing concentrations of compound 48/80 did not support the conclusion that the release of histamine was brought about by simple lysis of a membrane.

Riley/

Riley (1959) also argued against a theory of lysis when he showed that histamine was released from mast cells by a solution of toluidine blue in saline without disruption of the cells.

West (1955) found that when subcutaneous tissue of the mouse was treated by fractionation, the fraction which contained the greatest number of mast cell granules also yielded most histamine. When these granules were treated with histamine-releasing compounds, histamine was liberated into the supernatant fluid. These findings led West to suggest that histamine was normally present in the mast cell granules of the mouse,

Schayer (1956) investigated the method by which histamine is incorporated into mast cells. He found that mast cells suspended in rat peritoneal fluid were able to decarboxylate C 14 labelled histidine to form histamine. He further showed that this process was not influenced by previous incubation of the suspension with histamine, and concluded that mast cells did not take/

take up exogenous histamine.

To summarise the evidence so far presented, it has been shown that the occurrence of histamine in many tissues is associated with the presence of mast cells. Histamine is located in the granules of these cells and probably exists in a "free" or chemically unbound state (Riley 1959).

It should not be supposed that all tissue histamine is contained in mast cells. For example, Mota, Ferri, Beraldo and Junqueira (1956) have found that although the histamine content of the fundus and duodenum of the rat is high, it is not depleted after injection of compound 48/80. Since these regions contained very few mast cells, Mota et al. concluded that histamine must be located at some other site in the mucosa of this species. Riley & West (1955) found that in the pyloric region of the pig stomach, the mast cells are situated chiefly in the submucosa which has a high histamine content (up to $100 \mu\text{g/G}$). The overlying mucosa, however, also has a high histamine content (up to $80 \mu\text{g/G}$) but few mast cells/

cells. Riley has resolved this anomaly by suggesting that in the latter tissue, histamine may be bound in the mucin of the goblet cells of the pyloric glands (Riley 1959).

Histamine has also been found in the eosinophil blood leukocytes of the dog, and Code (1956) has suggested that most of the histamine in the blood of this species was associated with the eosinophils. It seems more probable, however, in view of the previous evidence that the eosinophils may be concerned more with the transport, neutralisation or destruction of histamine, rather than with its storage (Archer 1956 & 1960). Previous reference has been made to the presence of eosinophils in the mucosa of the rat stomach (Mota et al. 1956). Although no connection has been found between these cells and the histamine content of that tissue, it may be that such a relation does, in fact, exist.

Investigation of Histamine Content of the Stomach.

As the sheep stomach consists of four anatomically different compartments and as the abomasum has been shown to be more sensitive to histamine than the others, it was considered important to determine the histamine content of the tissues of each compartment.

Method.

Histamine was extracted from the tissue by the method described by Földberg and Harris (1953). Strips of tissue approximately 3 cm. long by 0.5 cm. wide were dissected from the stomach wall. Omasal laminae were cut off at a height of 2 mm. from the surface, and projecting folds of abomasal mucosa were also cut off. The strips were blotted on filter paper and accurately weighed on a torsion balance. They were then transferred to a 10 ml. beaker containing 3 ml. N/3 hydrochloric acid. The beaker was heated for/

for 2 - 3 minutes on a boiling water bath, which made subsequent grinding of the tissue easier. The contents of the beaker were then poured into a mortar and ground with sand. The tissue and sand, together with washings totalling 5 ml. N/3 hydrochloric acid, were then transferred to a flask and heated on a boiling water bath for 60 minutes. Each flask was then stoppered and stored in a refrigerator at 4°C until assayed.

The neutralised extract was assayed for histamine on the isolated guinea pig ileum suspended in atropinised Tyrode's solution, using a standard solution of histamine prepared from histamine acid phosphate. A 4-point assay was carried out by the conventional technique in which test and standard solutions were added at intervals of 2 minutes. Each solution was allowed to act for 30 seconds. All extracts contracted the ileum and these contractions were antagonised by mepyramine maleate in the same concentration as that which antagonised the action of histamine.

Table IX

Histamine Content of the Four Compartments of the
Sheep Stomach.

Expressed as μg histamine base /G of tissue.
Each figure is the average of three samples.
S.E. = standard error of the mean.

	<u>Abomasum</u>	<u>Omasum</u>	<u>Rumen</u>	<u>Reticulum</u>
	19.17	1.82	2.98	2.08
	3.78	1.84	1.61	1.91
	14.85	3.23	2.32	4.54
	13.72	8.66	2.23	2.29
	11.12	2.00	1.84	2.76
	18.57	2.41	2.30	2.44
Mean.	13.54	3.33	2.21	2.67
S.E.	± 2.31	± 1.09	± 0.19	± 0.39

Results.

Histamine content of each compartment.

Three adjacent strips of tissue were taken from each compartment of the sheep stomach and the histamine contained in each sample was extracted and estimated. Table IX summarises the results obtained from the assay of six stomachs. Each figure is the mean result obtained from the separate estimation of three specimens. It can be seen that, with one exception, the abomasum contained more histamine than any of the other compartments. The histamine content of the abomasum ranged from 3.78 - 19.17 $\mu\text{g/G}$ with a mean value of $13.54 \pm 2.31 \mu\text{g/G}$.

The mean values obtained from the assay of omasum, reticulum and rumen were respectively $3.33 \pm 1.09 \mu\text{g/G}$, $2.21 \pm 0.19 \mu\text{g/G}$ and $2.67 \pm 0.39 \mu\text{g/G}$. Statistical analysis by means of a t test showed a significant difference between the histamine content of the abomasum and the omasum. ($t = 4.001$ $P < 0.01$). The differences in histamine content of reticulum, rumen and omasum/

Table X

Distribution of Histamine in the Abomasum

Expressed as μg histamine per gram of tissue.
Each figure is the average of three samples.

	<u>Mucosa.</u>		<u>Muscle.</u>	
	<u>Body</u>	<u>Pylorus</u>	<u>Body</u>	<u>Pylorus</u>
	35.9	23.2	7.8	4.7
	23.5	9.9	3.2	3.1
	31.4	28.2	6.9	2.4
	14.6	8.6	4.9	0.6
	12.4	8.6	1.7	1.2
	20.1	13.1	3.9	0.8
Mean	23.0	15.3	4.7	2.1
S.E.	\pm 3.78	\pm 3.55	\pm 0.9	\pm 0.6

omasum were not statistically significant.

It is clear from these results that the abomasum has a greater content of histamine than any of the other compartments.

Histamine Content of the Tissues of the Abomasum.

In view of the finding that the abomasum contained more histamine than any of the other compartments, it was decided to study the distribution of histamine in the mucosa and muscle layers. Tissue from the body of the abomasum was also compared with that from the pylorus to determine whether there was any difference in the histamine content of these two regions.

Strips of tissue were taken from the body of the abomasum and from the pylorus. The mucosa was carefully dissected with scissors from the underlying muscle and histamine was extracted and assayed as previously described. Specimens of tissue taken from six stomachs were examined. The results are summarised in Table X in which each figure represents the mean result from the estimation of three samples.

It/

It will be seen that the histamine content of the mucosa of the body as well as of the pylorus was significantly higher than that of the corresponding muscle layers. There was also a significant difference ($P < 0.01$) in the histamine content of muscle from the body and from the pylorus. The fact that the mucosa contained five to six times more histamine than the muscular wall and the additional evidence that the mucosa of the body had a greater histamine content than that of the pylorus suggested a further examination of this tissue. It was hoped by this means to determine whether there was a preferential storage of histamine in the mucosa of the body. In the dog, for example, Gavin, McHenry and Wilson (1953) and Feldberg and Harris (1953) reported that mucosa from the body of the stomach contained more histamine than pyloric mucosa.

Distribution of Mast Cells in the Sheep Stomach

The relation between the histamine content of tissues and the presence of mast cells has already been discussed. The evidence reviewed has shown that there is a close relation between the number of mast cells and the histamine content of many tissues. In planning a study of the distribution of mast cells in the sheep stomach, it was essential to secure specimens of tissue immediately after the animals were killed. This was necessary in order to fix the tissue rapidly for the subsequent satisfactory staining of mast cells. As early as 1895, Hardy and Westbrook emphasised the need to avoid aqueous fixatives, and a number of authors have subsequently confirmed this.

Various fixative solutions have been described which have been designed to reduce the amount of water present. Smith and Atkinson (1956) described a fixative solution of 10 mls. formalin in 90 mls. of 95% ethylalcohol to which 1g of calcium acetate was added and in which tissue was left for twenty-four hours. It was then transferred/

transferred to 95% ethyl alcohol before dehydration in the conventional manner. In testing this method, some difficulty was experienced in obtaining satisfactory staining of mast cells with the solution of acidified toluidine blue which they recommended.

Attention was then directed to a method suggested by Watt (1959)^{*} in which the tissue was fixed by immersing it in 50% methyl alcohol for twenty-four hours. Subsequent dehydration was then carried out using increasing concentrations of methyl alcohol. This method of fixation has been used throughout the investigation and has given very satisfactory results.

For identification of mast cells, various stains have been used, most of which depend on the use of toluidine blue. Watt (1959) described a method in which sections were placed in a 0.6% solution of eosin in methyl alcohol for 20 minutes, and were then counterstained in a 1% solution of toluidine blue in $2\frac{1}{2}$ % ethyl alcohol for 30 seconds. In the present investigation, it was/

* personal communication.

was found that the background stain produced by eosin was too intense, but that this could be reduced to some extent by immersing sections in eosin solution for 1 - 3 minutes only, and by leaving them in toluidine blue for 15 - 30 minutes. Subsequent experiments, however, showed that the tissues could be recognised without background staining, and the use of eosin was discontinued.

In exploring the best conditions for staining with toluidine blue, the method of improving this dye described by Robinson and Baesich (1958) was adopted. This method depends on precipitating the dye from an aqueous solution by mercuric chloride or potassium iodide. Details of this are described in appendix V. A saturated solution of precipitated dye was used and was allowed to remain in contact with the sections for 10 - 15 minutes. This stain produced marked metachromasia of the granules which enabled the mast cells to be easily distinguished from the surrounding cell nuclei.

Methods./

Methods.

Preparation and Fixation of Tissue.

