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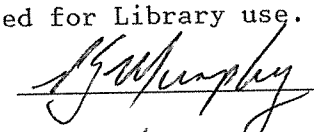
Signed 

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AN INVESTIGATION INTO THE STRUCTURE AND FUNCTION OF THE ILEOCAECAL JUNCTION OF THE SHEEP

A Thesis presented in fulfilment of
the requirements for the degree of
MASTER OF SCIENCE
by thesis only
at Massey University.

Peter Grant Murphy
B.Sc. Dip. Sci.
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Abstract

The sheep ileocaecal junction has been shown to have sphincter-like properties, fulfilling the criteria for a gastrointestinal sphincter identified by Fisher and Cohen (1973). However the short zone of elevated pressure within the Ileocaecal Junction (ICJ) could be due to venous engorgement rather than tonic muscular activity, as the tip of the ileum examined histologically reveals an irregular musculature interspersed with a vascular loose connective tissue. A similarity is suggested to the human ileocaecal sphincter as described by Quigley, Borody, Phillips, Weinbeck, Tucker and Haddad (1984). This short (less than 5mm) zone of elevated pressure in the sheep would appear to work in conjunction with a valvular action as described by Kuman and Phillips (1987) for the human ICJ, preventing retrograde digesta flow from the caecum to the ileum.

EMG recordings of the ileocaecal region in the conscious sheep showed approximately 70-80% of the MMC phase 3 activity that reaches the distal ileum progresses as far as the ICJ. This appears to be the main motility pattern present in this region in the conscious sheep, and very little coordination was observed between patterns of caecal and ileal activity.

Feeding was found to decrease the amount of MMC activity in the distal ileum by lengthening the interval between successive MMC phase 3 patterns of motility. This effect of feeding on MMC activity appears not to be due to the levels of circulating gastrin, and could be due to either a reflex inhibition such as an intestino-intestinal reflex mediated by noradrenaline as has been described for cats, or humoral mechanisms involving another agent such as cholecystekinin, VIP or Substance P. Both the latter substances were shown to be present in nervous tissue within the ileum, though their role remains unclear.

Preface

The digestive tract of ruminants has long been of interest to biologists, chiefly due to the modified stomach or rumen (Hofmann, 1973). Studies of the rumen have resulted in many publications describing its function and physiology in detail (Kay, 1983).

There has been much less interest in the small intestine of the ruminant, probably because this part of the ruminant resembles that found in other mammals (Wyburn, 1981). The junction between the ileum and colon, in particular, is the least explored part of the gastrointestinal tract of any species, even though it represents an important transition zone (Quigley, Borody, Phillips, Weinbeck, Tucker and Haddad, 1984). The ileocaecal junction separates the abundant caecal flora from the ileum, and the failure of this function results in well documented pathological conditions (Gorbach, Plant, Nahas, Weinstein, Spanknebel and Levitan, 1967). In the sheep, activity in the distal small intestine and large intestine has been examined by means of electromyographic recordings (Fioramonti, 1981), and radiographically (Reid and Dellow, 1972). Both these studies were primarily concerned with large intestine activity, and gave only brief reference to the sheep ileocaecal junction. Current understanding of the function of the ileocaecal junction is due mainly to studies in dogs (Kelly, Gordon and DeWeese, 1966; Kruis, Azpiroz and Phillips, 1985), cats (Pahlin, 1975; Conklin and Christensen, 1975) and Humans (Quigley, Phillips and Dent, 1984; Quigley, Borody, Phillips, Weinbeck, Tucker and Haddad, 1984.)

These species are monogastric, and small intestine motility and digesta flow in such species differs from that seen in the ruminant. Regular, cyclic activity in the small intestine is only seen between meals in most monogastric species, but occurs continuously in the sheep, irrespective of feeding (Ruckebusche, 1970; Bueno, Fioramonti and Ruckebusche, 1975). Such a difference in small intestine activity could indicate differences in function. Code and Schlegel (1974) suggested the interdigestive activity of the

small intestine gave it a `housekeeper' function, preventing bacterial overgrowth between meals. In species such as sheep, which show this type of activity constantly, this regular pattern of activity is responsible for the transit of digesta through the small intestine to the caecum. With digesta being propelled through the small intestine by different motility patterns in different species, it would not be prudent to extrapolate results from experiments on the small intestine of monogastric species to ruminants such as sheep. The ileocaecal junction (ICJ) of the sheep, about which very little has been published, warrants further investigation.

Acknowledgements

The development of this thesis has been a protracted affair which began even before its formal initiation in 1987, when my supervisor, Dr. Dave Carr made a remark about the lack of published material on this topic. In launching into this part-time project, I had little idea that I had embarked on an undertaking that would lead down many interesting paths and take until 1992 to complete! Many thanks to Dr Carr for his always supportive comments and patience. Thanks too to Dr. Gordon Reynolds for his help, particularly during Dr Carr's sabbatical absence; Dr Heather Simpson for having a gastrin assay running, and John Elgar and Jane Candy for running my samples through the assay. In the histology lab. I would have been lost without Roy Sparksman, a fount of wisdom and good humour, to whom I owe whatever expertise I have developed in histology.

I also would like to thank my wife Heather, and family: Andrew, Simon and Nicola. It can't have been much fun with Dad always juggling their needs with work commitments and THE THESIS. Thanks - it's done.

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CHAPTER 1
Literature Review

1.1 The Ileocaecal Junction - Valve or Sphincter?

Pahlin (1975) attributes the first anatomical description of the ileocaecal region to Variolus, writing in 1573, although Bauhin in 1579 was the first to propose a valve-like function to the terminal part of the ileum. This valve action was thought to prevent the backflow of digesta from the caecum to the ileum, since it prevented the retrograde flow of water from the colon to the ileum in dead animals.

Much later, in 1904, Elliot reported that the ileocaecal junction (ICJ) in the cat lost its ability to separate ileal and caecal contents after it had been denervated. This suggested a muscular action, implying sphincter-like qualities within the terminal ileum since denervation led to a loss in muscle tone and thus incompetence. This raised a question as to whether the junction between the ileum and the caecum would be more appropriately described as a valve or as a sphincter. Certainly the description of the region in humans (Elliot, 1904), cats (Rosenberg and Dio Dio, 1969) and sheep (Fioramonti, 1981), as a protrusion of the terminal ileum into the colon, surrounded by colonic wall, would support the impression of a valve - like function (Figure 1.1).

A recent report from Kuman and Phillips (1987) further supports this concept. Using postmortem human specimens, Kuman and Phillips found that caeco-ileal reflux was prevented as long as the fibrous tissues responsible for the angulation between the ileum and the caecum were intact. They described these fibrous structures as the superior and inferior ileocaecal ligaments. ICJ valvular competency was maintained in all specimens up to 40

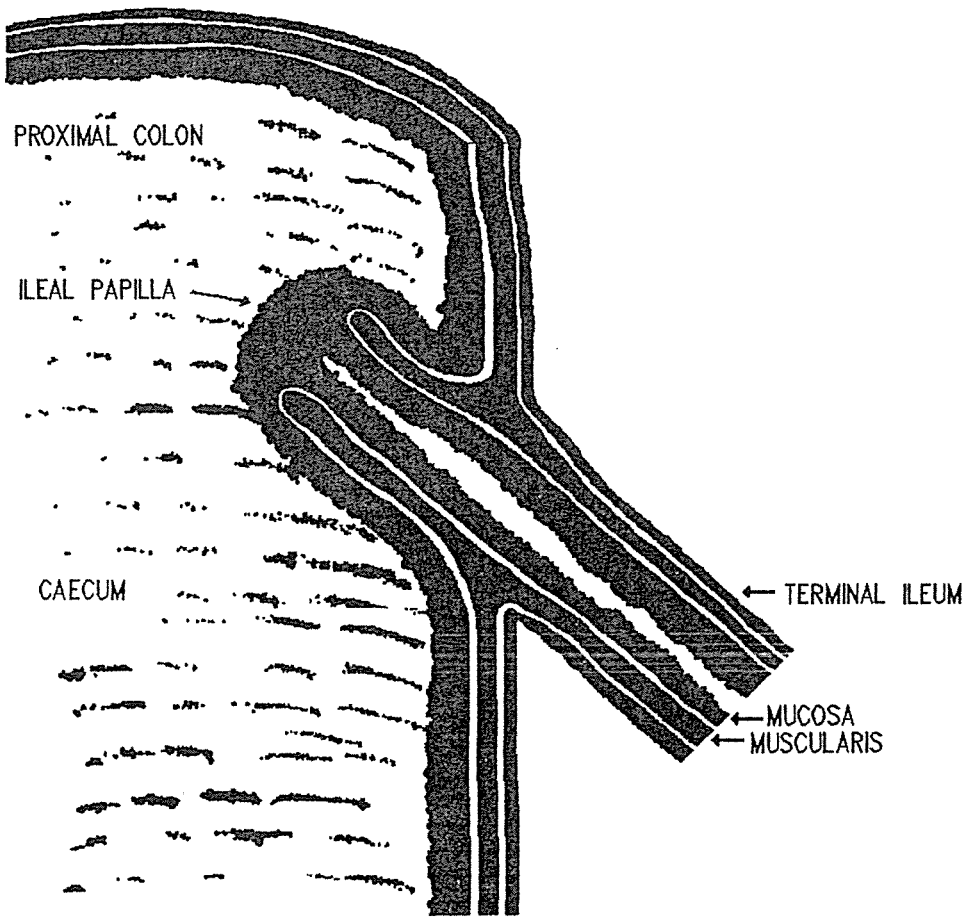


FIGURE 1.1 Schematic diagram of a section through the sheep Ileocaecal Junction.

mm Hg, and in most (10 of 12), 80 mm Hg with the ileocaecal ligaments intact. Cutting the ligaments rendered the junction incompetent, but surgical reconstruction of the ileocaecal angle restored competence.

Those species with an ileocaecal junction anatomically similar to humans, such as cats and sheep, could use the valve-like characteristics of the junction to prevent reflux of caecal contents into the ileum.

This valvular theory is not supported by the findings of Elliot, or by Rendleman, Antony, Davis, Buenger, Brooks and Beattie (1958), who reported that the ileal reflux of caecal contents increased with increasing intracaecal pressure in dogs and humans. This observation, supports the view that the ileocaecal junction has a sphincter - like action rather than a valvular action which would close more positively with an increase in pressure, until very high pressures were reached. Although this observation is at variance with Kuman and Phillips with respect to humans, in the dog the ileum is continuous with the proximal colon while the caecum protrudes as a blind sac from the side of this tube (see Figure 1.2). Such a structure would suggest sphincter action would be needed to separate colonic and ileal contents, and does not suggest a valvular action. Using direct observation in the dog, Hinrichsen and Ivy (1931) described a contracted ring around the ileocolic junction which remained closed except when the ileum was discharging its contents. This evidence is strongly supportive of a sphincter action.

Besides the ability to prevent retrograde digesta flow through either sphincter or valvular action (or a combination of both), the distal ileum has been shown in some species to use distinct patterns of motility to assist in this function. Kruis et al (1985) reported in

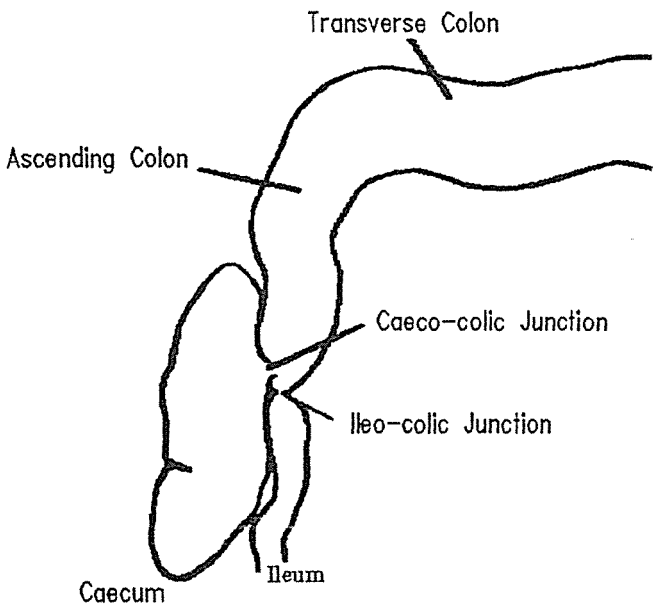


FIGURE 1.2 Schematic diagram of the canine ileocaecal region. (After Fioramonti, 1981).

dogs that the canine stool, when introduced into the ileum, provoked powerful propulsive contractions of the ileum. This would appear to be a mechanism utilizing the motility of the distal ileum to limit the amount of reflux from the large intestine.

In addition to preventing retrograde flow into the ileum, the ICJ also has been implicated in affecting the rate of flow of digesta from the ileum into the large intestine. Singleton, Redmond and McMurray, (1964) found only a small decrease in digesta transit time through the small intestine after ICJ excision in humans. However these workers also found that if the ICJ remained intact, up to 50% of the small intestine could be removed with digesta transit being largely unaffected, while the removal of both the ICJ and a large part of the small intestine results in a marked decrease in transit time through the small intestine.