Immediately after killing, the sheep stomach was dissected and, without any washing, specimens of tissue 10 x 3 mm. were placed in 50% methyl alcohol. They were then transported to the laboratory and fixation was carried out according to the following scheme.

Tissue was transferred to 70% methyl alcohol for one hour and then to 90% methyl alcohol for a further hour. Following that, it was subjected to three changes of absolute methyl alcohol, each for periods of $1\frac{1}{2}$ hours. Afterwards, the tissue was placed in chloroform and left overnight. At this stage, the tissue could safely be left in chloroform for periods of 2 - 3 days. Before embedding in paraffin wax, the tissue was transferred to a second change of chloroform for two hours.

Preparation and Staining of Sections.

Preparations 5 - 6 μ thick were cut on a standard Cambridge rocking microtome and mounted on microscope/

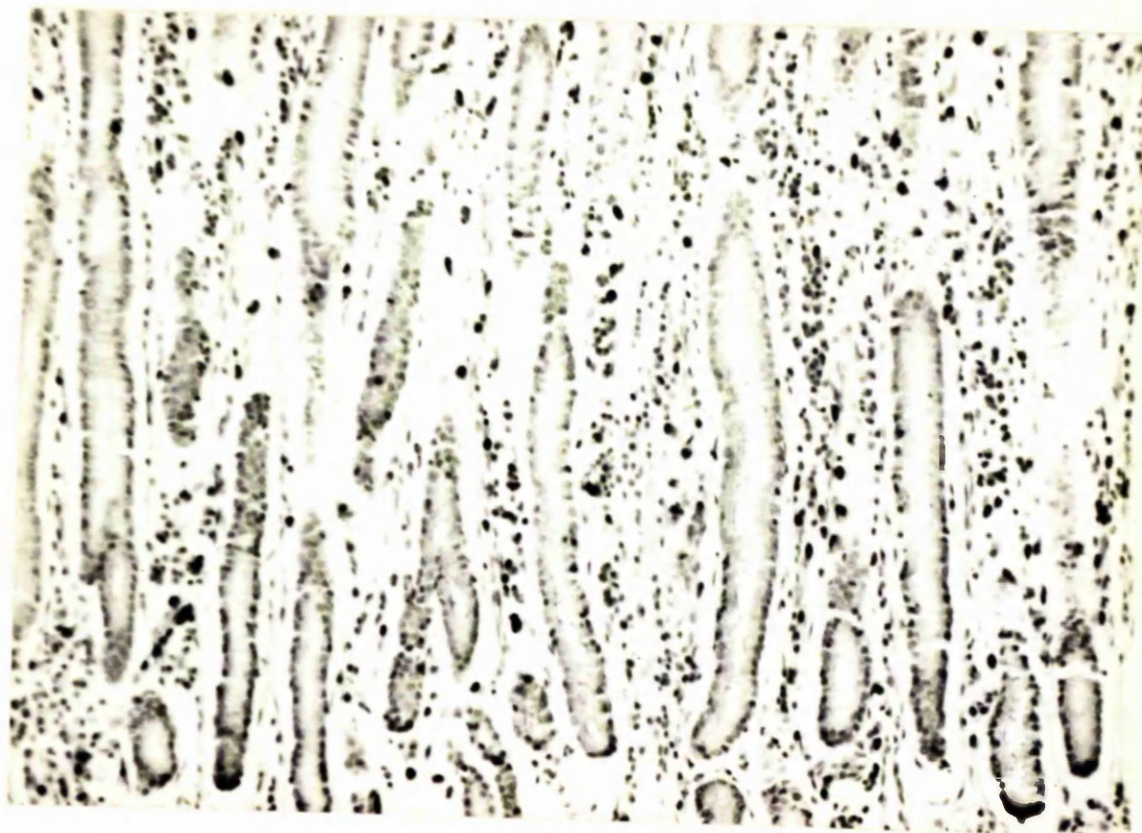
microscope slides. They were then transferred to xylol, then to absolute methyl alcohol and then to 90% methyl alcohol.

Sections were stained by placing in toluidine blue solution for 10 - 15 minutes, after which they were rinsed in tap water for approximately one minute. They were then rinsed in 90% methyl alcohol for 1 - 5 minutes until the blue colour started to fade. Rinsing in a second change of 90% methyl alcohol was continued until the blue colour was barely visible. Sections were then rinsed in two changes of absolute methyl alcohol and were blotted and transferred to xylol for 5 - 10 minutes. After rinsing in a second change of xylol, they were mounted in DPX mountant.

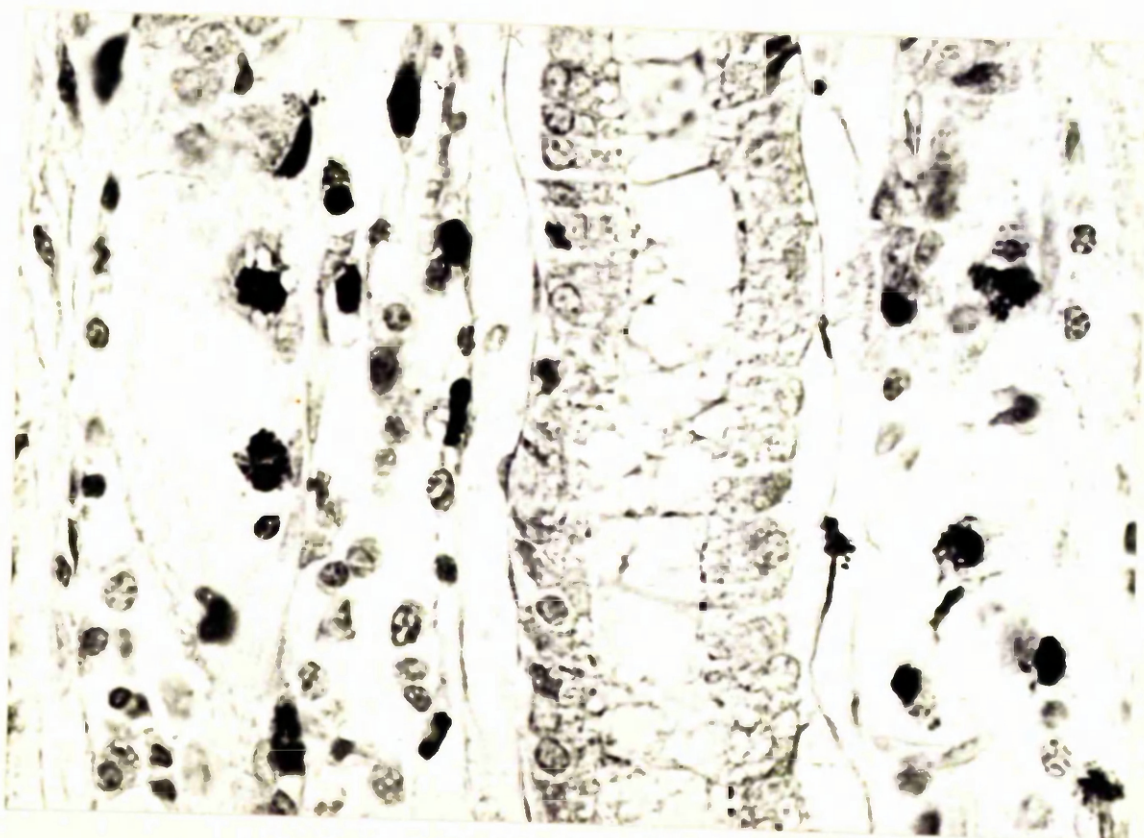
Technique of Cell Counting.

Sections were examined under a binocular microscope using a $\frac{1}{6}$ " (x 40) objective and x 8 eyepieces. Twenty-five to fifty fields were examined in each section, and a total of 150 fields were counted for each specimen of tissue. Only cells showing definite metachromasia were counted/

Fig. 50. Mast cells in abomasal mucosa stained with toluidine blue x 220.



X 800



counted, and cells which were out of focus with respect to the rest of the field were ignored. Cytoplasm in the cells of the deep mucosal glands of the pylorus also stained metachromatically with toluidine blue, but these cells were quite different in appearance and did not contain granules, so they could not be confused with mast cells. The typical appearance of mast cells in the sheep stomach is shown in fig.50.

Table XI

Mast cell counts per 50 microscope fields.

Results from 5 stomachs, three sections from each region.

<u>No. of stomach</u>	<u>Reticulum</u>	<u>Rumen</u>	<u>Omasum</u>	<u>Abomasum (Body)</u>	
				<u>Mucosa</u>	<u>Muscle</u>
1.	33	46	11	71	20
	31	35	41	42	15
	34	21	26	80	24
2.	13	12	34	80	24
	29	17	49	124	19
	14	16	16	98	25
3.	44	22	20	93	16
	41	31	10	50	42
	31	38	20	72	14
4.	8	16	30	258	31
	15	19	27	241	27
	11	17	25	278	20
5.	22	12	26	118	25
	27	22	22	93	18
	16	19	4	68	33
Mean	24.6	22.9	24.1	117.5	23.5

Results.

Mast Cell Content of the Four Compartments of the Sheep Stomach.

Tissues from eight sheep stomachs were used. Specimens from reticulum, rumen, omasum and abomasum were stained and examined for mast cells, and a comparative cell count was made of each compartment of five stomachs. No mast cells were observed in the epithelial layer in reticulum, rumen and omasum. In these compartments, they were found in the connective tissue, both of the sub-epithelial layer and of the smooth muscle. In general, they were more numerous in the connective tissue of projections, especially in the omasum.

Table XI is a summary of the cell counts obtained. Each figure represents the number of cells counted in 50 microscope fields. Inspection of the table shows that the greatest number of cells occurred in the mucosa of the abomasum, and this will be discussed in detail later.

There was some variation in the cell counts of sections from each specimen, but on the whole the/

the range of values found for each compartment was not very wide. In the reticulum, counts ranged from 8 - 44 with a mean of 24.6. In the rumen, the range was 12 - 46, and in the omasum it was 4 - 49. The mean cell counts for these were respectively 22.9 and 24.1. There were, therefore, no significant differences in the number of mast cells in these three compartments.

In the abomasum, cell counts were made of mucosa and muscle, and the table shows, as already pointed out, that the greatest number occurred in the mucosa in which the count ranged from 42 - 278, with a mean value of 117.5. On the other hand, the cell count of abomasal muscle closely resembled that found in the other compartments.