Spiller, Brown and Phillips, (1987) studied digesta movement through the ICJ of humans using radioactive markers. They reported that while postprandial digesta movement showed a steady flow rate, during interdigestive periods ileocaecal transit became erratic and, at low flow rates, the ileum was able to act as a reservoir, at least for up to two or three hours. In dogs Spiller, Brown, Phillips and Azpiroz (1986) showed a similar phenomenon even postprandially, with the terminal ileum being cleared of about half its contents every 90-120 minutes. This may indicate that the ICJ is a more effective barrier in dogs than in humans. This precept is supported by published evidence indicating that the ICJ in dogs has a high tonic pressure (Kelly, Gordon and Deweese, 1965; Quigley, Phillips and Dent, 1983) whereas the same region in the human shows less tone (Quigley, Phillips and Dent, 1984; Nasmyth and Williams, 1985).

From the evidence reviewed here, the ICJ could have a valvular function only in those species with an appropriate anatomical configuration in this region, as present in humans, cats and sheep. This does not preclude the junction from having sphincter-like qualities as well, and the reports of Singleton et al (1964); Spiller, Brown, Phillips and Azpiroz, (1986); and Spiller, Brown and Phillips, (1987) suggest that motility patterns of the distal small intestine may be important to ICJ function.

1.2 Evidence for sphincter action at the Ileocaecal Junction.

Pahlin (1975) described a gastrointestinal sphincter as "a muscle which surrounds and serves to close an orifice. This serves to keep the gastrointestinal contents in one part of the alimentary canal for an appropriate time by preventing too rapid a passage or preventing regurgitation".

While some gastrointestinal sphincters such as the pyloric sphincter at the distal end of the stomach can be recognised anatomically, others are difficult to visually identify and their classification as sphincters is a functional one (Higgs, Shorter, and Ellis, 1965). Such sphincters can be demonstrated physiologically if they fulfil the criteria suggested by Cohen, Harris and Levitan (1968) and refined by Fisher and Cohen (1973):

a) A sphincter exhibits an intraluminal pressure greater than that of the cavities separated by the sphincter.
b) Appropriate stimulation proximal to the sphincter results in a consistent fall in the elevated pressure within the sphincter.
c) Appropriate stimulation distal to the sphincter results in a prompt rise in sphincteric pressure.

In order to establish the existence of a sphincter, these criteria outlined by Fisher and Cohen (1973) need to be met.

Kelly et al (1965) reported an area of high pressure in the terminal ileum of the dog. The average basal pressure was 19 cm H_2O (13.6 mmHg) above that of the non-actively contracting ileum or caecum. The length of this high pressure zone was found to vary between 0.5 cm and 3 cm, averaging 1.0 cm. Quigley et al (1983), also working with dogs, found an average pressure of 28.5 cm H_2O (23.6 mmHg). A similar zone has been described in cats (Conklin and Christensen, 1975). This zone of elevated pressure occurs in a region of thickening of the circular muscle in the terminal ileum of the cat, though such a visible anatomical feature of the ICJ has not been described for any other species.

While there seems to be little argument over the presence of a zone of elevated pressure at the ileocaecal junction in cats and dogs, reports from other species are less conclusive. Kelly et al (1965) did note that the pressure developed by the sphincter in the dog was greater with increasing diameter of the devices used to measure pressure, as had been reported in work on other sphincter regions in the dog. With much of the early work on sphincter pressure being performed with "pull through" saline filled balloons, this explains some of the discrepancies between results obtained by different workers. For instance, the ICS intraluminal pressure of 20 cm H_2O (14.3 mmHg) recorded by Cohen et al (1968) in humans could not be substantiated by Quigley, Phillips and Dent (1984), who only found intermittent periods of elevated pressure in the order of 1 - 2 mm Hg (1.4 - 2.7 cm H_2O) using fine multilumen flexible catheters for pressure recording. Quigley and co-workers were of the opinion that the pressures recorded were sufficient to compartmentalize

the intestine adequately.

In order to be classified as a sphincter, though, the other two criteria proposed by Fisher and Cohen need to be examined - namely the effects of 'appropriate' stimulation proximal and distal to the ICJ.

Hannes (1920), Tönnis (1924) and Pahlin and Kerwenter (1975) found in cats that injecting fluids into ileal and colonic fistulas in dogs resulted in an increase in pressure of the proposed sphincteric region. Intraluminal pressure would, however, decrease before advancing waves of contraction as they moved down the ileum to the ICJ. Others working in this area in the dog reported that mean intraluminal ICJ pressure decreased with increasing volume of ileal contents, while distal ileal motor activity increased. (Short, (1919); Hinrichsen and Ivy, (1931); Kelly et al, (1966); Cohen et al, (1968); Quigley et al, (1983)).

Rubin, Cardwell, Ouyang, Snape and Cohen (1981) described the effects of ileal distension in the cat as being a consistent reduction in tonic pressure at the ICJ although this was frequently followed by a period of contraction and elevated pressure.

Although the intraluminal pressure at the ICJ has been reported by some as increasing in response to distention of the ileum proximal to the ICJ (e.g. Pahlin and Kerwenter, 1975) there is a consistent reported decrease (at least transiently) in pressure at the ICJ when an advancing wave of ileal activity approaches. Bueno et al (1975) described waves of activity in the small intestine pushing intestinal contents along before them. Such movement of digesta could provide the "appropriate stimulation proximal to the sphincter" that results in "a consistent fall in the elevated pressure within the

sphincter". Fisher and Cohen's second condition would seem to have been met, even if the ICJ response to simple distention or bolus injection into the ileum seems to be variable.

Reports on the effects of caecal or colonic distention are much more consistent. There is an increase in tonic pressure in the ICJ in response to colonic distention in humans (Cohen et al, 1968) cats (Rubin et al, 1981) and dogs (Kelly et al, 1966). This response would clearly inhibit retrograde flow from the caecum into the ileum. From available evidence in dogs, cats and humans, the ICJ does fulfil the criteria proposed by Fisher and Cohen in 1973, and in these species can be considered a functional sphincter.

No published evidence relating to the characteristics of the ICJ in the sheep is readily available.

1.3 Motility patterns of the distal small intestine.

1.3.1 Evidence from Myoelectric recordings.

In 1969 Szurszewski described a cycle of myoelectric activity in the canine small intestine. Carlson, Bedi and Code (1972) further described this pattern of canine myoelectric activity as the Interdigestive Myoelectric Complex, identifying four phases. The first phase was a period of quiescence, which was replaced by a period of seemingly random activity (Phase 2). This developed into a period of intense activity (Phase 3), after which the activity passed through a Transition Phase (4) reducing back to Phase 1 (Quiescence). Carlson's fourth phase appears to be of short duration and occurs on an irregular basis, and is not always used when describing the activity

(Weisbrodt, 1981). The cycle of activity has been shown to sweep down the length of the small intestine, originating proximally in the duodenum.

Weisbrodt (1981) reviewed the descriptions of the patterns of cyclic activity described by a number of investigators, and argues that the term "Interdigestive Myoelectric Complex" is too specific a descriptor, as the activity does not necessarily occur only as an "Interdigestive" phase, nor can it be recorded only as myoelectric activity. Weisbrodt maintains that the cyclic activity of the small intestine should be described as a "Migrating Motility Complex", or MMC.

The arrival of the MMC at the ileocaecal junction has been shown to alter ileocaecal sphincter (ICS) activity in humans (Quigley et al, 1983). While the ICS often has a basal tone higher than the surrounding ileum or colon, the arrival of the MMC results in pressure peaks of 70-80 cm H_2O (50-58 mmHg) within the sphincter lumen. This supports the observation by Weinbeck and Janssen (1974) that coordinated spike activity moves freely across the ICJ from ileum to colon, but contrasts with reports by Balfour and Hardcastle (1975, 1978) of an electrically silent zone at the ICS.

In a later report, Quigley, Phillips and Dent (1984) observed that 90% of the MMC's developing in the canine small intestine would reach the ICJ, and 86% of these would pass over the ICJ into the colon. Other species have been more difficult to categorise. In humans, 90% of recorded MMCs appear to `disappear' into the `random activity of the ileum' (Quigley, Borody et al, 1984). It does appear that there are a number of changes in MMC activity that are consistent across species, however. These develop as the MMC progresses distally into the terminal ileum. Firstly the phase I periods (quiescence),

which are typical of the duodenum, are much shortened and sometimes absent in the terminal ileum. Secondly, the random phase 2 contractions tend to group into discrete bursts. Quigley et al considered that both these features would give a more specialized propulsive function to phase 2 contractions in the distal small intestine. In addition, the phase 3 contractions of the MMC propagate more slowly in the distal regions of the small intestine compared to the proximal small intestine. Typical propagation speed in the ileum of the dog is 1.2 cm min^{-1}, as opposed to 2.1 cm min^{-1} in the duodenum. Bueno et al (1975) described a faster rate of MMC propagation (4.0± 1.5 cm min^{-1}) in the jejunum of the dog, and an even faster rate in the sheep jejunum (17.8± 5.7 cm min^{-1}), though these authors gave no data on rates of MMC migration in the ileum.

Kerlin, Zinsmeister and Phillips (1982) and Kruis et al (1985) correlated small intestine motor activity with chyme transit times, and found that flow is very slow during phase 1, and becomes progressively faster during phase 2 and phase 3. In addition to MMC activity, these workers and others (Quigley, Phillips and Dent, 1984) also identified two other contractile patterns that seem to be associated with the interdigestive phase of intestinal activity.

First, groups of clustered, rhythmic phasic activity were seen to propagate through the ileum during the phase 2 (irregular) activity of the MMC in dogs (Discrete Clustered Contractions, or DCC). Their occurrence was variable, from 3 per hour down to none during an 8 hour recording period. The DCC lasted from 30 seconds to 9.6 minutes, propagating at velocities of up to 60 cm min^{-1}. The effect of this pattern of contraction on chyme transit remains to be determined, since it is difficult to separate its effects from that of phase 2 activity of the MMC.

secondly, Kruis et al (1985) described a broad phasic wave of contraction which is rapidly propagated along the ileum. Speeds of up to 27 cm $min.^{-1}$ were recorded. Similar activity has been described by Quigley, Borody et al (1984) in dogs. Quigley et al described these as `Prolonged Propagated Contractions', or PPCs, although a similar event has been described as a `peristaltic rush' by White, Rainey, Monaghan and Harris (1934), and Code, Rogers, Schlegel, Hightower and Bargen (1957) in humans. The PPC has been shown to be evoked by ileal distention, and is able to empty the ileum in humans and dogs (Kruis et al 1985). The response is augmented when bile acid or fecal material is in the bolus creating the distention. Other possible equivalents of the PPC have been described in rabbits (Koch, Martin and Mathias, 1983) in response to cholera enterotoxin and other infectious agents. Others have simulated the electrical patterns typical of the PPC by ileal distention (Sjorgren, Wardolow and Charles, 1984) or by morphine administration (Sarna, 1984).

PPC's would appear to be very variable in occurrence. Quigley, Borody et al (1984) recorded none in some human subjects, up to a maximum rate of 3/hour in one subject. They occur in the last 20 - 30 cm of the ileum, with some crossing the ICJ. Their presence appears to ensure that ileal irritants such as cholera enterotoxin or colonic contents entering into the ileum are rapidly cleared into the large intestine, in a similar response to the secondary peristaltic waves clearing acid from the oesophagus following gastroesophageal reflux (Kruis et al 1985).

1.3.2 The ICJ of the sheep.

In sheep, the MMC occurs on a regular basis even after feeding (Bueno et al 1975). Since this cycle of activity is continuous in the sheep, the muscular activity it

represents is responsible for the movement of digesta through the small intestine. Fioramonti (1981) endoscopically observed the ileal protuberance into the caecum of the sheep, noting an average contraction frequency of about 13 per minute, though this ranged from every few seconds to once every 2 or 3 minutes. This movement of digesta corresponded to EMG activity measured by ileal serosal electrodes placed 1cm from the end of the ileum, with the higher rate of contraction occurring at the same time as Phase 3 of the MMC, and the lower rates with Phase 2. (Fioramonti describes Phase 2 and Phase 3 of the MMC as Irregular Spiking Activity (ISA) and Regular Spiking Activity (RSA)). With each contraction 0.5 to 2mls of digesta was ejected into the caecum.

Fioramonti (1981) also described a period of caecal activity prior to the arrival of the MMC at the ileocaecal junction in sheep, proposing this as a mechanism to evacuate the caecum which then typically reduces it's level of activity for approximately 10 minutes as digesta is squirted through the ileocaecal orifice prior to the arrival of phase 3 MMC. Following this, caecal activity resumes, mixing the newly arrived digesta with caecal and proximal colon contents.

Reid and Dellow (1972) observed the activity of the terminal ileum radiographically. They described bursts of activity lasting from a few seconds up to 10 minutes, repeating 12 - 20 times every 24 hours. Bueno et al (1975) reported 15-20 MMCs per 24 hours from EMG recordings from sheep jejunum.

The movement of digesta across the sheep ICJ would appear to be linked to the arrival of Phase 3 MMC activity in the distal ileum.

1.4 Pharmacology of the Ileocaecal region.

1.4.1 Pharmacology of the ICJ In Vitro.

After three criteria for determining the existence of a sphincter had been proposed by Cohen et al (1968), Bass, Ustach and Schuster (1970) suggested a fourth feature should be used to characterise gastro-intestinal sphincter regions: circular muscle strips should contract (in vitro) in response to adrenergic stimulation. (Non sphincteric gut muscle will not.)

The initial in vitro studies of muscle strips from the region of the ileocaecal junction were carried out by Gazet and Jarrett (1964). Their studies included samples from humans, monkeys, cats and dogs. Further work by Conklin and Christensen (1975) on the cat and opossum, and Cardwell, Rubin and Snape (1981) on the cat, consistently support the findings of Gazet and Jarrett. In summary, the isolated circular muscle strips from the ileocaecal sphincter (ICS) respond to sympathetic agonists such as adrenaline, noradrenaline and phenylephrine by contracting, while muscle from the adjacent regions of ileum and colon respond by relaxing. The ICS muscle response is blocked by phentolamine, suggesting an alpha-adrenergic receptor is involved in mediating this response. All the muscle strips (ileum, ICS and colon) are reported to respond to acetyl choline by contracting. In addition, Cardwell et al also reported that circular muscle strips from the ICS showed no response to various humoral agents - secretin, gastrin, glucagon and CCK, while colonic and ileal circular muscle strips did respond. Colonic muscle was inhibited by glucagon and secretin and contracted in response to CCK and Gastrin, while ileal muscle was inhibited by glucagon and contracted in response to CCK and gastrin.

1.4.2 Pharmacology of the ICJ in Vivo.

Pahlin and Kerwenter (1975) showed in anaesthetised cats that sympathetic amines elicited an excitatory response from the ICS. This response could be blocked by alpha receptor blocking agents, but remained after a beta receptor blockade. In fact, in two thirds of the experiments, sympathetic amines injected after ß - blockade (propanolol) resulted in an enhanced response, suggesting a tonic inhibitory innervation mediated by beta receptors in the intact animal.

Rubin, Fournet, Snape and Cohen (1980), also reported similar responses from the ICS to catecholaminergic agonists and blocking agents.

Johnson (1977) discussed the likely involvement of gastrin in increasing the tone of the lower oesophageal sphincter, and Polak and Bloom (1981) reported gastrin containing nerves in the distal small intestine and proximal colon, suggesting a possible sensitivity of this region to gastrin.

1.5 Innervation of the muscularis externa of the small intestine.

The small intestine is intrinsically supplied by two plexuses of nerves, containing a variety of neurotransmitters (Polak and Bloom, 1981). Within the wall of the intestine, these two nervous networks are concentrated in two distinct areas - between the two layers of the muscularis externa (Myenteric, or Auerbach's, plexus) and in the submucosa (Submucosal, or Meissner's, plexus).

Gabella (1987) describes the myenteric plexus as forming a continuous network from the oesophagus to the anal canal

in monogastric species. Harrison and Wathuta (1980) identified the myenteric plexus in the small intestine, colon and rectum of sheep.

The submucosal plexus is found right throughout the length of the ruminant intestine (Habel, 1956).

Within these plexuses are ganglia containing cell bodies of autonomic neurons which have been described as enteric neurons (Szurszewski and Weems, 1976). The two plexuses are interconnected, and the ganglia are thought to be able to integrate sensory input and contribute to intestinal motor control patterns (Wood, 1981). Recent studies have shown a variety of neurotransmitters are involved in this control (Polak and Bloom, 1981; Gabella, 1987). The actions of some of these in the intestine are fairly well established. Acetylcholine acting on smooth muscle cells has a stimulatory effect (Weisbrodt, 1987), while the effect of catecholamines is generally inhibitory except on sphincter muscle (see section 1.4). Other neurotransmitters, such as Vasoactive Intestinal Peptide (VIP), Substance P and Serotonin, are not so well understood.

VIP was discovered in 1970 by Said and Mutt in fractions of extract of porcine gut, and has since been found in large quantities throughout the entire length of the digestive tract of numerous species, including ruminants, with the majority of neurone cell bodies located in the submucosal plexus (Polak and Bloom, 1981; Harrison and Wathuta, 1980). VIP has been shown to relax intestinal smooth muscle (particularly circular muscle) in the cat (Sjöqvist and Fahrenkrug, 1987), and is thought to act as a neurotransmitter at interneuronal synapses (Costa and Furness, 1983), although its role as a neurotransmitter remains to be established.

Substance P is a powerful gut smooth muscle stimulant (Yau, 1978), and is thought to mediate non-cholinergic smooth muscle stimulation through interneurones within the myenteric plexus and branches into the circular muscle layer of the intestine (Katayama and North, 1978; Bornstein, North, Costa and Furness, 1984). However the lack of substance P in the interganglionic connections suggests that Substance P serves mainly a localized role within the muscularis of the intestine (Costa, Furness, Llewellyn-Smith and Cuello, 1981). Substance P may also be involved in sensory neurotransmission within the enteric nervous system (Polak and Bloom, 1981).

Serotonin (5-Hydroxytryptamine, or 5-HT) is thought to be the noncholinergic mediator of responses evoked relatively distant to ganglia within the enteric nervous system, as it is found in relative abundance in the plexuses between ganglia and has been shown to mediate contractile responses from intestinal muscle in response to nerve stimulation in guinea pigs (Erde, Sherman and Gershon, 1985). Wood and Meyer (1979) have also demonstrated that adrenergic stimulation of the small intestine inhibits serotonin release in the myenteric plexus.

CHAPTER 2

EMG and Pressure studies of the Ileocaecal Junction in the anaesthetised sheep.

2.1 Introduction

Sphincter properties have already been demonstrated at the Ileocaecal Junction (ICJ) of a number of species, including dogs (Hinrichsen and Ivy, 1931; Kelly *et al*, 1965; Jarrett and Gazet, 1966; Quigley *et al*, 1983), cats (Pahlin, 1975; Conklin and Christensen, 1975; Rubin, Fournet, Snape and Cohen, 1980) and humans (Cohen *et al*, 1968; Quigley, Phillips and Dent, 1984; Quigley, Borody *et al*, 1984; Nasmuth and Williams, 1985). The sphincter at the ICJ is thought to prevent the reflux of bacteria laden caecal contents into the ileum (Quigley, Borody *et al*, 1984; Gorbach *et al*, 1967). It may also control digesta movement from ileum to caecum (Spiller *et al*, 1987).

Reid and Dellow (1972) describe the movement of digesta through the ileocaecal orifice of sheep as being "pulsatile and vigorous". Fioramonti (1981) observed 'jet-like' pulses of digesta entering the caecum, suggestive of fluid being forced at pressure through a restricted orifice with a region of tonically elevated pressure. This study used acute experimentation to investigate the pressure and myoelectric characteristics of the sheep ICJ to determine if a tonically active region of elevated pressure existed, and whether the region shows the characteristics of a true physiological sphincter as defined by Fisher and Cohen (1973).

2.2 Materials and Methods

2.2.1 Electrode Manufacture

Electrodes for Electromyographic (EMG) recording were manufactured from a 1 metre length of multicored teflon coated stainless steel wire (Cooner Wire Company, USA). A knot was tied 5cm from one end, and the end of the wire inserted into the blunt end of a 2.5 cm long hollow needle made by cutting the end from a 25 gauge hypodermic needle. Once inserted, the base of the needle was crimped onto the wire and a 2mm section of insulation removed by careful scraping with a scalpel blade from the short end of the wire beside the knot (Figure 2.1).

2.2.2 Preparation of Animals

Five Southdown cross sheep of mixed sex were used in this experiment, ranging in weight from 20 to 33 kg (Table I). Anaesthesia was induced using a 5% halothane/ oxygen mixture administered through a rubber mask placed over the muzzle of the animal. Once anaesthetised as indicated by a loss of palpebral and limb withdrawal reflexes, the animal was placed in a supine position, and the trachea, femoral artery and femoral vein were cannulated. The tracheal cannulae were glass (18mm OD), with a single side arm for ventilation with oxygen in case breathing stopped. The arterial and venous cannulae were manufactured from 50 cm lengths of polyethylene tubing (ID 2mm, Dural, Australia), filled with 50 units ml^{-1} heparinised saline before insertion (Heparin Sodium, Leo Pharmaceutical products, Denmark). Anaesthesia was maintained by chloralose administered intravenously via the femoral vein cannula. (Initially 70 mg kg^{-1} live weight, 23 mg kg^{-1} every 2 hours maintenance.) Blood pressure was monitored via the arterial cannula either through a pressure transducer (Model 4327-I, Bell and Howell, Calif. U.S.A.) amplified

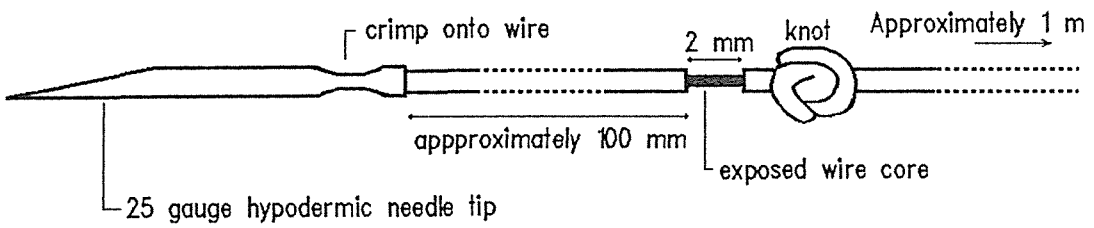


FIGURE 2.1 Diagram of electromyograph (EMG) electrode tip prior to insertion.

Animal Number	Live Weight (kg)	Sex
1	27	female
2	32	female
3	25	male
4	20	male
5	33	male

TABLE I: The live weight and sex of the Southdown cross sheep used in the experiment.

to the ink pen chart recorder (Gould Instruments, Ohio, USA), set at 0.25 volts full scale deflection and chart speed 0.25 mm sec^{-1}, or occasionally by attaching the cannula to a mercury manometer for observation.

The abdominal viscera were exposed by a 20 cm transverse incision in the ventral abdominal wall beginning from the midline and moving to the right of the animal, to a point slightly cranial to the iliac crest. The caecum and distal small intestine were then lifted from the abdominal cavity and placed in a warmed (37^{0}C) perspex tray (Figure 2.2). When not being manipulated, the exposed viscera were covered by a film of polyethylene (Glad, New Zealand) to prevent dehydration.

The electrodes were implanted in groups of 3, each electrode being placed 5mm from the other two in a triangular formation. Electrode groups were placed in the following locations:

1) Ileum, 10cm proximal to the ICJ
2) Ileum, 5cm proximal to the ICJ
3) At the ICJ
4) Caecum, opposite the ICJ

The needle tip of each electrode was passed through the serosal surface of the intestine wall and looped through the muscularis, but not the mucosa, until the exposed section of wire was located within the muscularis. The wire was then tied in a reef knot,and the needle end cut off leaving a 2-3 mm wire protrusion (Figure 2.3).

Each set of 3 electrode wires was connected to a twin core shielded cable. Two of the electrode wires were connected to the cable core wires, providing the EMG signal. The third electrode was connected to the earthed cable shield. The signal was then amplified 1000x (AC amplifier, Physiology and Anatomy Department, Massey University, N.Z.), and then recorded on the chart recorder using the

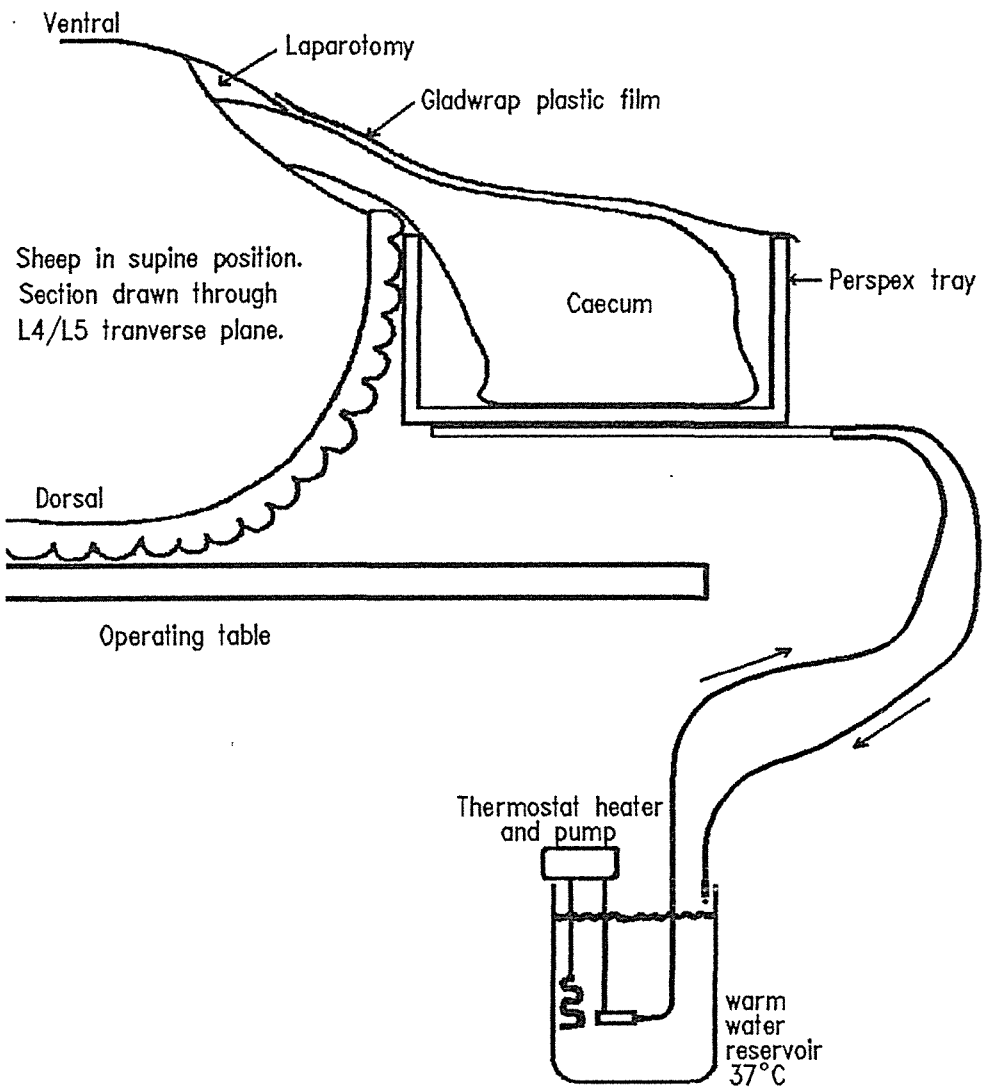


FIGURE 2.2 Schematic diagram showing the positioning of the caecum in a perspex tray and covered in gladwrap plastic film to prevent dehydration during the course of the experiment. Copper tubing attached to the underside of the tray allowed warmed water to circulate, helping to maintain the temperature of the caecum.