It is clear from the results that the population of mast cells in the abomasum is much greater than in the other compartments. Since the structure of the abomasum is more complex than that of the other compartments, it was thought desirable to make a more detailed study of the distribution of mast cells in the different tissues of this compartment. Cell counts were made of the mucosa/

Table XII. Distribution of Mast Cells in the Abomasum.

Mast cell counts per 50 microscope fields. Each figure is average to nearest whole number from 3 slides.
Results from seven stomachs.

<u>No. of stomach</u>	<u>Body</u>		<u>Pylorus</u>	
	<u>Mucosa</u>	<u>Sub-mucosa</u>	<u>Mucosa</u>	<u>Muscle</u>
1	64	33	105	8
2	101	77	37	14
3	72	46	51	16
4	258	61	43	6
5	182	40	79	8
6	131	31	56	4
7	249	110	204	7
Mean	151.0	56.9	82.1	8.3

mucosa, sub-mucosa and muscle layers.

The distribution of mast cells was investigated in the body and pylorus of the abomasum obtained from seven stomachs, and the results are shown in Table XII. It will be seen that the cell count of the body mucosa was greater than that of any of the other tissues, and ranged from 64 - 258, with a mean value of 151.0/50 fields.

There were fewer mast cells in the pyloric mucosa, the cell count of which ranged from 37 - 204, with a mean value of 82.1.

The sub-mucosa was rich in mast cells in both body and pyloric regions. In the body, the mean cell count was 56.9/50 fields, while in the pyloric region the mean value was 78.3/50 fields. In contrast, the muscle contained comparatively few mast cells. From these results, it is obvious that mast cells in both the body and pylorus are preferentially distributed in the mucosal and submucosal layers.

Histamine Profiles of Abomasal Mucosa.

As a result of the discovery of large numbers of mast cells in the abomasal mucosa it was considered worth-while to complete the examination of this tissue with a detailed survey of the distribution of its histamine and mast cells.

It was hoped, by this means, to reveal the presence of any non mast cell histamine as had been done by Riley and West (1956) in the pig.

Dog gastric mucosa was examined in detail by Feldberg and Harris (1953) using a technique for the estimation of histamine in serial sections of tissue. Frozen sections 10μ thick were cut parallel to the surface of the mucosa. Alternate sections were placed in tinfoil cups for extraction with HCl whilst the remainder were fixed in 10% formal for histological examination. The histamine content of individual sections was determined by biological assay and a profile was constructed to show variations in the distribution of histamine from the surface of the mucosa to the underlying smooth muscle. They found that the distribution/

distribution of histamine was not even and that definite "peaks" occurred. In the body of the stomach there were two peaks, one associated with the parietal cells and one with the muscularis mucosae. In the pyloric region, however, the profile showed only one peak corresponding to the zone of pyloric glands.

A similar technique was applied to hog pyloric mucosa by Riley and West (1956) as a means of detecting the location of non mast cell histamine. Frozen sections were cut and weighed and histamine was extracted by placing the sections in 10% trichloroacetic acid. Strips of tissue adjacent to the main block were fixed and stained to demonstrate mast cells. It was found that only in the submucosa was the high content of histamine paralleled by the density of mast cells. In the mucosa, however, there were peaks of histamine concentration but only few mast cells. The method used in the present investigation is essentially similar to that described by Riley and West (1956).

Method:

Blocks of tissue approximately 2 cm. square were cut from the wall of the sheep abomasum and stomach contents were removed from the mucosal surface by gentle blotting with filter paper. The tissue was mounted on the table of a Leitz microtome and was frozen hard in 30 - 60 seconds. It was then allowed to warm slightly before cutting was started so that sections did not fragment when cut. Overlapping mucosa was also trimmed from the edges of the block before cutting sections.

Sections 50 μ thick were cut, weighed on tinfoil cups and transferred to test-tubes each containing 1 ml. of N/3 HCl. The histamine content of each sample was then assayed by the method described for larger pieces of tissue except that grinding with sand was omitted.

Strips of tissue were also cut from the wall of the stomach at each side of the block and were fixed in 50% methyl alcohol. These were subsequently dehydrated and stained with toluidine blue.

Counts/

Counts of mast cells were made from 5 sections for each profile. As the mucosa was seldom more than three times the width of one microscope field seen under $\frac{1''}{6}$ objective, counts were made of $\frac{1}{2}$ fields with the division running parallel to the mucosal surface.

Table XIII

Histamine and mast cell profiles of mucosa from the body of the abomasum.

<u>No. of specimen</u> *	<u>Depth from Surface.mm.</u>	<u>Histamine content μg/100 mg.</u>	<u>Mast cells per 40 fields.</u>
1 (0.75)	0.0 - 0.1	3.08	
	0.1 - 0.2	4.25	101
	0.2 - 0.3	1.20	112
	0.3 - 0.4	2.33	
	0.4 - 0.5	2.13	122
	0.5 - 0.6	2.30	147
	0.6 - 0.7	3.30	
	0.7 - 0.8	2.50	148
2 (0.60)	0.0 - 0.1	0.83	
	0.1 - 0.2	2.63	117
	0.2 - 0.3	2.43	132
	0.3 - 0.4	3.08	
	0.4 - 0.5	0.70	108
	0.5 - 0.6	0.76	100
3 (0.80)			84
	0.0 - 0.1	1.80	
	0.1 - 0.2	2.80	87
	0.2 - 0.3	2.60	149
	0.3 - 0.4	2.40	
	0.4 - 0.5	2.80	117
	0.5 - 0.6	1.92	136
	0.6 - 0.7	2.05	
	0.7 - 0.8	1.72	123

*Average thickness of mucosa (mm) shown in brackets.

Table XIV

Histamine and mast cell profiles of mucosa from the pyloric region of the abomasum.

<u>No. of specimen*</u>	<u>Depth from Surface.mm.</u>	<u>Histamine content µg/100 mg.</u>	<u>Mast cells per 40 fields.</u>
4 (0.90)	0.0 - 0.1	2.33	127
	0.1 - 0.2	2.80	66
	0.2 - 0.3	7.60	
	0.3 - 0.4	3.67	
	0.4 - 0.5	3.33	74
	0.5 - 0.6	3.67	67
	0.6 - 0.7	3.26	
	0.7 - 0.8	2.69	89
	0.8 - 0.9	2.47	90
5 (0.55)	0.0 - 0.1	0.25	
	0.1 - 0.2	0.90	36
	0.2 - 0.3	3.00	24
	0.3 - 0.4	0.70	
	0.4 - 0.5	0.85	49
	0.5 - 0.6	0.70	59
6 (0.80)	0.0 - 0.1	3.68	205
	0.1 - 0.2	3.00	157
	0.2 - 0.3	2.93	
	0.3 - 0.4	3.27	
	0.4 - 0.5	5.67	124
	0.5 - 0.6	4.46	102
	0.6 - 0.7	5.60	
	0.7 - 0.8	3.54	93

*Average thickness of mucosa (mm) shown in brackets.

Fig. 51. Histamine and mast cell profile of abomasal mucosa.

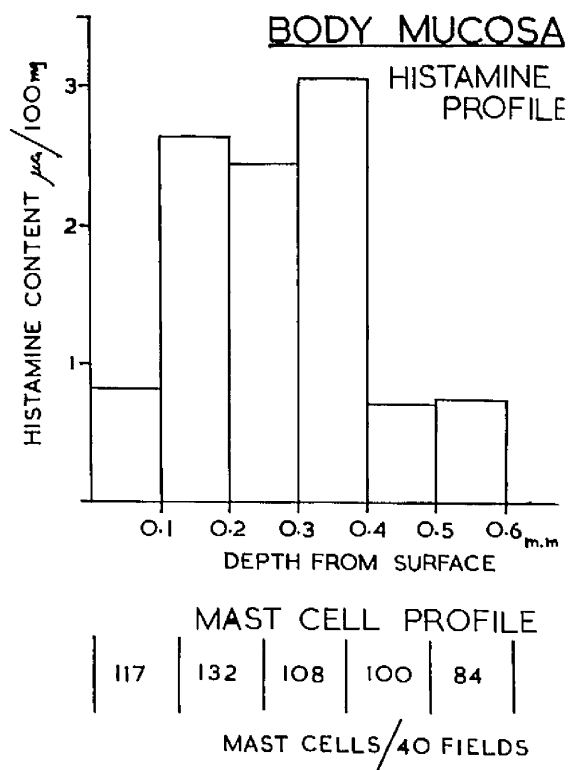
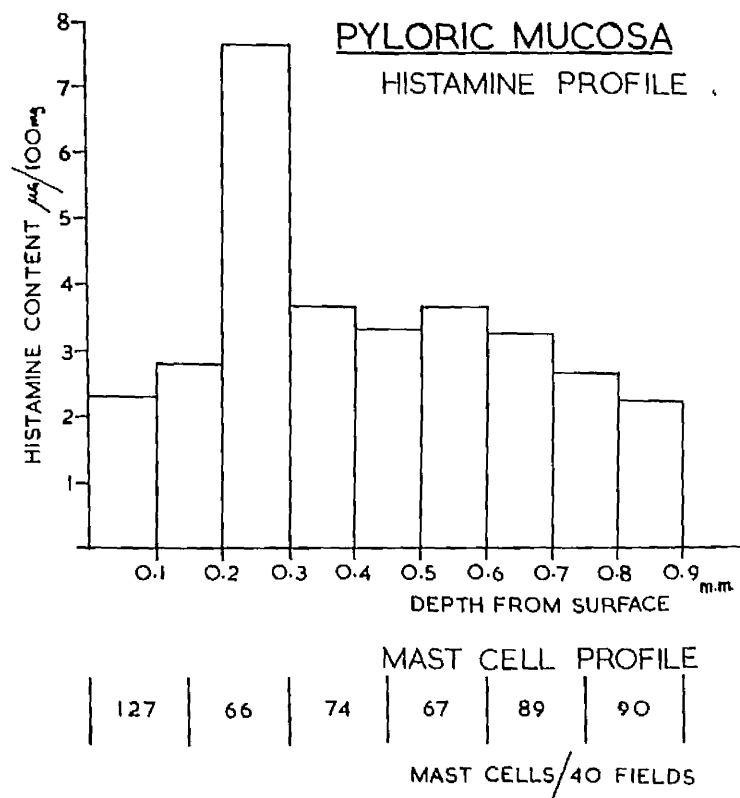


Fig. 52. Histamine and mast cell profile of abomasal mucosa.