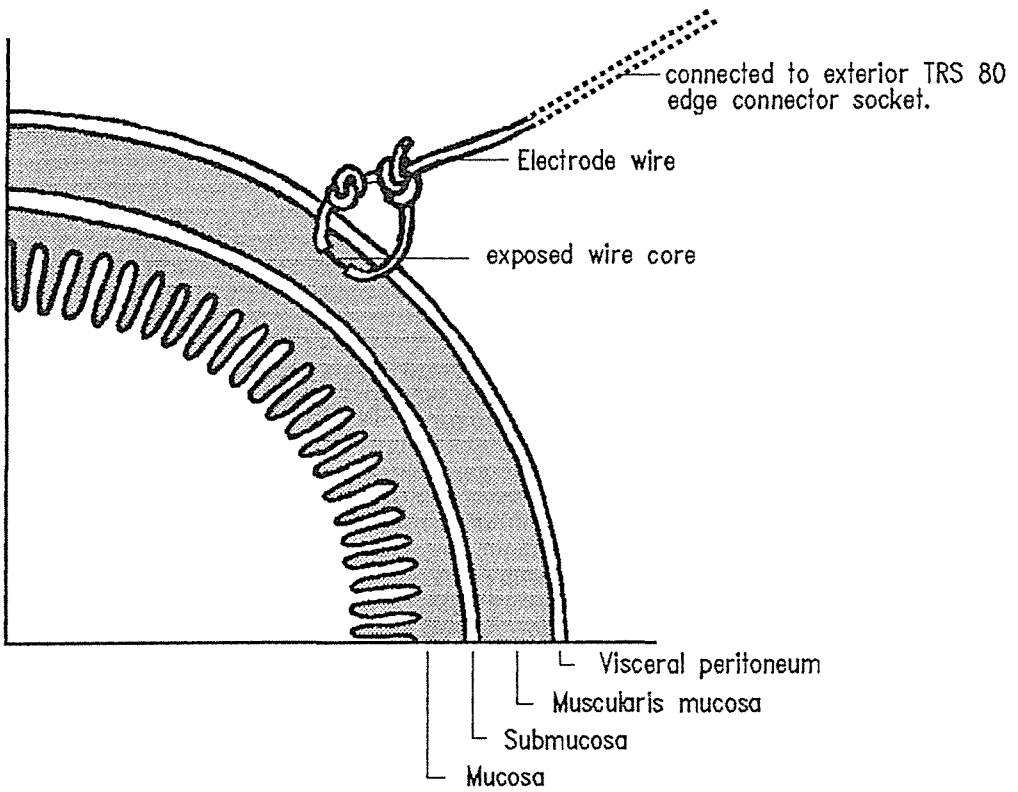


FIGURE 2.3 Schematic diagram of a transverse section through the intestine wall showing how the wire is inserted. The wire is tied in place, leaving the exposed section of wire in contact with the cells of the muscularis mucosa.

same settings as previously described for blood pressure recordings.

A pressure recording catheter was prepared for the intestine by occluding the distal end of polyethylene tubing (ID 2mm, Dural, Australia) for 3mm with plasticine, and cutting a 2mm diameter side hole 5mm from the tip. The catheter was filled with isotonic saline and introduced into the ileum through the ileal wall 20 cm proximal to the ICJ. The tip was then moved distally into the caecum. Leakage of ileal contents was prevented by a purse-string suture around the catheter entry point.

The catheter was attached to another pressure transducer which was positioned at the same vertical height as the catheter tip to avoid pressure artifact.

Pressure transducers were calibrated to atmospheric pressure (0 mm HG), 10 and 20 mm Hg at the beginning and end of each experiment. No calibration changes were observed in the recording equipment after the initial warm-up period of 10 to 15 minutes.

2.2.3 Experimental procedure.

Handling the intestines was kept to a minimum after it was observed to reduce intestinal motility for up to 5 minutes. A pause of at least 5 minutes post handling was allowed before the experimental procedure commenced. The pressure recording catheter was gently manipulated manually to pass through the terminal ileum into the caecum. After waiting for motility to resume, the catheter was slowly withdrawn in 5mm steps, with 1 to 2 minutes pause at each step. This was repeated at least three times for each animal.

If a zone of intraluminal pressure (anything greater than zero mm Hg) was located, the side hole of the catheter was located within this region during subsequent procedures. Where no zone of elevated pressure was detected, the side hole of the catheter was located as near as possible to 5mm from the end of the ileum within the ileal papilla, as determined by manual palpation of the catheter tip through the intestine wall.

A caecal distending balloon was prepared using a rubber balloon approximately 50mm x 50mm deflated tied over the end of a 30 cm long polyethylene tube (5mm ID, Dural, Australia), (Figure 2.4). A saline filled open tipped catheter (2mm ID Dural, Aust.) was attached to the balloon inflation tube and used to record intraluminal pressure. The balloon and catheter tip were introduced into the caecum through an incision made in the caecal wall approximately 5cm from the apex. Leakage of caecal contents through the incision site was prevented by a purse-string suture pulled tight around the tubing.
A similar procedure was used to distend the ileum 10cm proximal to the ICJ, using 2mm ID tubing with a segment of a condom (Durex, NZ) tied over the end. The ileal balloon measured 20mm x 10 mm deflated.

The balloons were then inflated with air : 100 - 200 mls (caecal balloon) or 10 mls (ileal balloon). Balloon inflation was maintained for 2 to 5 minutes. Each procedure was repeated at least 5 times, at intervals of at least 5 minutes.

2.3 Results

2.3.1 General Observations

Intraluminal pressures in the resting distal ileum and caecum were equal to atmospheric pressure, and any

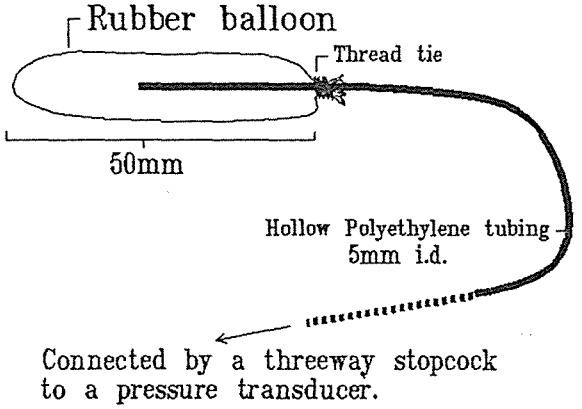


FIGURE 2.4 Diagram of the balloon inflation tube used for caecal distention.

increase in pressure was recorded as mm Hg above this. Increased contractile activity of the ileum coincided with increased EMG activity and intraluminal pressure (Figure 2.5a-e) in all the animals studied, although no coordinated sequences of contractions similar to the Migrating Motility Complex (described in chapter 1.3.1) were noted during the course of these experiments. There were discrete bursts of activity (duration around 20-30 seconds) typified by the recording shown in Figure 2.5 (b). Pressure peaks of up to 30 mm Hg were recorded during such EMG burst activity.

2.3.2 Intraluminal Pressure at the Ileocaecal Junction.

Withdrawal of the saline filled catheter from the caecum through the ICJ produced equivocal results. In the first animal the general activity of the distal ileum was such that it was difficult to determine resting pressures with any degree of accuracy, although the pressure within the ICJ in intervals between burst activity remained higher than the pressure recorded simultaneously from the ileum, 10cm proximal to the ICJ and the caecum (Figure 2.6).

Subsequent repeats of this procedure on the other animals showed no zone of elevated pressure in two cases (2nd and 4th animals) and a distinct zone of elevated pressure in the other two cases (3rd and 5th animals). Careful withdrawal of the catheter tip through the ICJ in the latter two animals showed the length of the zone to be quite short - between 5 and 10 mm in animal 3, and no more than 5 mm in animal 5. Figure 2.5 shows recordings obtained from intraluminal catheters in the ICJ.

2.3.3 Pressure changes with intestinal distention.

Ileal distention generally had no effect on ICJ activity as ascertained by both EMG and intraluminal pressure

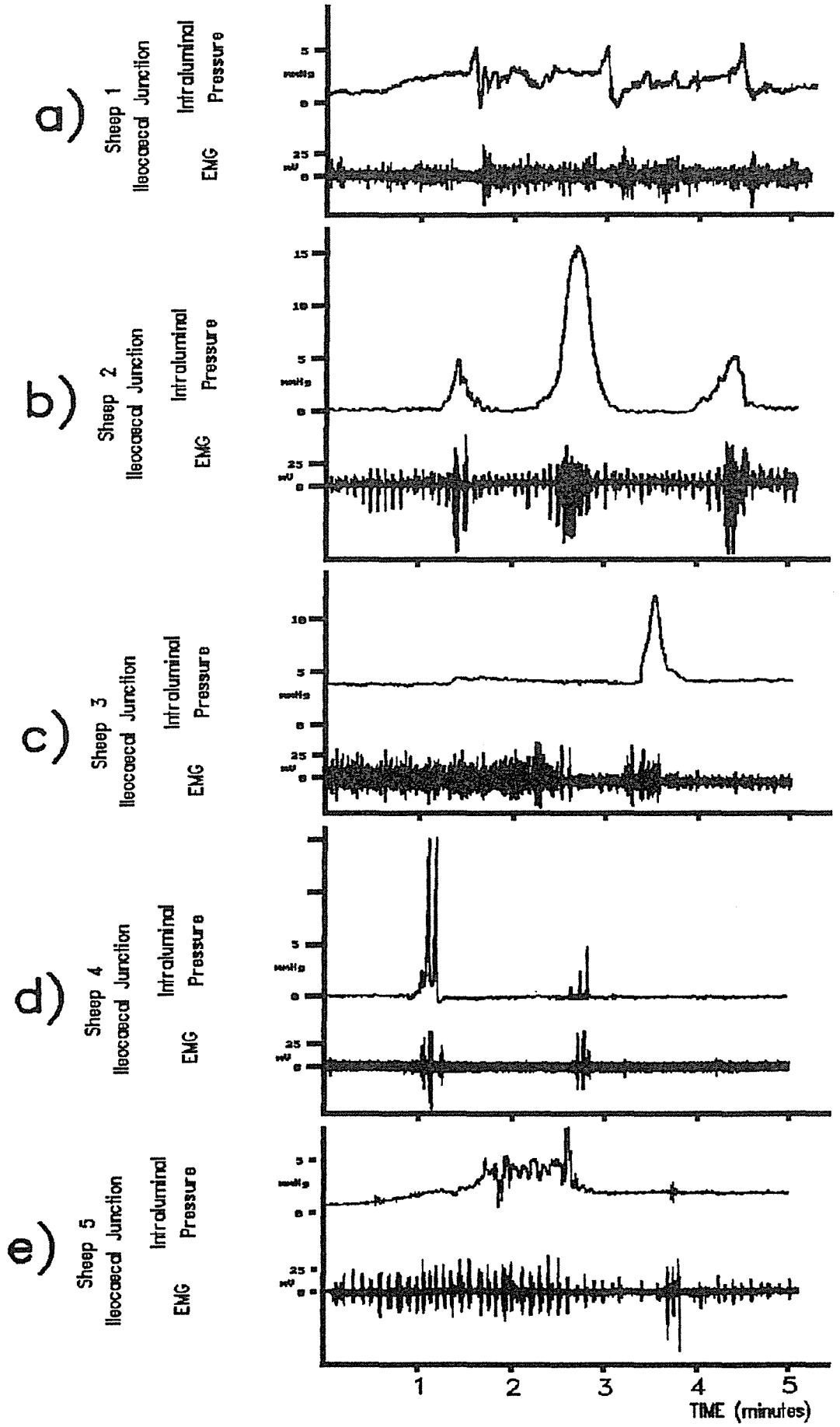


FIGURE 2.5 EMG and intraluminal pressure recordings from the ileocaecal junction of 5 different sheep, illustrating the relationship between the two.

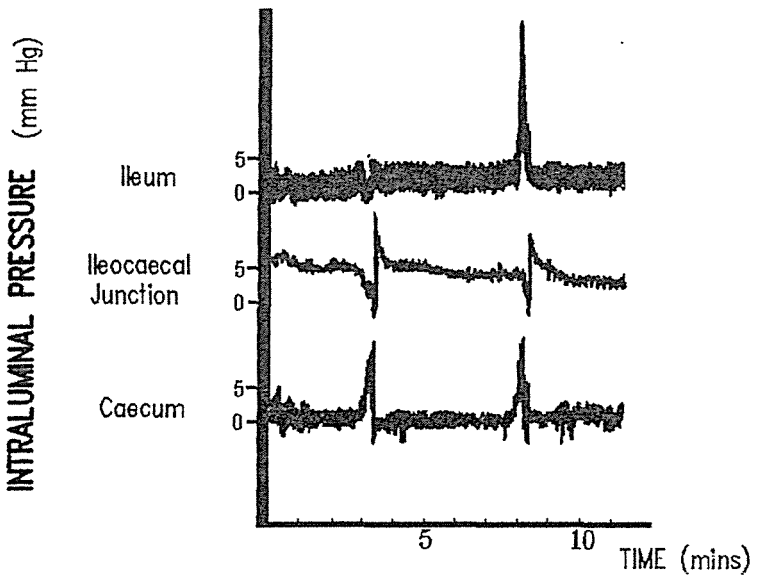


FIGURE 2.6 Sheep 1: simultaneous intraluminal pressure recordings from the caecum, ileocaecal junction (ICJ) and ileum (5 cm proximal to the ICJ).

recordings. The exception was sheep 3, which in 4 of the 5 experiments showed a transient (10 to 15 second) decrease in ICJ pressure, followed by a return to resting pressure (Figure 2.7).

Inflation of the caecal balloon with 150-200 mls of air generally resulted in an increase in ICJ intraluminal pressure. The response, when it occurred, was accompanied by an increase in EMG activity as recorded from the ICJ (see Figure 2.8).

The third animal in the series did not show the same consistency as the others. The caecum was distended 12 times using 200 ml inflation of the caecal balloon. The ICJ pressure increased on only 4 occasions. On three occasions there was no response observed in the ICJ, while the remaining 5 times saw an associated transient (20 - 30 second) slight decrease in ICJ pressure. This response was always associated with a subsequent rise in ICJ pressure to slightly above resting pressure before the caecal balloon was deflated.

2.4 Discussion

This study has demonstrated by means of a pull-through technique the existence of a zone of tonically elevated pressure within the ileocaecal junction of three of the five sheep studied. The pressure in this zone is in the order of 2 to 5 mm Hg above the intraluminal resting pressure on either side of the ICJ, and is very short (approximately 5mm in length), apparently located within the ileal papilla. That a zone of tonically elevated pressure could not be consistently demonstrated in all the animals studied suggests that in sheep, as in humans (Quigley, Borody et al; 1984) the ICJ only maintains a low tonic elevation in pressure on an intermittent basis. Quigley, Borody et al did consider that this would be

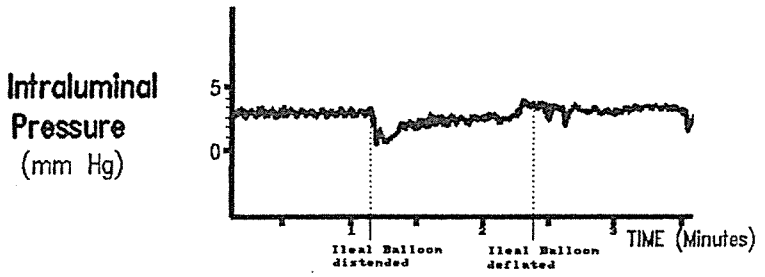


FIGURE 2.7 Sheep 3: Intraluminal pressure recording from the ileocaecal junction (ICJ). The ileum was distended 10 cm proximal to the ICJ with an intraluminal balloon inflated with 10 ml of air.

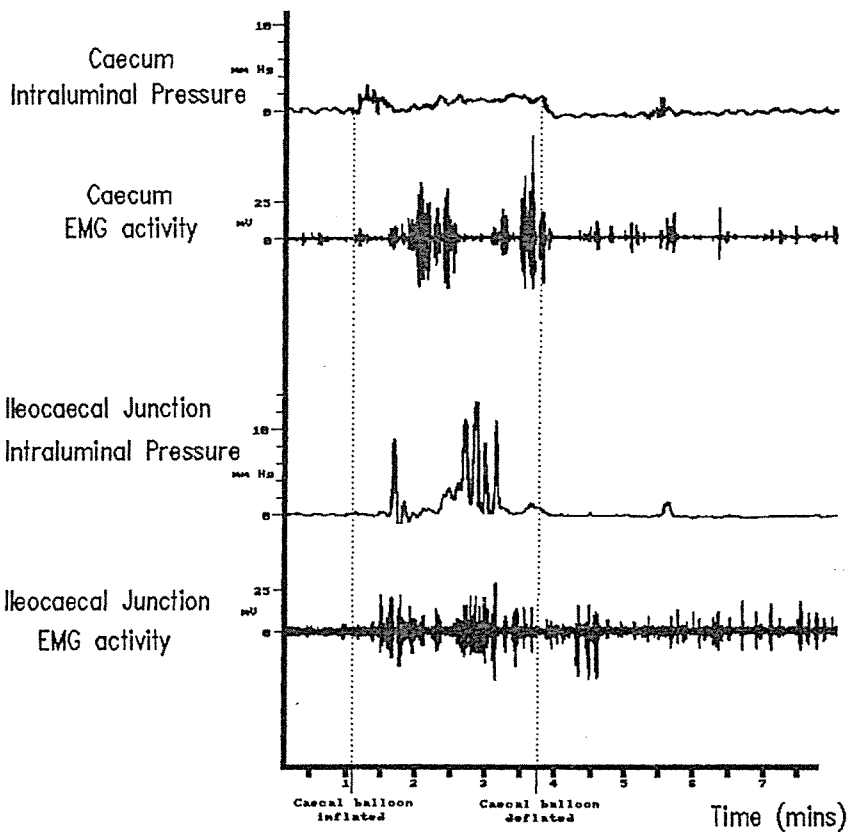


FIGURE 2.8 Sheep 4: Caecal distention related to increased EMG activty at the ileocaecal junction. The caecal intraluminal balloon was inflated with 100 ml of air.

sufficient to adequately compartmentalize the small and large intestines.

Fioramonti (1981) reported a similarity in structure between sheep and human ICJ. The similarity in recorded resting pressures at the ICJ suggests a functional similarity as well.

The response of the ICJ to caecal distention is consistent with the presence of a sphincter as defined by Fisher and Cohen (1973). The contractile response and the rise in tonic pressure in the ICJ following caecal distention was clearly a physiological response, as the EMG recording showed increased muscular activity concomitant with the recorded pressure changes. An increase in pressure alone could have been due to compression of the ileal papilla by a rise in intracaecal pressure.

The response of the ICJ to ileal distention was much less conclusive, since a sphincter at the ICJ would be expected to respond to ileal distention by showing a decrease in pressure. With only three animals demonstrably showing a tonic baseline pressure elevation, the expected response was only possible from three of the five animals studied (Sheep 1, 3 and 5). Recordings from sheep one showed spontaneous irregular bursts of activity in the ileum, making close study of changes in the resting pressure across the ICJ impossible. Of the other two sheep, only sheep 3 showed a consistent transient decrease in pressure in response to ileal distention, similar to the results reported by Kelly *et al* (1966) in the dog. It would appear that a control mechanism does exist in the sheep that causes a change in ICJ activity in response to ileal distention, although the results in this study can hardly be regarded as conclusive in this regard, and further experimental work is needed to confirm this.

The results of these experiments do suggest that the ICJ of the sheep does have the characteristics of a sphincter region as suggested by Fisher and Cohen (1973). The pressures within the sphincter region are much less than those recorded for other species, particularly the dog, in which much of the study of the ICS has been performed. However the possible valvular function of the ICJ should be borne in mind when comparing pressure recordings from sheep (and humans) with the dog, which anatomically shows no sign of valvular action in this region. Higher ICS resting pressures would presumably be needed in dogs to prevent reflux of digesta from the caecum.

In addition, the possibility of coordinated ileal and caecal activity in the sheep as suggested by Fioramonti (1981) would have implications for any sphincter action at the ICJ. A clearing of the caecum before ileal emptying would mean intraluminal ICJ pressure would vary with trans-ileal activity rather than be maintained at a high tonic level. As no regular patterns of activity such as the MMC were recorded in any of the anaesthetised animals in this study, no conclusions can be drawn about the effect of such activity on tonic sphincter pressure.

Maintaining the sheep under chloralose anaesthesia clearly had some effect on small intestine motor activity, as the regular pattern of the MMC was not observed in these acute experiments.

A pleasing feature of this experiment was the coordination observed between EMG activity and the intraluminal pressure recordings at the same level of the intestine (Figures. 2.5, 2.6). These data, in conjunction with the report by Bueno et al (1975) relating EMG activity to the digesta flow in the small intestine of sheep, would suggest that EMG recordings provide an accurate

representation of intestinal activity in terms of both intraluminal pressure and digesta flow in the sheep. Such a method could be used with confidence to monitor activity in the region of the ICJ to determine the involvement of the ICJ with the coordinated patterns of intestinal activity in the ileocaecal region of the conscious sheep.

CHAPTER 3

Myoelectric activity of the distal ileum

3.1 Introduction

The Migrating Motility Complex (MMC) occurs regularly in the sheep small intestine (Bueno et al, 1975). It therefore is accepted by many as the principle activity responsible for the movement of digesta through the small intestine of these animals. Reported observations of the movement of digesta through the Ileocaecal Junction suggest this movement is linked to MMC activity in the distal ileum (Fioramonti, 1981; Reid and Dellow, 1972).

This experiment was designed to monitor EMG activity in the terminal ileum over periods of some hours and, intermittently, days, in order to determine activity patterns at the Ileocaecal junction both between and during meals.

3.2 Materials and Methods

3.2.1 Electrode Manufacture

Twelve electrodes for Electromyographic (EMG) recording were prepared as described in Chapter 2.2.1. These were sterilized by soaking overnight in 2% Zephryn antiseptic (Bayer, New Zealand).

3.2.2 Preparation of Animals

Three Southdown cross sheep (one female and two male) ranging in weight from 25 to 27 kilograms, were used in the experiment. The animals were dosed with an anthelmintic (Nilverm, ICI) on, or shortly after arrival at the laboratory livestock pen. They were then housed indoors for at least two weeks pre-operatively in a 2m by

2m pen on wooden duckboards in order to accustom them to an indoor environment. They were fed chaffed lucerne hay once daily.

To fit the electrodes, each animal was prepared by clipping the right flank closely, using an Oster size 40 blade (Sunbeam Corporation, Wisconsin, USA). Anaesthesia in the animals was induced with sodium thiopentone ('Intraval' sodium, May and Baker Ltd, England) at a dose rate of 30 mg kg^{-1} intravenously administered. A 9.0 mm endotracheal tube (Oral medishield, Magill, UK) was then inserted and the anaesthesia was maintained with 2% halothane in oxygen (Fluothane, I.C.I., England). Aseptic conditions were maintained throughout the procedure, with the animal's right flank being scrubbed with hibitane antiseptic (I.C.I.).

The right side of the peritoneal cavity was opened by a dorsoventrally oriented laparotomy approximately 10cm long, beginning 3-4 cm anterior to the iliac crest. The caecum and terminal ileum were located, and four groups of electrodes were implanted in the manner and location described in the previous chapter (2.2.2). The twelve wires from the electrodes were then formed into a loose loop of approximately 75 mm diameter in the peritoneal cavity, and then passed through the subcutaneous fascia to emerge from a mid-dorsal location at the approximate level of the seventh thoracic vertebra. A hollow stainless steel probe was used to punch through the fascia and skin. The probe was 10mm in diameter and 0.5 m in length. A rounded stainless steel tip was screwed into the end of the probe to enable it to be pushed easily through the subcutaneous fascia, and once through the tip was unscrewed and the loose end of the wires were pulled through the probe, which was then withdrawn.

Following surgery, each animal was closely watched until

it was capable of maintaining sternal recumbency unaided and the danger of inhalation of regurgitated stomach contents reduced.

A day after recovery, each animal was placed in a metabolism crate. The free ends of the wires were soldered onto an electrical connector (TRS-80 edge connector, Dick Smith Ltd., Australia). This was held in place by an elastic body net (Systenet, International Surgical Netting SPA, Italy).

Recordings commenced no earlier than 3 days post operatively, and before recording sessions began an indwelling 16 gauge 24 inch jugular catheter (E-Z Cath, Deseret Pharmaceutical Co., Utah, USA) was put in place under local anaesthetic (2% Lignocaine HCl, Astra Pharmaceuticals PTY Ltd, Australia). This enabled the withdrawal of blood samples during the course of each experiment. The jugular catheter was filled with sterile heparinised saline (500 units per ml Sodium Heparin, Leo Pharmaceutical Products, Denmark) between recording sessions. Prior to each experiment the catheter was flushed with sterile saline (0.9%).

Each catheter lasted approximately 7 - 10 days before blockage required replacement.

3.2.3 Recording Protocol

During the recording sessions EMG traces were observed for the MMC pattern of activity, as shown in Figure 3.1. Once phase three activity was completed, a 5ml blood sample was taken via the venous catheter. This was repeated every 15 minutes for 225 minutes (until 15 samples had been obtained). 3 mls of 0.9% sterile saline was injected into the catheter after each sample to reduce the likelihood of blood clots forming in the tubing.