Results.

Profiles were constructed for either the mucosa of the body or of the pylorus from six stomachs. The results are shown in Tables XIII and XIV and typical individual profiles are illustrated in figs. 51 and 52. In each profile of mucosa from the body of the abomasum there was a peak of histamine concentration just below the surface. In two profiles (nos. 1 and 2) there was also a second peak deeper in the tissue. Mast cell counts tended to vary with histamine content in profiles 2 and 3 but in profile 1 the high level of histamine just below the surface was not related to an increased number of mast cells.

In two profiles of pyloric mucosa (nos. 4 and 5) there was a marked peak of histamine concentration just below the surface of the mucosa. In the third profile a broader zone of high histamine content occurred deep in the mucosa. In none of these profiles was there a rise in mast cell counts corresponding to these peaks. It was also apparent that the ratio of histamine to mast cells was greater in the pyloric mucosa than in/

in the mucosa from the body of the abomasum.

It was concluded that there was clear evidence of non mast cell histamine in the pyloric mucosa. It was possible also that non mast cell histamine was present throughout the abomasal mucosa but the proportion would not be very high. In general, the large number of mast cells in the mucosa of both regions of the abomasum indicated that much of the histamine in this tissue was probably associated with mast cells.

Table XV Histamine and mast cells in 12 tissues from adult sheep.

Values are given for individual sheep. - = Not done.

Mast cell counts expressed as cells /50 fields.

0 - 20 cells = + 50 - 100 = +++
 20 - 50 " = ++ 100 - 200 = ++++

Tissue	Histamine content $\mu\text{g/g}$			Average mast cell population	
Lung	41	105	259	70	++++
Heart	1.0	0.6	-	-	0
Aorta	1.9	2.5	-	-	0
Diaphragm	0.3	0.7	-	-	+
Oesophagus	0.8	1.5	-	-	+
Duodenum	-	-	61	31	+++
Ileum (mid)	32	36	24	-	++
Colon	4.0	7.0	6.1	-	+
Liver	12	7.7	-	-	++
Spleen	2.7	2.0	-	-	+
Mammary gland.	-	-	5.0	39	++
Skin (abdominal)	1.4	2.4	1.2	2.4	+
(ear)	-	2.2	1.5	2.2	+

Histamine and Mast Cells in Other Tissues.

The results obtained in the detailed investigation of histamine and mast cells in the sheep stomach have shown that considerable amounts of histamine are present in abomasal mucosa. There was little available information, however, about the histamine content of other sheep tissues so a limited number of experiments were carried out to obtain some idea of the general distribution of histamine in the sheep.

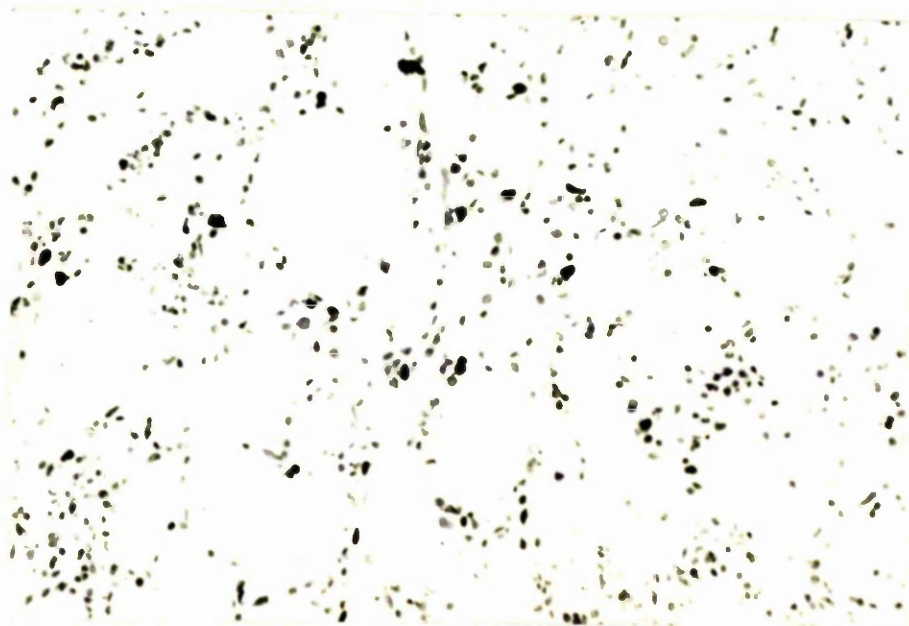
The histamine content of samples of tissue weighing 0.5 - 1.0 G was estimated by the method already described for the stomach. Samples of tissue were also fixed and stained with toluidine blue and mast cell counts were made. The tissues selected were mostly those previously studied in other species (Mongar & Schild 1952, Riley & West 1953, Feldberg 1956) so that comparisons could be made.

Results.

The histamine content of each tissue and an indication of its mast cell population is shown in Table XV. Lung was especially rich in histamine/

Fig. 53. Mast cells in sheep lung stained with toluidine blue.

X 220



X 800

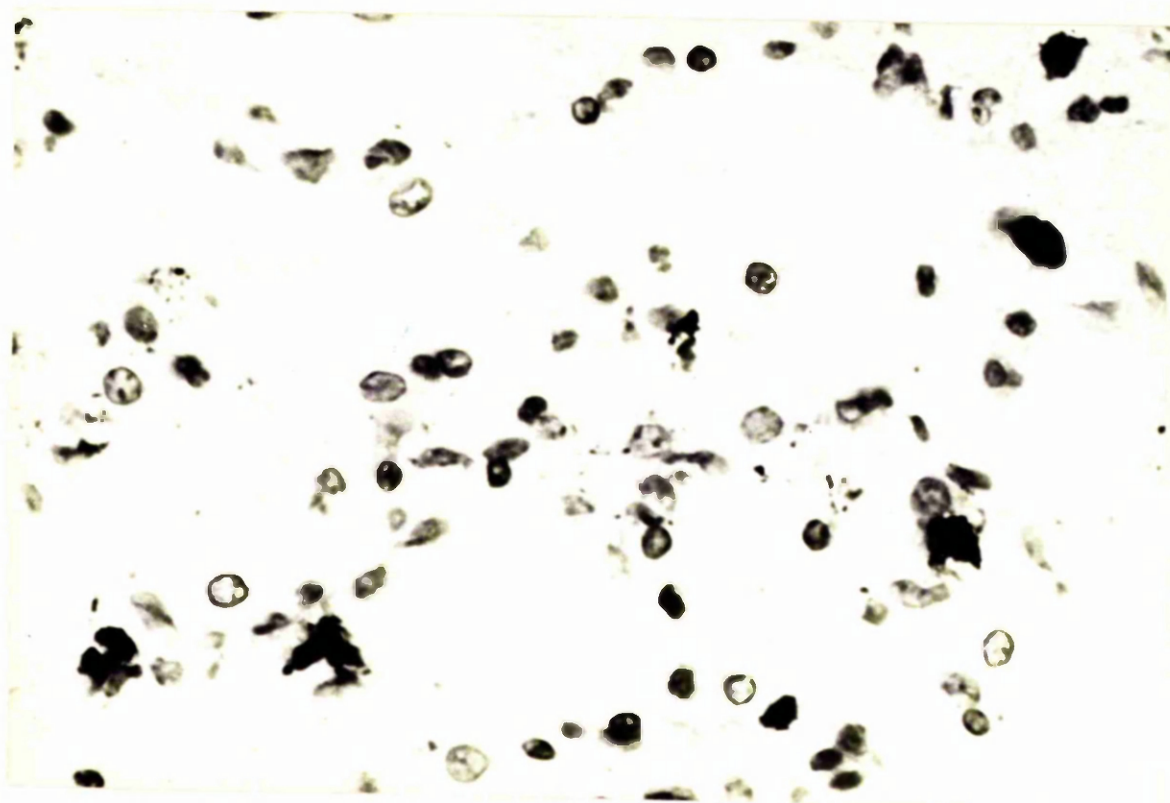
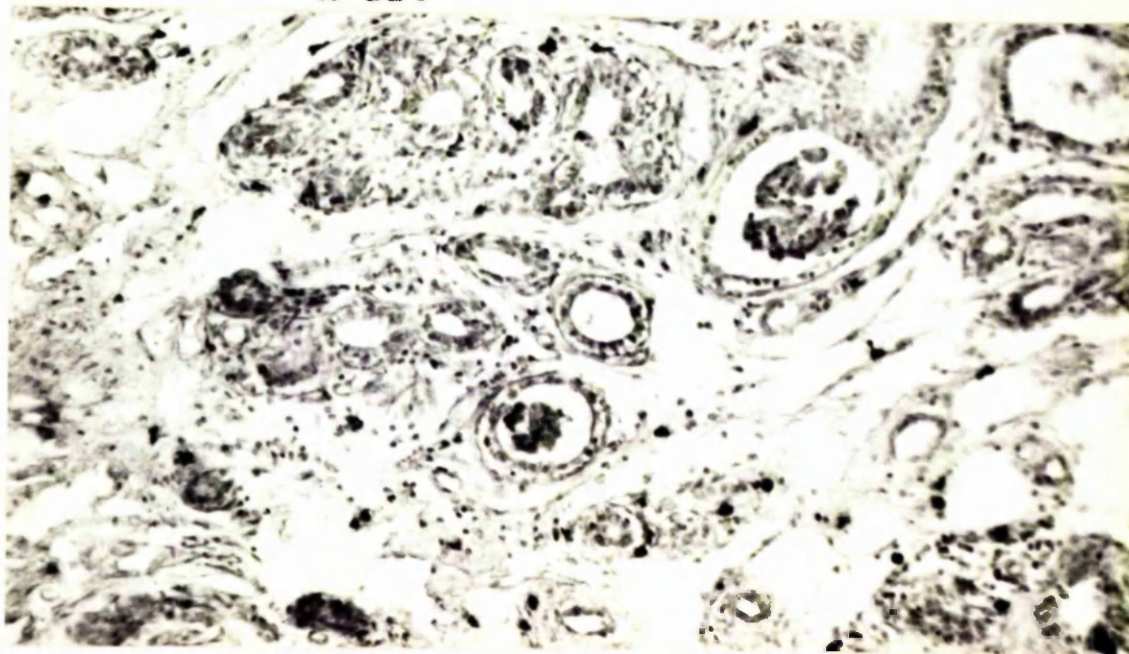
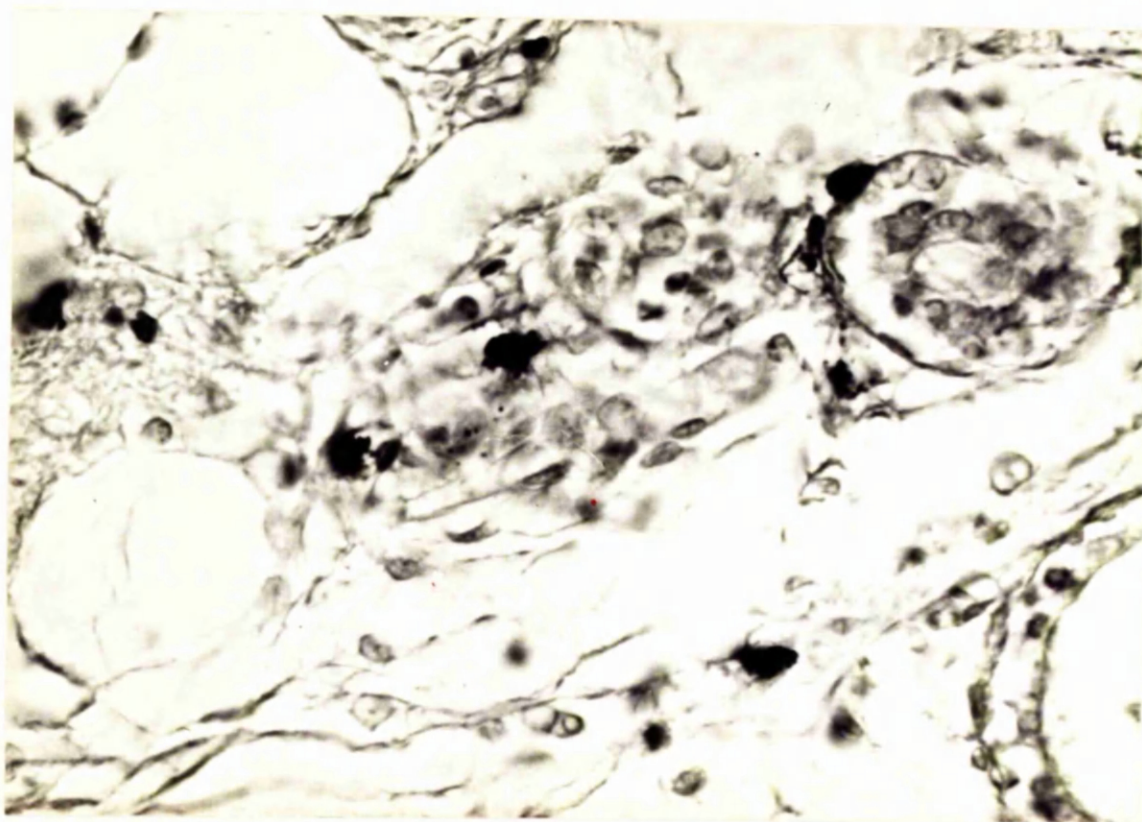


Fig. 54. Mast cells in sheep mammary gland stained with toluidine blue.

X 220



X 800



histamine having values ranging from 41 - 259 $\mu\text{g}/\text{G}$. Mammary gland and liver also had a high histamine content and both these tissues had correspondingly high mast cell counts although these were less than those obtained from sections of lung. Illustrations of mast cells in these tissues are shown in figs. 53 and 54.

Elsewhere in the body only the intestinal tract had a high histamine content and both histamine and mast cells became less towards the colon. Skin contained little histamine and there was no difference between samples from the abdomen and those taken from the ear. In all tissues, however, a correlation existed between the amount of histamine and the number of mast cells present.

Discussion.

The results of the experiments described in this part of the thesis show clearly that, of the four compartments of the sheep stomach, only the abomasum contains an appreciable quantity of histamine. Although wide variations have been found in the histamine content of this compartment, Feldberg (1956) has pointed out that this is not an uncommon finding in other studies of the histamine content of animal tissues. During the earlier stages of this investigation, when these variations were becoming obvious, the possibility was considered that they might be accounted for by a rapid loss of histamine from the tissues between death of the sheep and extraction of the tissue. In order to test this possibility strips of tissue were dissected, weighed and placed in acid as soon as the abdomen had been opened, whilst other strips were taken from the same stomach on arrival at the laboratory and treated in a similar manner. When the histamine content of the specimens in the two groups was estimated, no significant differences were/

were observed. It was concluded that any loss of histamine which may have occurred during transport of the stomach to the laboratory is negligible. A similar comparison was also made in respect of mast cells in specimens of stomach. Here it was found that those which were not fixed until arrival at the laboratory showed clear evidence of disruption of mast cells. It was evident, therefore, that any histamine released as a result of this disruption still remained in the specimens of tissue.

The mechanism by which histamine is stored in tissue is not fully understood. It has been observed, however, that the histamine content of tissues varies with the age of the animal. Riley and West (1953) have shown that as the age of an animal increases, so also does the histamine content of its tissues. There are, however, exceptions to this, as Rocha e Silva (1940) has reported a decrease in the histamine content of rabbit skin with increasing age. The sheep used in the present investigation were at least 12 months old, but no records were available as to their/

their date of birth or of history prior to slaughter. It is not possible to say whether the variations in histamine content already mentioned were due, even in part, to the ages of the sheep.

The finding that abomasal mucosa contained more histamine than any other tissue of the stomach was not unexpected, in view of the results of similar investigations which have been reported in other species. Gavin, McHenry and Wilson (1933), Douglas, Feldberg, Paton and Schachter (1951) and Feldberg and Harris (1953) have each shown that in the dog stomach most of the histamine was contained in the mucosa, and that the mucosal content of histamine was greater in the body than in the pylorus. Douglas et al. considered that in the dog stomach, the body mucosa contained approximately twice as much histamine as the pyloric mucosa, but it appears that, in the sheep, this regional difference in histamine content is less marked.

The experimental results have shown that the distribution/

distribution of histamine in the sheep stomach closely parallels the mast cell population of each region.

Mast cells were very sparse in the reticulum, rumen and omasum and tissue from these compartments contained very little histamine. In the abomasum, on the other hand, the histamine content was high and mast cells were plentiful. Although such a correlation might be expected for many tissues, the mast cell population of gastric mucosa has been found to vary widely according to the species examined. Mota et al. (1956) found large numbers of mast cells in the gastric mucosa of the dog, cat and guinea-pig but in the rat there were few of these cells in either the stomach or small intestine. There was, in fact, no correlation between histamine and mast cells in the gastro-intestinal tract of the rat. In the pig, Riley & West (1955) found large numbers of mast cells in the fundic mucosa but few cells in the pyloric region.

Although the histamine profiles of sheep gastric mucosa demonstrated that there was some non/

non mast cell histamine in the pyloric region, the ratio of histamine to mast cells in this tissue was not much greater than that of mucosa from the body of the abomasum (See Tables X and XII). Thus it may be concluded that most of the histamine stored in abomasal mucosa is associated with mast cells.

Amounts of histamine recovered from other tissues were of the order given for several species in the survey by Feldberg (1956). Although this author gave no figures for the sheep, he reported that both the lungs and the mucosa of the gastro-intestinal tract had a high histamine content in several species.

The histamine content of sheep liver (approx. 5 - 10 $\mu\text{g/G}$) was similar to the values given by Feldberg for cattle but was much less than the figures for the dog, rabbit and horse (up to 40 $\mu\text{g/G}$). The small amount of histamine recovered from sheep skin was rather surprising in view of the large amounts occurring in many other species. It was thought that skin from an extremity such as the ear might contain more histamine as had been/

been shown in the guinea pig by Feldberg and Miles (1953). It was found, however, that the histamine content of ear skin was no greater than that obtained from the abdomen.

The proportion of stored histamine released either by a chemical releaser or in an anaphylactic reaction has been shown to vary with both the tissue and species involved. Mongar & Schild (1952) found no correlation between the histamine content of guinea-pig tissues and the fraction which could be released. Of 13 tissues studied by these authors, the greatest percentage of histamine was released by the diaphragm but the greatest absolute quantity was obtained from the lung. The stomach yielded the smallest amount of histamine but this represented a larger fraction than that released from the small intestine.

In a similar investigation in rats, Feldberg & Talesnik (1953) found that compound 48/80 liberated little histamine from the stomach although it depleted skin and skeletal muscle. In the oat stomach also, compound 48/80 released only part of/

of the stored histamine (Smith 1953) and Mota et al. (1956) considered that this might be the proportion contained in the mast cells.

Since the quantity of endogenous histamine released by any tissue depends not only on the percentage released but also upon the original content, the availability of stored histamine in the sheep can only be determined by an examination of histamine release from specific tissues.

GENERAL SUMMARY AND CONCLUSIONS

Summary and Conclusions.

The normal cycle of contractions in the ruminant stomach depends on the propagation of stimuli which are transmitted from a localised region of the medulla oblongata, and which travel to the stomach through the vagus nerves. There is evidence to suggest that acetylcholine is released at the vagal nerve endings and causes contraction of the muscle. Stasis of the stomach may result when the transmission of these stimuli is blocked or when contraction of the muscle is inhibited.

A method has been developed for investigating the action of a number of drugs on specimens of each compartment of the sheep stomach. Preparations of isolated strips of muscle from each compartment were suspended in oxygenated Tyrode's solution at 37°C and the responses of these strips to acetylcholine, adrenaline, 5-hydroxytryptamine and histamine have been studied. It has been shown that acetylcholine contracted strips of all four/

four compartments, but there were no marked regional differences in sensitivity to this drug. The action of acetylcholine was antagonised by atropine and was potentiated by neostigmine.