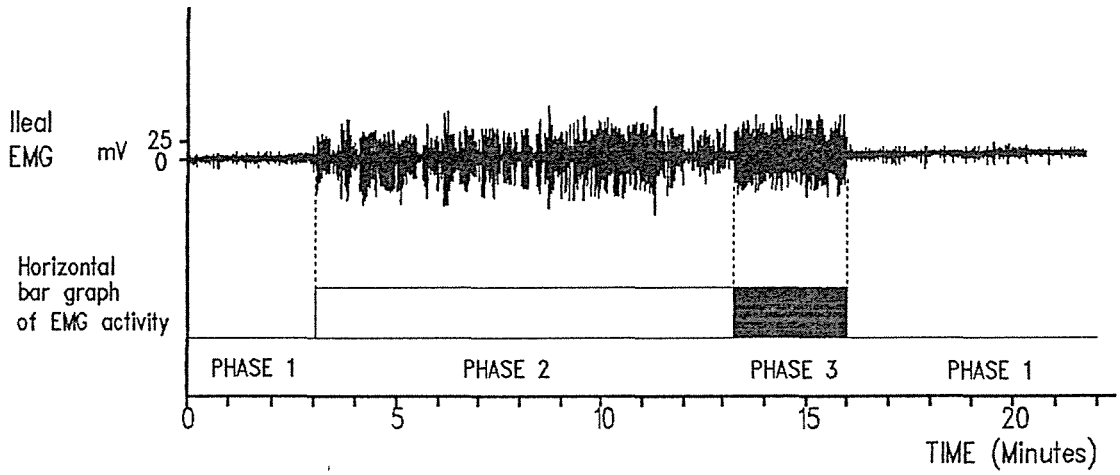


FIGURE 3.1 Sheep 1 EMG activity recorded from the ileocaecal junction (upper trace) converted to a horizontal bar graph to show the three phases of the Migrating Motility Complex (MMC).

After each blood sample had been taken it was allowed to clot over a 10 minute period and then stored in a refrigerator at 4°C. After the experiment was complete the samples were centrifuged at 3000 rpm for 10 minutes. 2-3 ml of serum was then drawn off and frozen at -10°C for later gastrin analysis.

This sequence of blood sampling and EMG recording was repeated 5 times as control experiments for each animal. Interspersed randomly with these control experiments were 5 experiments in which 1 kg of dried lucerne chaff was provided for the animal immediately following the withdrawal of the 3rd blood sample (feeding experiments). The sequence was repeated a further 3 times with Sheep 1 being injected subcutaneously with 7 μg kg^{-1} pentagastrin (peptavlon, ICI) after the 3rd blood sample of the series was taken.

This series of experiments was not performed on sheep 2 as available pentagastrin stocks were exhausted, and the radioimmunoassay technique used for analyzing gastrin levels was found to be unable to detect pentagastrin (see 3.2.5).

Sheep 3 was injected with 7 μg kg^{-1} pentagastrin in the same fashion as sheep 1 while EMG recordings were being made, though no blood samples were taken for gastrin analysis.

In the second experimental animal a nematode infestation was detected after six experiments, four of which were controls (see Results 3.3.2). The animal was then treated with an anthelmintic (Valbazen, Smithkline Beecham Animal Health, NZ). Three months later a further 3 control and 2 feeding experiments were performed on the uninfected animal, before a deterioration in the quality of the EMG recordings meant further experiments could not be

successfully carried out.

Experimental protocol summary:

1) Ileal EMG activity was monitored. Following the identification of phase three MMC activity, a 5 ml blood sample was taken and stored in a refrigerator at 4°C. (Time = 0 minutes).

2) Blood samples taken every 15 minutes until 15 samples collected.

3) Immediately following the 3rd blood sample being taken, the experimental manipulation was carried out:

either a) feeding the animal with 1kg of dried lucerne chaff,

or b) subcutaneous injection of 7 μg kg^{-1} pentagastrin,

or c) no treatment (control).

4) The procedure (steps 1-3) was repeated 15 times for each animal, with steps 3a, b and c being repeated 5 times each in random order.

3.2.4 Statistical analysis of results.

Statistical formulae used to compare data sets generated by this experiment are as follows:

$$s = \sqrt{\frac{\Sigma(X-\bar{X})^2}{n-1}}$$

$$SEM = \frac{s}{\sqrt{n}} \times 1.96$$

s = Standard Deviation
SEM = Standard Error of the Mean (95% confidence limit)

When comparing means for significant differences, a pooled variance (s^2) was calculated, and the means compared using the T test:

$$s^2 = \frac{1}{n_1 + n_2 - 2} \times \left(\Sigma X1^2 - \frac{(\Sigma X1^2)}{n_1} + \Sigma X2^2 - \frac{(\Sigma X2)^2}{n_2}\right)$$

$$t = \frac{\bar{X}1 - \bar{X}2}{s\sqrt{[1/n_1 + 1/n_2]}}$$

Probability was estimated from the value of t using $n_1 + n_2 - 2$ degrees of freedom. Values of $P < 0.05$ indicated a significant difference between means. (Parker, 1975)

3.2.5 Gastrin Radioimmunoassay

The procedure used by Mr J. Elgar and Miss J. Candy was that described by Hansky, Soveny and Korman (1971) using Hansky's gastrin antiserum 74.

Gastrin I^{125} was prepared by the chloramine - T method and purified by sequential affinity and ion-exchange chromatography. Incubation tubes contained 100 µl standard, or 100 µl of unknown serum, 400 µl antibody 1/40,000, and 500 µl tracer (1000-1200 counts min^{-1}). All reagents were made up in 0.02 M veronal buffer (pH 8.6) containing 0.5% bovine serum albumin (Armour Pharmaceutical Co., Eastbourne, England), thimerosil (Sigma) and neomycin sulphate (Sigma). Each determination was set up in triplicate, mixed and incubated at 4°C for 2 days. Separation of antibody-bound gastrin from free gastrin was achieved with a second antibody raised in sheep against rabbit τ globulin.

Assay Variation: Inter-assay pool variation <17%
Intra-assay variation <13%
(see Appendix A)

3.3 Results

3.3.1 Myoelectric Recordings

Periods of EMG recordings were classified into three phases of MMC activity as suggested by Weisbrodt (1981):

phase 1 (no activity)
phase 2 (random activity)
phase 3 (regular sustained activity).

The three activity phases were represented in horizontal bar form for ease of interpretation (see Figure 3.1). A fourth category was used to represent regular burst activity (RBA) typical of the caecum, consisting of bursts of activity approximately 30 seconds long, recurring every 60 - 90 seconds. Fioramonti (1981) reported this type of activity in the large intestine of most species, identifying it as Short Spike Burst (SSB) activity. This pattern of activity was also observed in the recordings from the ICJ of sheep 3 (Figure 3.2).

3.3.2 Control EMG recordings.

A graphic representation of the data from the first control experiment using sheep 1 can be seen in Figure 3.3. Data and graphs of other experimental recordings are presented in Appendix B.

In sheep 2 after 5 experiments blood gastrin analysis showed serum gastrin levels of the order of 100 fmol ml^{-1}. Subsequent microscopic examination of weighed fecal samples from this animal showed 850 strongylate eggs per gram, indicative of a nematode worm infestation. Such infestations have been linked with increased serum gastrin levels (Anderson, Hansky, and Titchen, 1981). Treatment of the infected animal with Valbazen anthelmintic reduced the egg count to zero by seven days post treatment. In later

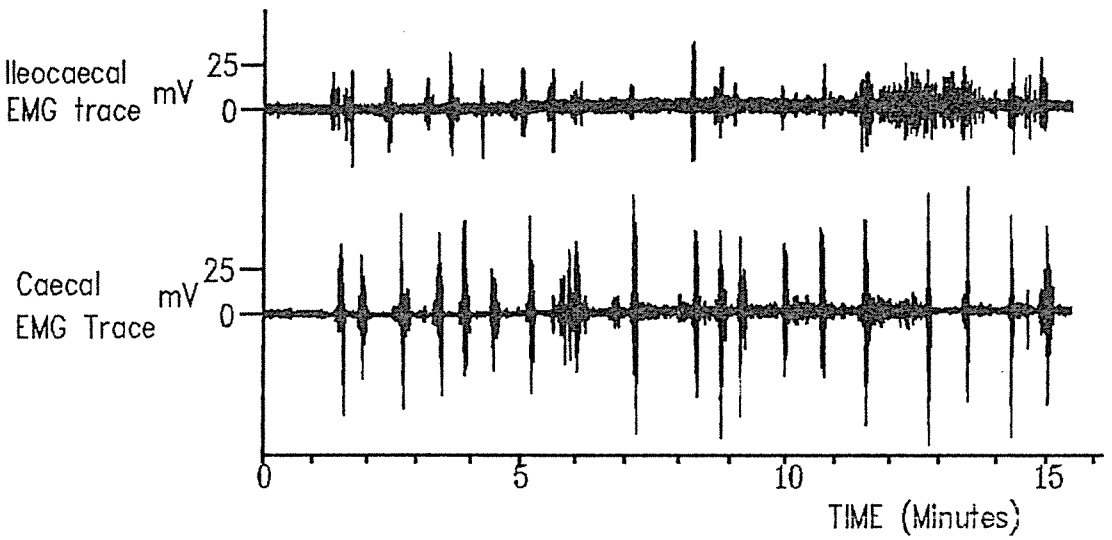


FIGURE 3.2 Sheep 3: EMG recordings from the ileocaecal junction and caecum. This Regular Burst Activity (RBA) was observed in caecal EMG recordings from all three animals used in the experiment.

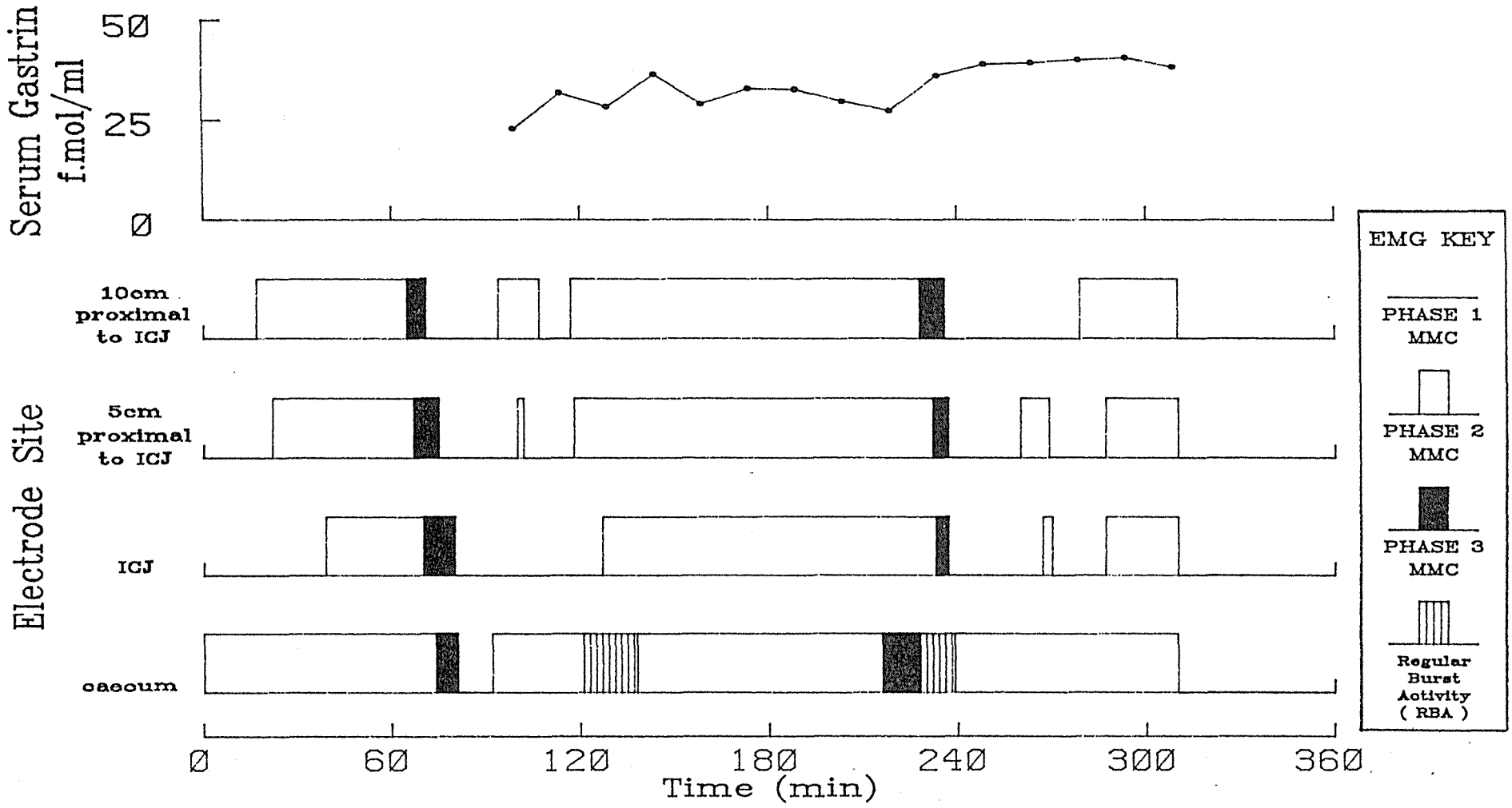


FIGURE 3.3 Sheep 1, first control experiment. EMG activity from the distal small intestine was monitored. After an MMC (Phase 3) was identified, blood samples were taken every 15 minutes, and serum gastrin levels measured. EMG recording ended at T = 310 minutes.

experiments serum gastrin levels were much lower, in the range 15 - 40 fmol ml^{-1}. A further 3 control and 2 feeding experiments were performed using this animal.