The action of adrenaline varied according to the site from which the preparation was taken. Strips of rumen and abomasum were generally relaxed by low concentrations and contracted by high concentrations of adrenaline. Some strips of reticulum and omasum contracted in response to this drug whilst others were unaffected. Contractions of reticulum, rumen or omasum produced in response to acetylcholine were inhibited by the previous addition of adrenaline to the tissue bath.

5-hydroxytryptamine invariably contracted strips of reticulum, rumen and omasum. Strips of omasum, however, were usually insensitive to this drug. The action of 5-hydroxytryptamine was antagonised by lysergic acid diethylamide.

Histamine did not contract any of the preparations/

preparations of sheep stomach muscle, but, on the contrary, usually caused relaxation of strips of rumen and abomasum. Only strips showing spontaneous motility were relaxed by histamine, and preparations of abomasum were generally much more sensitive to histamine than preparations of rumen. Contractions of the abomasum in response to acetylcholine were inhibited by the previous addition of histamine to the tissue bath. Similar but less marked inhibition by histamine of contractions in response to acetylcholine was also observed with rumen strips. Histamine did not inhibit the action of acetylcholine on reticulum or omasum. Mepyramine maleate antagonised the action of histamine, but this occurred only when the antagonist was present in high concentrations. These results suggest that, in the sheep stomach, histamine has a direct relaxant and inhibitory action on abomasal smooth muscle.

Intravenous injections of histamine caused inhibition/

inhibition of movements of the reticulum, rumen and abomasum in conscious sheep. This has been demonstrated in sheep prepared with ruminal and abomasal fistulae in which stomach movements were transmitted via inflated balloons to water manometers or electronic transducers. The reticulum appeared to be somewhat less sensitive to histamine than the other compartments in that after a period of inhibition, contractions returned rapidly to their original amplitude. The rumen recovered more slowly and succeeding contractions gained gradually in force. The inhibitory action of histamine was readily prevented by mepyramine.

Evidence was obtained in an acute experiment to show that the action of histamine on the reticulum was indirect. Contractions of the reticulum in an anaesthetised sheep were produced by electrical stimulation of the peripheral end of the cut vagus nerve. These contractions were not affected by intravenous injections of histamine but/

but were reduced by an injection of atropine.

In addition to the effect on stomach movements, histamine given intravenously, increased secretions of the respiratory tract, affected respirations and caused defaecation. The effects of larger doses were examined in four sheep. The effects seen included, facial oedema, excessive salivation, great respiratory distress, and prostration. The action of these non-fatal doses was short-lived, however, indicating that, as in other species, histamine is rapidly cleared in the sheep.

The action of histamine on blood pressure and respirations was investigated in anaesthetised sheep. Intravenous injections of histamine caused a fall in blood pressure. With doses exceeding $2.0 \mu\text{g/Kg}$. this usually occurred in two stages with an intermediate period in which the blood pressure/

pressure rose to its previous level. Respirations were depressed in force and with larger doses, a period of apnoea sometimes occurred. Continuous intravenous infusion of histamine had only a slight depressant effect on the blood pressure.

Free histamine may originate in the sheep either by absorption from the alimentary tract or by release from the tissues. These two possibilities were discussed in Parts 3 and 4 of this thesis. Previous reports suggested that histamine might be formed in the rumen and absorbed directly from that compartment. Incubation of ruminal fluid with L-histidine did not result in any marked formation of histamine, nor was this enhanced by the addition of substances known to activate tissue histidine decarboxylase. Incubation of ruminal fluid containing 2% of added glucose at 37°C led to the formation of histamine after 2 - 3 days. This was accompanied by a fall in pH to 4.0. Histamine was not destroyed either by incubation with ruminal fluid for 3 hours or by addition to ruminal contents in a cannulated sheep/

sheep. In the latter case the histamine content of the ruminal fluid appeared actually to increase to reach a peak at $1\frac{1}{2}$ - 3 hours after dosing. It was not possible to account for this apparent increase.

Histamine put into the rumen had started to pass to the abomasum within one hour of dosing and the abomasal concentration of histamine reached a peak at the same time as that seen in ruminal contents.

Sheep dosed with histamine per cannula showed no response and it was concluded that little if any absorption of free histamine occurred in the rumen. Experiments were carried out in which samples of portal and peripheral venous blood were taken from anaesthetised sheep. Solutions of histamine were then put into the rumen but these produced no consistent effect on plasma histamine levels. This procedure did not effect blood pressure or respirations of the sheep.

The lack of response of sheep to high concentrations of histamine in the rumen did not support the/

the hypothesis that the formation of histamine in the rumen can, in itself, be a cause of disease. This does not, however, rule out the possibility that histamine might be associated with other factors, such as excess lactic acid formation or the multiplication of pathogenic organisms.

Secretion

A detailed investigation has been made of the distribution and content of histamine in the stomach. It was found that abomasal mucosa contained more histamine than any other tissue of the stomach, but there was no difference in the histamine content of each of the fore-stomach compartments.

A study was also made of the distribution of mast cells in each compartment, and evidence was found of a direct relation between the histamine content and mast cell count of each of the four compartments. In a more detailed examination of abomasal mucosa histamine and mast cell "profiles" were constructed. By this means evidence of non-mast cell histamine was obtained in the pyloric mucosa of the sheep. A correlation between histamine content and the distribution of mast cells has also been demonstrated in other sheep tissues.

APPENDICES

Appendix I

Electronic Recording Apparatus

Ink-on-paper records were made with an A.E.I. 4 channel pen recorder type E.P.R. Signals were fed to the recorder from a twin channel D.C. amplifier type R 2324 having a gain of X 300.

Pressure Transducers.

A Statham strain guage transducer was used in conjunction with an MR 501 pre-amplifier (Southern Instruments Ltd.). Although this was calibrated to record pressure changes in mm. of Hg. it was quite suitable for measuring pressures of 0 - 25 cm. water.

A second channel was provided by a capacitance micromanometer (Infra Red Development Ltd.) calibrated to register pressure changes of -20 to +20 cm. of water. It was not possible to record a standing pressure with this instrument and equal pressures were applied to both sides of the capacitance diaphragm at the start of each experiment. In spite of this the leak across the diaphragm/

diaphragm was slow taking several minutes to run from +20 cm. to zero so that contractions of the stomach were recorded without noticeable distortion when compared to the other recording channel.

Appendix II.

Recording of Blood Pressure and Respirations in Anaesthetised Sheep.

Apparatus.

Mercury manometer with float and writing pointer (Palmer Ltd.) was connected by pressure tubing to a glass cannula with side-arm. (Cannulae were made in the laboratory). The apparatus was filled with heparinised saline and a pressure of 120 mm. Hg was applied before connecting to the sheep.

A Brodie tambour with writing arm was connected by means of pressure tubing to the side arm of a T-piece at the end of the endotracheal tube.

Drugs and Reagents.

Heparin (Boots) 1000 units/ml.

Heparinised saline contained 100 units heparin/ml.

Histamine was dissolved in saline to give a concentration of 0.5 mg/ml.

Method of Cannulating Carotid Artery.

The carotid artery was exposed in the cervical region/

region and two ligatures of thread were applied separated by a distance of 5 cm. The cranial ligature was tied whilst the caudal was looped loosely round the artery. A Difenbach's clip was applied caudal to the loose ligature to produce temporary occlusion of the artery. An incision was then made in the wall of the artery and a cannula inserted and held in position by tying the caudal ligature.

During the course of an experiment it was sometimes necessary to clear small clots from the mouth of the cannula by injecting heparinised-saline through the side-arm.

Appendix IIIThe Estimation of Histamine in Plasma.

Based on the method described by Adam,
Hardwick & Spencer (1957).

Materials.

Glass columns 0.6 cm. int. dia. and 30 cm. long were constructed. The upper 10 cm. of the tube was widened to 2 cm. int. dia. to form a cylindrical bulb. A ground glass socket and cone (B 19) were fitted to the upper and lower ends respectively.

Amberlite C.G.50 chromatography resin, Type 1 (100 - 200 mesh). (B.D.H.)

This was treated to remove small particles by suspending 30 G in 1 L of distilled water. This was poured off after 10 minutes and the resin was re-suspended 6 times after which it was dried at 50°C for 24 hours.

Powdered Cellulose. (Whatman Chromatography Grade)

Phosphate buffer (pH 7.92) This was made from stock solutions and contained 53 ml. of 0.2M NaH_2PO_4 and 947 ml. of 0.2 M Na_2HPO_4 per litre. The mixture was adjusted to pH 7.92 and stored in a refrigerator. Before use, the buffer was diluted by adding 438 ml. to 542 ml. of distilled water.

6% trichloroacetic acid.

0.1 N hydrochloric acid.

0.1 N and 1.0 N sodium hydroxide.

0.01% neutral red solution.

Heparin (Boots) 1000 units/ml.

Methods.

1. Preparation of Columns. 300 mg. powdered cellulose and 50 mg. dry resin were mixed in a 25 ml. conical flask. 4.0 ml. 0.1 N NaOH was added accurately from a burette and the flasks were gently shaken to ensure thorough mixing. The mouth of each flask was then covered with aluminium foil and the flasks were allowed to stand overnight.

Each glass column was set up in a rack and fitted with a glass wool plug. A 100 ml. top funnel was fitted at the upper end and 4 ml. diluted buffer was poured onto the column. 10 ml. diluted buffer was added to the activated resin and the contents of the conical flask were poured into the column forming a composite column of resin and cellulose about 4 cm. in length.

Further quantities of buffer were then added slowly from the tap funnel until the fluid leaving the column had reached pH 7.92.

2. Preparation of Plasma Samples. Samples of plasma were taken either by siliconed syringes or via polythene cannulae and were run into 15 ml. nylon centrifuge tubes each containing 100 units heparin. Blood was centrifuged at 900 g for 10 mins and the plasma was then drawn off and re-centrifuged for 60 mins. at 2,200 g. 5 ml. plasma was placed in a 15 ml. siliconed centrifuge tube and 5 ml. 6% trichloroacetic acid was added. The mixture was allowed to stand for one hour and the precipitate was then removed by centrifugation at 2,200 g. for 30 mins. The yield of supernatant was then measured and invariably lay within the range 7.0 - 7.4 ml.

5 ml. of centrifuged supernatant was transferred to a 10 ml. stoppered graduated cylinder and one drop of neutral red indicator was added. The solution was neutralised with 1.0 N NaOH added from a 5 ml. burette and the end-point was adjusted as necessary with small quantities of 0.1 N HCl and 0.1 N NaOH. 1 ml. of phosphate buffer was then added and the volume made up to 10 ml. with distilled water.

3. Absorption and Elution. The prepared sample was poured into the tap funnel and was allowed to run onto the column at a rate of 0.3 ml/min. The sample was followed by 5 ml. distilled water and the column was allowed to run dry. Preliminary experiments showed that, under these conditions, histamine would be entirely retained by the composite column.

Columns were eluted with 0.1 N HCl. The method of elution differed slightly from that used by Adam and his colleagues in that a larger amount of eluate was collected. Preliminary experiments showed that only part of the histamine was eluted by the 2 ml. of HCl and 1.5 ml. of distilled water recommended in their paper. The modification was determined by collecting eluate from histamine-containing columns in several fractions and determining how the histamine was distributed between them. It was found that with 3 x 4 ml. fractions all the histamine was in the second fraction. It was also found that histamine left the column when the pH of the eluate fell to 4.5. In all subsequent experiments 4 ml. of solution was collected starting when the pH of the eluate reached 4.5.

4. Assay. Samples were neutralised with 1 N NaOH and were diluted and assayed on the guinea-pig ileum preparation using the automatic assay apparatus (Boura, Mongar & Schild, 1954). As the concentrations of histamine recovered were seldom less than $0.025 \mu\text{g/ml}$. there was no need to resort to a more sensitive method of assay. When samples were assayed at dilutions less than X 5 a mixture of salts was added to provide a similar composition to that of Tyrode's solution. Details of the amounts used are given below:

Solution A. 0.05 ml. added /ml. of eluate.

" B. 0.1 ml. " " " "

" C. 0.05 ml. " " " "

Solution A contains 80 G/L NaCl

" B " 1.2 G/L KCl

1.2 G/L CaCl_2

60 mg/L MgCl_2

0.3 G/L NaH_2PO_4

Solution C contains 20 G/L NaHCO_3 .

Table XVI

Recovery of Histamine from Blank Solutions.

<u>Details of Sample.</u>	<u>Total histamine content μg</u>	<u>Histamine recovered μg.</u>	<u>% recovery</u>
22.1.62 A	0.25	0.237	95.0
B	0.50	0.514	103.0
7.2.62 A	0.25	0.203	81.2
B	0.25	0.238	95.2
C	0.25	0.216	86.4
D	0.25	0.369	147.6
15.2.62 A	0.25	0.245	98.0
B	0.25	0.279	111.6
C	0.25	0.290	116.0
20.3.62 A	0.25	0.228	91.2
B	0.25	0.337	135.0
C	0.125	0.170	136.0
D	0.125	0.122	81.0
31.5.62 A	0.50	0.484	97.8
B	0.50	0.490	98.0

Mean recovery = $104.9\% \pm 5.1 \text{ S.E.}$

Table XVII

Recovery of Histamine Added to Plasma.

<u>Details of Sample</u>	<u>Amount added μg.</u>	<u>Amount re-covered μg.</u>	<u>% Recovery</u>
15.11.61 A	0.50	0.252	50.4
B	0.25	0.240	96.0
C	0.25	0.105	42.1
26.4.62 A	0.50	0.264	52.9
B	0.50	0.307	61.4
C	0.50	0.222	44.5
28.4.62 A	0.20	0.180	90.0
B	0.20	0.269	134.5

Mean recovery = $71.4\% \pm 11.5$ S.E.

Table XVIII

Histamine Content of Sheep Plasma.

No. of Sheep	Plasma histamine μ g/ml
S5	0.092
S21	0.065
S12	0.055
S15	0.027
S16 (1st)	0.090
S16 (2nd)	0.063

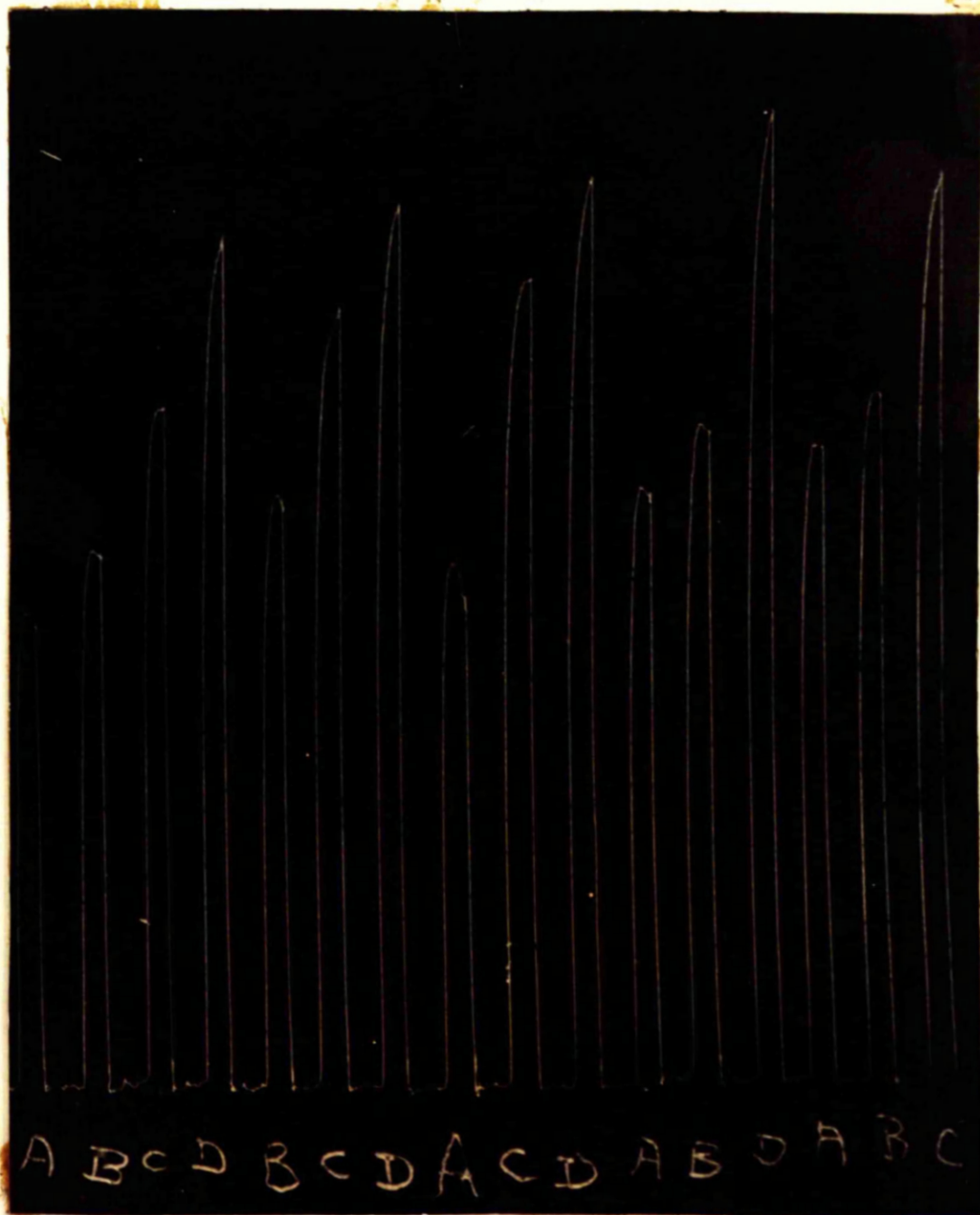
Results.

1. Recovery of histamine from blank solutions. The efficiency of the method was tested using solutions of histamine in Tyrode solution in place of plasma samples. These were extracted and estimated and results for 15 samples, containing 0.125 - 0.5 μ g. of added histamine are shown in Table XVI. The mean recovery was $104.9\% \pm 5.1$ S.E.
2. Recovery of histamine added to plasma. The recovery of histamine added to plasma was determined in 8 samples and the results are shown in Table XVII. The mean recovery was $71.4\% \pm 11.5$ S.E. It was clear from these results that not all the added histamine would be recovered from plasma but the mean recovery was not greatly different from that obtained by Adam et al. (80%). No correction has been made for this loss in subsequent estimations.
3. Histamine Content of sheep plasma. The histamine content of duplicate samples of plasma was estimated in 5 normal sheep and the results are shown in Table XVII. Values obtained ranged from 0.027 - 0.092 μ g/ml. and there was close agreement between duplicate samples from the same animal.

Fig. 55. Four-point assay of extract of abomasal muscle.

A = 0.04 ml. extract. B = 0.08 ml. standard.
C = 0.06 ml. extract. D = 0.12 ml. standard.

Standard solution contained 0.2 μ g/ml.
histamine base.



Appendix IVAssay of Tissue Extracts for Histamine.