The control data presented does not include data from Sheep 2 during the nematode infestation. This data will be reported separately in section 3.3.5.

In the control experiments in the three sheep, a total of 36 MMC sequences were recorded. The mean intervals between phase 3 contractions for each animal is shown in Table II. The overall mean interval in all the control experiments was 112± 16 SEM minutes, an average of 13 MMCs per 24 hours.

The rate of propagation of these MMCs through the distal ileum was 2.7± 0.4 SEM cm min^{-1}.
Of the MMCs observed in the control experiments, 82% of phase 3 contractions reached the ICJ but only 27% appeared to pass through to the caecum.

Caecal quiescence was not generally associated with the arrival of an MMC at the ICJ, as suggested by Fioramonti (1981). It usually occurred while the ileum also showed inactivity (18 of 24 observed periods of reduced caecal activity). Only 20% of MMC arrivals at the ICJ were preceded by a period of intense caecal EMG activity.

The caecum of all three animals showed a distinct pattern of regular activity (regular Burst Activity, or RBA). Fioramonti (1981) described this as SSB activity, identifying it as typical of the large intestine of most species. In the present study RBA was often distinguishable even in caecal traces showing predominantly irregular activity (i.e. during phase 2 - like activity). When phase 2 activity declined the RBA became quite distinct, with an average interval

Control Experiments: MMC Phase 3 data.				
Sheep Expt	MMC speed cm min^{-1}	To ICJ	To Caecum	Time Interval
1/1	2.0	yes	yes	
	1.7	yes	no	163
1/2	1.4	yes	yes	
	2.5	yes	yes	117
1/3	2.5	yes	no	
	3.3	yes	no	185
1/4	1.7	yes	yes	
	2.5	yes	yes	129
	nsd	no	no	116
1/5	2.5	yes	yes	
	1.3	yes	no	97
	0.7	yes	yes	83
2/5	2.0	no	no	
	nsd	no	no	106
	6.0	yes	yes	212
2/6	3.0	yes	no	
	5.5	yes	yes	86
	nsd	no	no	116
2/7	4.5	yes	yes	
	2.5	yes	no	196
3/1	4.5	no	no	
	2.0	yes	no	102
	1.6	yes	no	80
3/2	5.0	yes	no	
	2.5	no	no	69
	2.7	yes	yes	81
	2.5	yes	no	69
3/3	1.7	no	no	
	2.5	yes	no	89
	2.0	yes	no	81
3/4	2.5	no	no	
	4.0	yes	no	116
	4.5	yes	no	130
3/5	2.0	yes	no	
	2.0	no	no	82
	1.7	no	no	90
	1.7	yes	yes	99
13 Expts.	MEAN 2.7±1.1 SEM cm min^{-1} n=34	82% of MMCs reaching caecum n=37	27% of MMCs reaching caecum n= 37	MEAN INTERVAL 112±39SEM minutes n=24

TABLE II Data from control EMG recordings of MMCs in sheep 1-3. The speed of migration of the MMC (Phase3) was calculated, and it's progress through the ileocaecal region was monitored. Where possible the time interval between successive MMC's was measured. The data summarised here are presented in full in Appendix B.
(nsd = not sufficient data to calculate velocity).

of 56± 9 SEM seconds. In sheep 3 the same type of activity was recorded from the terminal ileum at the same time as it occurred in the caecum (see Figure 3.2).

3.3.3 Ileal activity in fed animals.

The data from the experiments involving feeding is presented in Table III.

The interval between MMC phase three contractions in these recordings was taken as the time from the first observed MMC phase three (after which blood sampling and feeding occurred) to the appearance of the next MMC phase 3. The results from sheep 2 during the nematode infestation are not included in the data presented here.

The mean interval between MMCs while feeding was 141± 22 SEM minutes, equivalent to 10 MMCs per 24 hours. This is a substantial interval increase when compared with the interval between MMCs recorded during the control experiments ($P=0.02$). The rate of propagation of the MMCs through the distal ileum was 2.1± 0.7 SEM cm min-1. This average speed is not significantly different from the control values. ($P>0.05$)

Of the MMCs recorded post feeding, 73% passed through to the ICJ, and 25% reached the caecum. In less than 20% of instances was there phase three-like activity by the caecum preceding the arrival of an MMC at the ICJ. These results for MMC speed and progress across the ICJ are comparable to those in the control experiments.

3.3.4 Ileocaecal activity and Gastrin Levels.

The results of the gastrin analysis are recorded graphically against EMG activity for the first control experiment using sheep 1 in Figure 3.3. The data and

Feeding Experiments: MMC Phase 3 data.				
Sheep/ Expt. No.	MMC speed cm min^{-1}	To ICJ	To Caecum	Time to next MMC
1/1*	nsd	no	no	192
1/2	1.4	yes	no	102
1/3*	1.0	yes	yes	174
1/4*	1.5	yes	no	172
1/5	nsd			115#
2/3	1.3	yes	no	74
2/4*	3.3	yes	no	142
2/5	4.0	yes	no	100
3/1*	nsd	no	no	173
3/2*	3.0	yes	yes	172
3/3*	3.4	yes	yes	148
3/4	1.2	no	no	91
3/5	1.2	yes	no	163
13 Expts.	MEAN 2.1±0.7 SEM cm min^{-1} n=10	73% of MMC's reaching ICJ. n=12	27% of MMC's reaching ICJ. n=12	Mean Interval 141±22SEM minutes. n=12
Using * 7 Expts. only	MEAN 2.4±1.0 SEM cm min^{-1} n=5	71% of MMC's reaching ICJ. n=7	43% of MMC's reaching ICJ. n=7	Mean Interval 168±12SEM minutes. n=7

TABLE III

Analysis of EMG data from feeding experiments. Sheep 1-3 were fed 45 minutes after an MMC (Phase 3) was recorded in the distal ileum. The time interval to the next phase 3 was recorded, its speed calculated, and its progress through the ileocaecal region monitored.

* indicates those experiments in which an increase in serum gastrin was noted following feeding.

During the 5th experiment using sheep 1, normal MMC Phase 3 activity did not develop in the period recorded, though some regular activity did occur at the time indicated. This data was not used when calculating mean values.

(nsd = not sufficient data to calculate velocity).

The data summarised here are presented in full in Appendix B.

graphs of the other experiments are recorded in Appendix B.

As reported in 3.3.3 the interval between MMCs increased in the fed animals. Analysis of serum gastrin levels showed a noticeable increase within 30 minutes post feeding (a 'feeding response') occurred in 7 of 13 feeding experiments. (Sheep 1 feeding experiments 1,3 and 4; Sheep 2 feeding experiment 4; Sheep 3 feeding experiments 1,2 and 3.). If the mean interval between MMCs is considered for just these 7 experiments, a mean MMC interval of 168± 12 SEM minutes is obtained (8.6 MMCs per 24 hours). This very significantly different from the mean of the control values (P= 0.0007).

In comparison the mean MMC interval in the feeding experiments NOT showing a gastrin 'feeding response' was 92± 11 SEM minutes. When compared with control mean MMC intervals, there was no significant difference (P= 0.37).

3.3.5 Results from the nematode infected sheep 2.

As mentioned in section 3.3.2, sheep two was found to have been infected with nematodes. The results from sheep 2 during this period are summarised in Table IV. The data are presented fully in Appendix B.

These results show a higher than average frequency of MMCs - 18 per 24 hours, (mean interval of 81± 13 SEM minutes), compared to only 10 per 24 hours (mean interval 143± 45 SEM minutes) after treatment. This is a significant difference (P=0.004).

3.3.6 Results following Pentagastrin administration.

The results from experiments during which pentagastrin was injected subcutaneously into sheep 1 and 3 are shown in

A

Sheep 2 "control" experiments during worm infestation. MMC (Phase 3) data.				
Sheep/ Expt. No.	MMC speed cm min^{-1}	To ICJ	To Caecum	Time Interval
2/1	1.3	yes	yes	126
	3.7	yes	yes	81
	2.5	yes	no	
2/2	5.5	yes	yes	
	6.0	yes	no	61
	4.0	yes	no	48
	2.5	yes	no	85
2/3	3.4	yes	no	
	2.8	yes	no	76
	2.2	yes	no	68
2/4	4.5	yes	no	
	3.0	yes	no	77
	3.4	yes	no	109
	3.7	yes	no	78
4 Expts.	MEAN 3.3±1.3 SEM cm min^{-1} n=14	100% MMC's reaching ICJ n=14	21% of MMC's reaching Caecum n=14	Mean Interval 81±14SEM minutes n=10

B

Sheep 2 "feeding" experiments during worm infestation. MMC Phase 3 data.				
Sheep/ Expt. No.	MMC speed cm min^{-1}	To ICJ	To Caecum	Time to next MMC
2/1	3.0	yes	no	84
2/2	1.2	yes	no	59
2 Expts.	MEAN 2.1 cm min^{-1} n=2	100% of MMC's reaching ICJ n=2	0% of MMC's reaching Caecum n=2	Mean Interval 72 minutes n=2

TABLE IV (A and B)
Analysis of EMG data from sheep 2 during the nematose infestation, while serum gastrin levels were high (around 100 f mol ml^{-1}). The speed of migration of the MMC (Phase 3) was calculated, and its progress through the ileocaecal region monitored. Where possible, the time interval between successive MMCs was measured. Tha data summarised here are presented in full in Appendix B.

Table V. The data and graphs are presented fully in Appendix B.

No significant difference was found between these recordings and the control recordings with respect to MMC propagation velocity or the frequency of MMCs.

3.4 Discussion.

3.4.1 EMG sequences of the distal ileum and caecum.

Myoelectric activity of the ICJ appears predominantly dependant upon the arrival of the MMC, since very little activity was observed in this area that could not be categorized as part of the MMC.

Bueno et al (1975) reported a mean interval between phase three contractions of 88± 18 SEM minutes in the sheep jejunum. This is not significantly different from the control results obtained in these experiments (112± 16 SEM) ($P>0.01$).

From this comparison it would appear that a high percentage of the MMCs progressing down the sheep small intestine reach the last 10 cm of the distal ileum. Bueno et al (1975) recorded a very high rate of MMC migration along the jejunum of the sheep (17.8± 6.5 SEM cm min^{-1}). The rate of MMC migration in the ileum in this experiment was expected to be slower than the jejunal speed recorded by Bueno et al, as the MMC generally travels more slowly as it moves distally (Quigley, Phillips and Dent, 1984). The mean rate of propagation in control recordings in this experiment was 2.7 cm min^{-1}, though some phase 3 sequences were measured at speeds of up to 6 cm min^{-1}. In the light of the report by Bueno et al, the movement of the MMC through the sheep ileum at these rates seems reasonable. However, as pointed out by

Pentagastrin treatment experiments. MMC Phase 3 data.				
Sheep/ Expt. No.	MMC speed $cm\ min^{-1}$	To ICJ	To Caecum	Time Interval
1/1	2.2	yes	no	178
1/2	1.3	yes	no	91
1/3	1.3	yes	yes	126
3/1	4.0	yes	no	116
3/2	2.4	yes	no	121
3/3	2.1	yes	no	107
6 Expts.	MEAN 2.2±0.8 SEM minutes n=6	100% of MMCs reaching ICJ n=6	17% of MMCs reaching Caecum n=6	Mean Interval 123±23SEM minutes n=6

TABLE V

Analysis of EMG data from sheep 1 and 3. Pentagastrin was administered after the arrival of an MMC (Phase 3) at the ICJ. The migration speed of the next phase 3 activity was calculated, its progress through the ileocaecal region monitored and the time interval to the next phase 3 measured. The data summarised here are presented in full in Appendix B.

Weisbrodt (1987), interpretation of rate of movement along the intestine is dependant upon the distance between recording sites, and this can vary considerably depending upon the tone and activity in the longitudinal muscle layer of the intestine wall between the electrodes at the time of recording. What may be 10 cm or 50 cm as measured during implantation of the electrodes could be considerably different at a later time, and any data on the rate of propagation of activity along the intestine must be viewed with this consideration in mind. Nonetheless, the figures obtained in this experiment do appear consistent with those reported in the literature for the ileum, being somewhat faster than in other species, but slower than that reported for sheep jejunum.

The transmission of the MMC across the ICJ into the caecum appears to occur at a rather low frequency compared to the dog (just over 20% passing to the sheep caecum compared to 86% transmission in dogs). The coordination between caecal and distal ileal activity in the sheep as reported by Fioramonti (1981) was not observed in this experiment. Inactive periods of the caecum tended to occur at the same time as periods of ileal inactivity, not during or slightly before an MMC (Phase 3) arrived at the ICJ. However 20% of the MMCs arriving at the ICJ were preceded by a high level of myoelectric activity recorded from the caecum, and this could be interpreted as a means of moving digesta into the proximal colon in order to "make room" for the arrival of more from the ileum, as suggested by Fioramonti (1981). The effect does not appear to occur consistently, however.