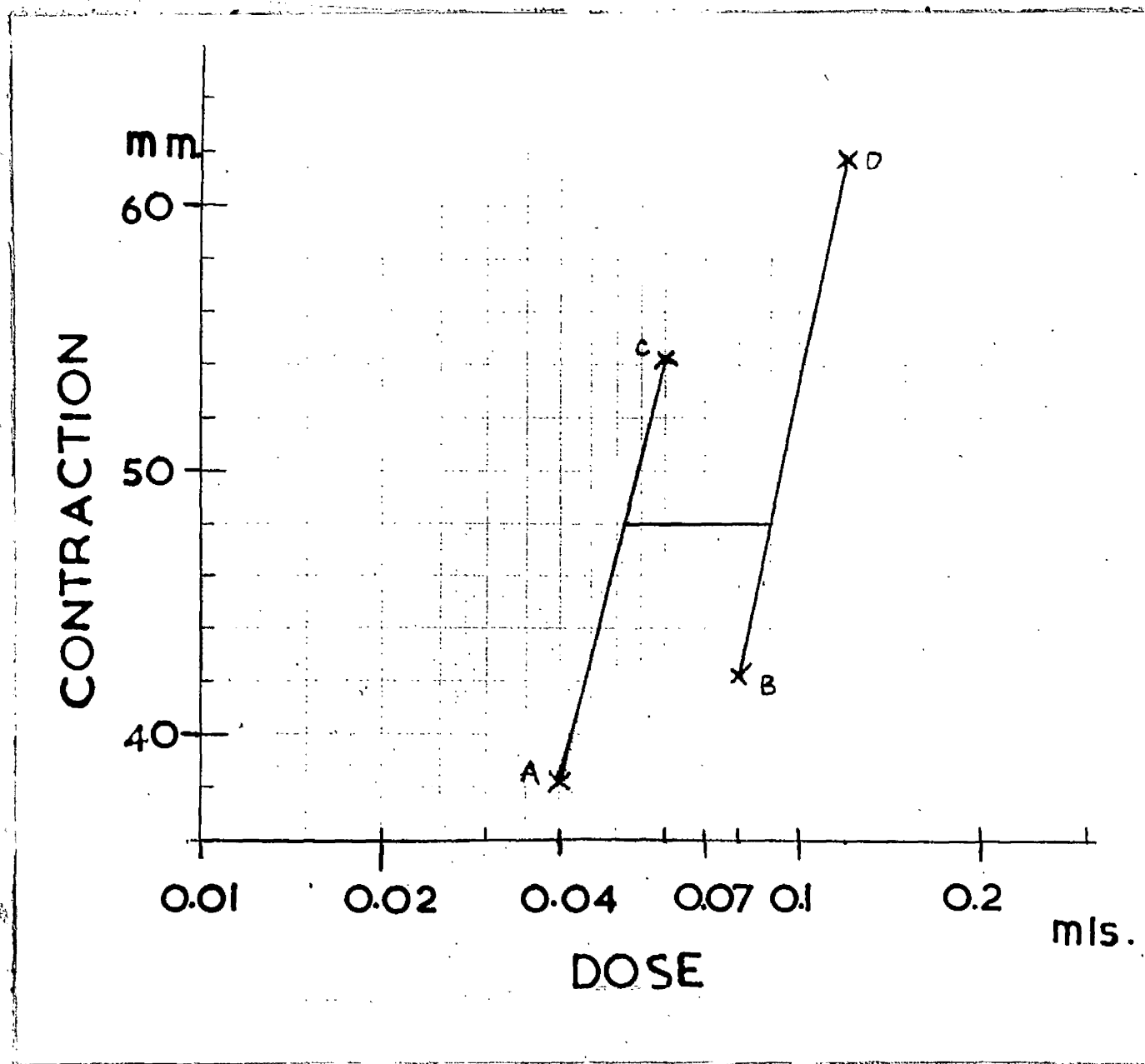
A guinea pig was killed by a blow on the head, the abdomen opened and the distal 10 cm. of ileum removed and placed in Tyrode's solution. A segment approximately 2 cm. long was cut and the intestinal contents washed out by means of a Pasteur pipette. The isolated segment was then suspended in oxygenated Tyrode's solution at 37°C in a tissue bath of 10 ml. capacity. The preparation was connected by means of a thread to an isotonic writing lever, movements of which were recorded on a rotating smoked drum.

Using this preparation, a four-point assay was carried out as shown in Figure 55. Doses of each solution were selected to produce approximately equal contractions of the muscle, and two doses each of standard solution and extract were used.

The sixteen contractions were then measured and the mean contraction of the ileum for each dose was/

Fig. 56. Result of four-point assay expressed graphically.

Points A, B, C and D represent mean heights of contraction for doses shown in Fig. 39.



was calculated. These results were plotted graphically as shown in figure 56, and a direct comparison of the potency of each solution was made.

In many experiments in which an accurate estimation of histamine content was unnecessary the four point assay was replaced by a simple 3 point assay using 2 doses of the unknown solution. This method was much quicker and a test carried out on 6 unknown solutions of histamine showed that the results were accurate to within 10%.

Calculation

Standard solution of histamine contained $0.2 \mu\text{g/ml}$. histamine base. From figure 56.

0.09 ml. histamine solution 0.0515 ml. extract,
and 1 ml. histamine solution 0.572 ml. extract of
abomasal muscle.

1 ml. histamine solution contains $0.2 \mu\text{g}$ histamine base, and 0.572 ml. extract contains $0.2 \mu\text{g}$ histamine base.

There was a total quantity of 15 ml. of extract, and 15 ml. extract contain $\frac{0.2 \times 15}{0.572}$

$$= \underline{5.24 \text{ g histamine base.}}$$

5.24 g histamine was obtained from 630 mg. of tissue

$$\begin{aligned} \text{and histamine content} &= \frac{5.24 \times 1000}{630} \\ &= \underline{8.317 \text{ g/G of tissue.}} \end{aligned}$$

Appendix 5.Preparation of Improved Toluidine Blue (Robinson & Baesich 1958).

A 1% aqueous solution of toluidine blue is prepared and filtered. 4 - 5 ml. of saturated potassium iodide solution is then added drop by drop, and a dark precipitate forms. This is collected by filtration and is washed repeatedly with small quantities of distilled water applied to both sides of the filter paper until the drippings are distinctly blue.

The filter paper is then allowed to dry at room temperature and the precipitate can then be scraped off and stored until required. To prepare the stain, 0.3 G of precipitate are added to 100 ml. of distilled water. This gives a saturated solution which stains most sections satisfactorily in 10 - 15 minutes.

Appendix VIProtocols of Experimental Sheep.

All cannulated sheep were half-bred ewes, at least 3 years old, weighing 44.5 - 87 Kg. Three were fitted with both ruminal and abomasal cannulae although the latter were successful in only 2 of these animals.

The period of survival following cannulation varied from 3 weeks to more than 9 months. Two sheep were found dead, and four others were destroyed for the following reasons:-

Pneumonia.
Chronic subcut. abscess in region of
ruminal cannula.
Broken abomasal cannula.
Abomasal cannulation failed due to wound
breakdown.

The three remaining sheep are still in use at the time of writing. Typical protocols for 2 sheep are given below:-

S2 Half-bred ewe. Weight 60 Kg.

15.1.61. Anaesthesia induced with 25 ml. 6% pento-
barbitone I.V. and maintained with cyclo-
propane-oxygen. Rumen adhesion prepared.
3 ml/

- 3 ml. procaine penicillin suspension I.M.
- 16.1.61. Eating food. 3 ml. procaine penicillin suspension I.M.
- 17.1.61. Eating food. 3 ml. procaine penicillin suspension I.M.
- 25.1.61. Sutures removed.
- 2.2.61. Anaesthesia as above. Adhesion incised and rumen cannula inserted. 4 ml. procaine penicillin suspension I.M.
- 3.2.61. Eating food. 3 ml; procaine penicillin suspension I.M.
- 4.2.61. Eating food. 3 ml. procaine penicillin suspension I.M.
- 17.2.61 - 27.4.61. Used approximately once weekly for recording stomach movements.
- 28.4.61. Lamé on L. hind leg. Lower end of leg was swollen and sheep was not eating. Foot was examined and hoof trimmed but there was no evidence of foot-rot. 5 ml. procaine penicillin suspension I.M.
- 29.4.61. Took some food but lameness unchanged. 5 ml. procaine penicillin repeated.
- 30.4.61. 5 ml. procaine penicillin I.M. No change.
- 1.5.61. 5 ml. procaine penicillin I.M. Ate about 50% of concentrates.
- 3.5.61/

- 3.5.61. Much less lame. Eating normally.
Subsequently recovered and used for recording of stomach movements until 19.7.61.
- 28.7.61. Not eating. Subcut. swelling developed ventral to ruminal cannula.
- 30.7.61. Abscess opened ventral to cannula.
Cheesy purulent material removed. Cavity ran ventrally for 5 - 10 cm. Opening enlarged, clean with cetrimide solution and filled with penicillin ointment.
- 1.8.61 - 30.8.61. The lesion was cleaned every two days and penicillin ointment applied.
There was constant contamination from leakage round the cannula and the tissue round the cavity became necrotic.
- 5.9.61. It was decided that the lesion was unlikely to heal well within a reasonable space of time and the sheep was destroyed.
No cause for this lesion was discovered but it may have originated from an infected suture. The abscess cavity was entirely subcutaneous and did not communicate with the rumen or abdominal cavity although the body wall eventually became very much reduced in thickness in this region.

S8 Half-bred ewe. Weight 44.5 Kg.

- 23.3.62. Anaesthesia induced with 20 ml. 6% pentobarbitone I.V. and maintained with cyclopropane-oxygen. Rumen adhesion prepared.
- 24.3.62. - 26.3.62. Uneventful recovery. 5 ml. procain penicillin daily.
- 30.3.62. Adhesion sutures removed.
- 17.4.62. Anaesthetised as described above. Rumen adhesion incised and rumen cannula inserted. 5 ml. procaine penicillin I.M.
- 18.4.62 - 20.4.62. Uneventful recovery. 5 ml. procaine penicillin daily.
- 14.5.62. Vaccinated with 2 ml. "Ovilin" subcut.
- 1.5.62 - 22.5.62. Samples of ruminal fluid withdrawn for incubation experiments.
- 23.5.62. Anaesthetised as described above. Laparotomy carried out and abomasal cannula inserted. 5 ml. procaine penicillin I.M.
- 24.5.62. Eating a little hay. 5 ml. procaine penicillin I.M.
- 25.5.62. Abdominal ring of cannula was unscrewed slightly. 5 ml. procaine penicillin I.M.
- 26.5.62/

- 26.5.62. Eating hay and some concentrates. 5 ml. procaine penicillin I.M.
- 15.6.62 - 28.6.62. Used twice weekly for recording of ruminal and abomasal movements.
- 29.6.62. Retaining plate of rumen cannula broken and cannula partially split. This was removed and new cannula fitted without difficulty under local infiltration anaesthesia with 2% lignocaine.
- 30.6.62. Retaining plate missing. It was noted that the sheep had apparently learned to rub the cannula against projection in its pen. The skin round the cannula was examined but there was no sign of local irritation. The sheep was removed to another pen without projections and no further trouble was experienced.
- 1.7.62 - 1.9.62. Used for experimental procedures.
- 25.8.62. It was noticed that there was no flow of fluid when the cap was removed from the abomasal cannula. Access to the abomasum was readily obtained, however, by passing a rubber tube up the cannula to lift a fold of mucosa which covered the upper end.
- N.B. This sheep is alive and in use at the time of writing.

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