The regular burst activity (RBA) noted in the ileum of the 3rd sheep has some similarities with the 'minute rhythm' described by Fleckenstein, Bueno, Fioramonti and Ruckebusche (1982) for other species, although this type of activity has only been reported in the proximal small

intestine, not in the ileum. It was not possible to estimate the rate of propagation in these experiments, as the activity appeared to occur simultaneously in the last 5-10 cm of the ileum and the caecum. In sheep 3 this activity occurred at the ileocaecal junction as well, and was coordinated with caecal activity. This could be interpreted as a mechanism to prevent backflow of digesta during periods of caecal activity.

During the course of this experiment, no other patterns of activity such as the Discrete Clustered Contractions or Prolonged Propagated Contractions described for other species was observed.

3.4.2 Serum Gastrin and EMG activity in the distal ileum.

The lack of correlation between serum gastrin levels and EMG activity in the distal ileum of the sheep studied indicate that gastrin has no direct effect on ileal motility. This is supported by the observation that the administration of pentagastrin to these animals made no noticeable difference to ileal EMG activity. Unfortunately the inability of the gastrin assay to detect pentagastrin meant that it was not possible to determine if the administration of the hormone actually had an appreciable effect on serum gastrin levels, so the significance of these results cannot be properly evaluated. Further work using either an assay sensitive to pentagastrin or the injection of the 17 amino acid form of gastrin (G17) would be required.

Of particular interest in the results from these experiments was the increase in the mean interval between MMCs following feeding. This was highly significant when just those experiments showing a gastrin `feeding response' were considered. Since the injection of pentagastrin did not demonstrably affect the frequency of

MMCs in the sheep, the high correlation between the gastrin feeding response and the lengthening of interval between MMCs would indicate that a common causative factor resulted in both the change in MMC frequency and the elevation in serum gastrin. That the change in MMC frequency is not directly due to serum gastrin changes is supported by results obtained from the nematode infected sheep, where high serum levels of gastrin were associated with a decrease in interval between MMCs, rather than an increase. (The decrease in interval between MMC phase 3 activity was presumably due to some effect of the parasites on intestinal motility, raising questions on the effect of nematodes on sheep intestinal activity patterns and opening another avenue worthy of further study).

The link between the change in MMC interval and the gastrin `feeding response' could be due to the release of hormones other than gastrin (such as insulin or CCK) but released concurrently, or due to the physical presence of the digesta as detected by the enteric nervous system. It may be a combination of these and other factors.

In monogastric species feeding causes the disruption of the MMC pattern (Reinke, Rosenbaum and Bennett, 1967), and the same has been shown to occur in overfed sheep (Ruckebusch and Bueno, 1975). The lengthening in the MMC interval observed in these experiments might be due to some MMC disruption in the small intestine similar in origin to the effect of feeding on monogastric intestinal activity. Whatever the cause, the reduction in the frequency of MMCs arriving at the ICJ during feeding would reduce the rate of transfer of digesta from small to large intestine.

The effect of feeding on small intestinal motility may be a transient effect only occurring in sheep fed distinct 'meals', and may or may not occur in sheep feeding *ad*

lib.. It's significance cannot yet be determined, and will require further investigation.

CHAPTER 4
Pharmacological studies of the ileocaecal region in the sheep.

4.1 Introduction

It has been demonstrated in vitro in humans, monkeys, cats and dogs, that isolated circular muscle strips from the ICJ respond to sympathetic agonists such as adrenaline by contracting, while muscle from the adjacent regions of ileum and colon respond by relaxing. This response is blocked by phentolamine, suggesting an alpha-adrenergic receptor is involved in mediating this response (Gazet and Jarrett, 1964; Conklin and Christensen, 1975; and Cardwell et al, 1981).

In vivo results by Pahlin and Kerwenter (1975) supported these findings, showing in anaesthetised cats that sympathetic amines elicited an excitatory response from the ICJ which could be blocked by alpha receptor blocking agents, but remained after a beta receptor blockade.
In this study an attempt was made to examine in vitro and in vivo the response of the sheep ICJ to various pharmacological agents.

4.2 Materials and Methods

4.2.1 In Vitro

Specimens were obtained from 10 southdown cross sheep killed by a captive bolt and subsequently exsanguinated. The abdomen was opened along the linea alba, and the terminal ileum and caecum identified and removed as soon as possible. The specimen was then rinsed with saline (0.9% NaCl) and the last 6cm of ileum removed. This was placed in approximately 25 ml of Ringer solution in a 100 ml conical flask. (Tyrode Ringer solution: 8.0 g l^{-1} NaCl;

0.2 g l^{-1} KCl; 0.2 g l^{-1} $CaCl_2$; 0.1 g l^{-1} $MgCl_2$; 1.0 g l^{-1} $NaHCO_3$; 0.05 g l^{-1} NaH_2PO_4; 1.0 g l^{-1} glucose). The flasks were cooled by storage on ice for transport to the laboratory, which took up to 20 minutes. The flasks were then aerated by gently bubbling Carbogen gas (95% oxygen and 5% carbon dioxide) through the Ringer solution. Later, each specimen was removed from its flask and trimmed to provide two transversely oriented strips of intestinal wall, one 5cm from the ICJ and the other from the ICJ itself. The resultant strips were approximately 0.5 cm wide and 1.5 cm long, with circular muscle fibres oriented longitudinally. These were placed in a 20 ml gut bath, attached to a writing lever, suspended in Ringer solution at 39°C, with Carbogen gas continually bubbling through it. (See Figure 4.1).

Drugs used were applied to the gut bath via a 1 ml graduated syringe. For example, a 0.1 ml sample of a drug (concentration 0.5 mg ml^{-1}) in the 20 ml of Ringer solution would give a final concentration of 2.5 µg ml^{-1}.
The following drugs were used in this part of the investigation:
Noradrenaline (Levophed, Winthrop); Propanolol (Inderol, ICI); Phentolamine (Regitine, Ciba Geigy Ltd, Switzerland); Acetyl Choline (Sigma); Atropine (Sigma); Isoprenaline (Isoprel, Winthrop).

After the administration of a drug, the muscle activity was recorded for a period 2-3 minutes or until a normal pattern of activity resumed. The gut bath was then flushed with 2 changes of Ringer solution and allowed to stabilize for several minutes before the next drug was applied.

4.2.2 In Vivo (Anaesthetised sheep)

Four of the sheep prepared as previously described in Chapter 2.2 - 2.4 were used in this experiment. With the

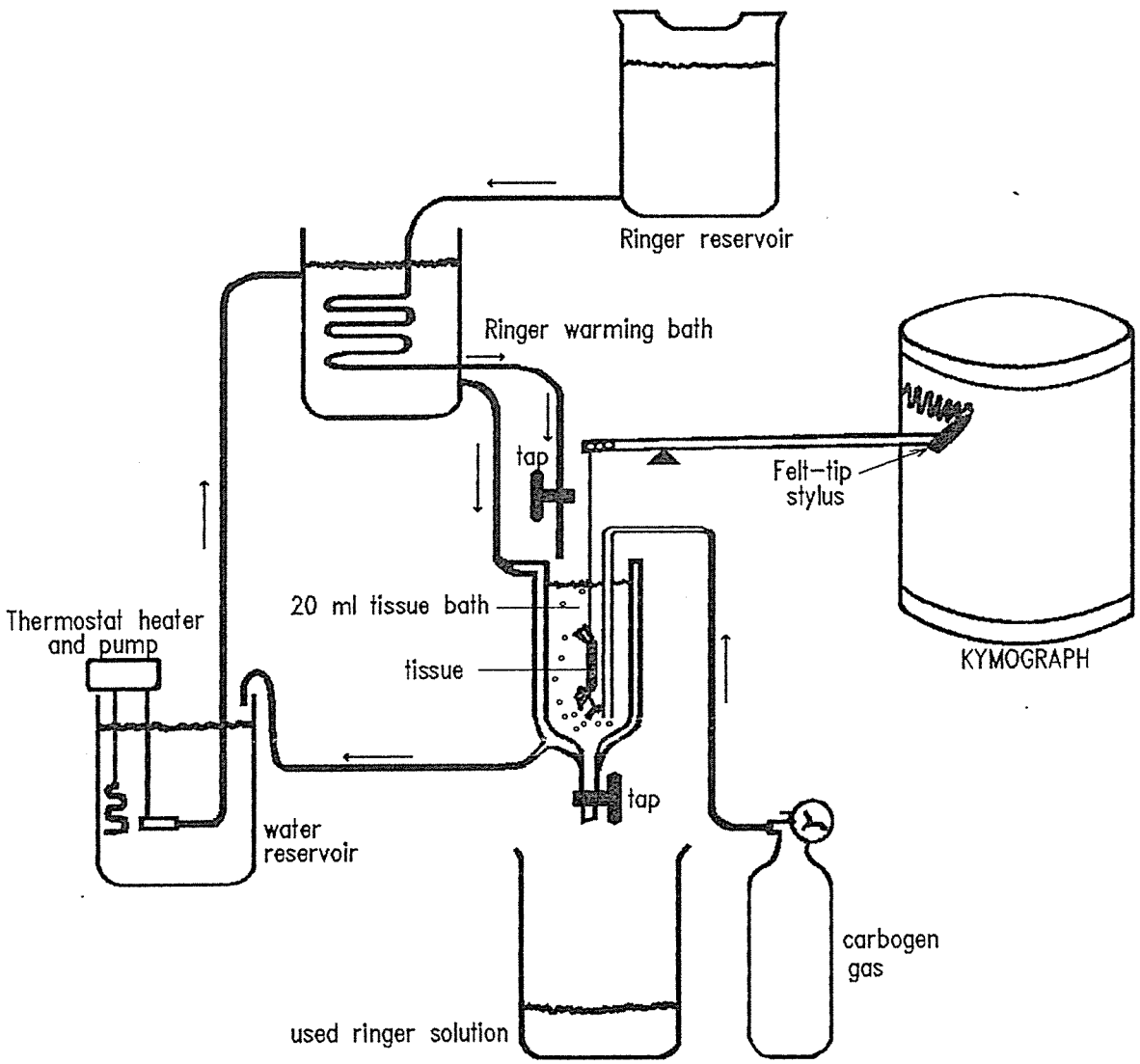


FIGURE 4.1 Schematic drawing of the in vitro experimental apparatus.

caecum and distal ileum in a warmed perspex tray, EMG and pressure recordings were made from the distal ileum and ICJ.

The following drugs were used in this part of the investigation:
Adrenaline (May and Baker Ltd, England) 1-4 μg kg^{-1} bolus
or 1.8 to 4.0 μg kg^{-1} min^{-1} infusion;
Propanolol (Inderol, ICI) 0.5-1 mg kg^{-1} bolus;
Pentagastrin (Peptavlon, ICI) 0.05 μg kg^{-1} min^{-1} infusion;
Carbachol (May and Baker Ltd, England) 5 μg kg^{-1} bolus.

All drugs were introduced into the animal by means of a femoral venous cannula, and in the case of a bolus, were followed with 2-3 mls of saline solution to flush the cannula.

The drugs were administered in the following sequence, with recovery periods of approximately 5-10 minutes after the end of the infusion or bolus injection:

a) Pentagastrin infusion.
b) Adrenaline bolus or infusion 3-5 minutes duration, at least 3 repeats.
c) Propanolol bolus, followed by adrenaline infusion or noradrenaline infusion.
d) Carbachol. This was the last drug administered to each animal before euthanasia.

Random control infusions of saline (0.9%) were made during the course of the experiments.

4.3 RESULTS

4.3.1 In Vitro Pharmacology of the ICJ.

Of the two specimens obtained from each of 10 sheep, less than half (9 tissue sample, 6 from the ileum and 3 from the ICJ) contracted in response to acetylcholine when

administered at a rate of 2.5 μg ml^{-1}. Only in two cases did sustained, rhythmic contractions develop in the muscle strips. These belonged to different animals, the third and the seventh in this series of in vitro experiments. In all other responding tissue strips any effects of acetylcholine disappeared within seconds following its application, and the recording returned to baseline levels. Application of adrenaline and noradrenaline had no noticeable effect on these tissue samples.

In the two samples which did produce consistent rhythmic contractions, the tissue from the third animal was from the ICJ, while the sample from the seventh animal was from the ileum 5cm proximal to the ICJ. Both tissue samples responded in the same manner. Both samples averaged 11 contractions per minute (range 10-12).

Acetylcholine - both responding muscle strips contracted vigorously in response to this drug at concentrations ranging from 2.5 μg ml^{-1} down to 0.1 μg ml^{-1} (Figure 4.2). This effect was blocked by the administration of atropine (30 μg ml^{-1}) to the tissue bath before the application of the acetylcholine. Of the other muscle strips which showed some response to acetyl choline, it was consistently excitatory and blocked by atropine.

Noradrenaline - both responding muscle strips were inhibited by noradrenaline. Concentrations as low as 0.05 μg ml^{-1} caused a slight reduction in contraction amplitude for 6-10 cycles. Concentrations of 0.1 μg ml^{-1} led to inhibition for 2-3 minutes, and concentrations of 2.5 μg ml^{-1} or more caused a sustained (5 minutes plus) inhibition of activity. The response to noradrenaline at 1 μg ml^{-1} was not greatly affected by the prior application of Propanolol (30 μg ml^{-1}). Phentolamine at concentrations of 30 μg ml^{-1} or more did reduce the inhibitory response of the muscle to Noradrenaline. (Figure 4.3).

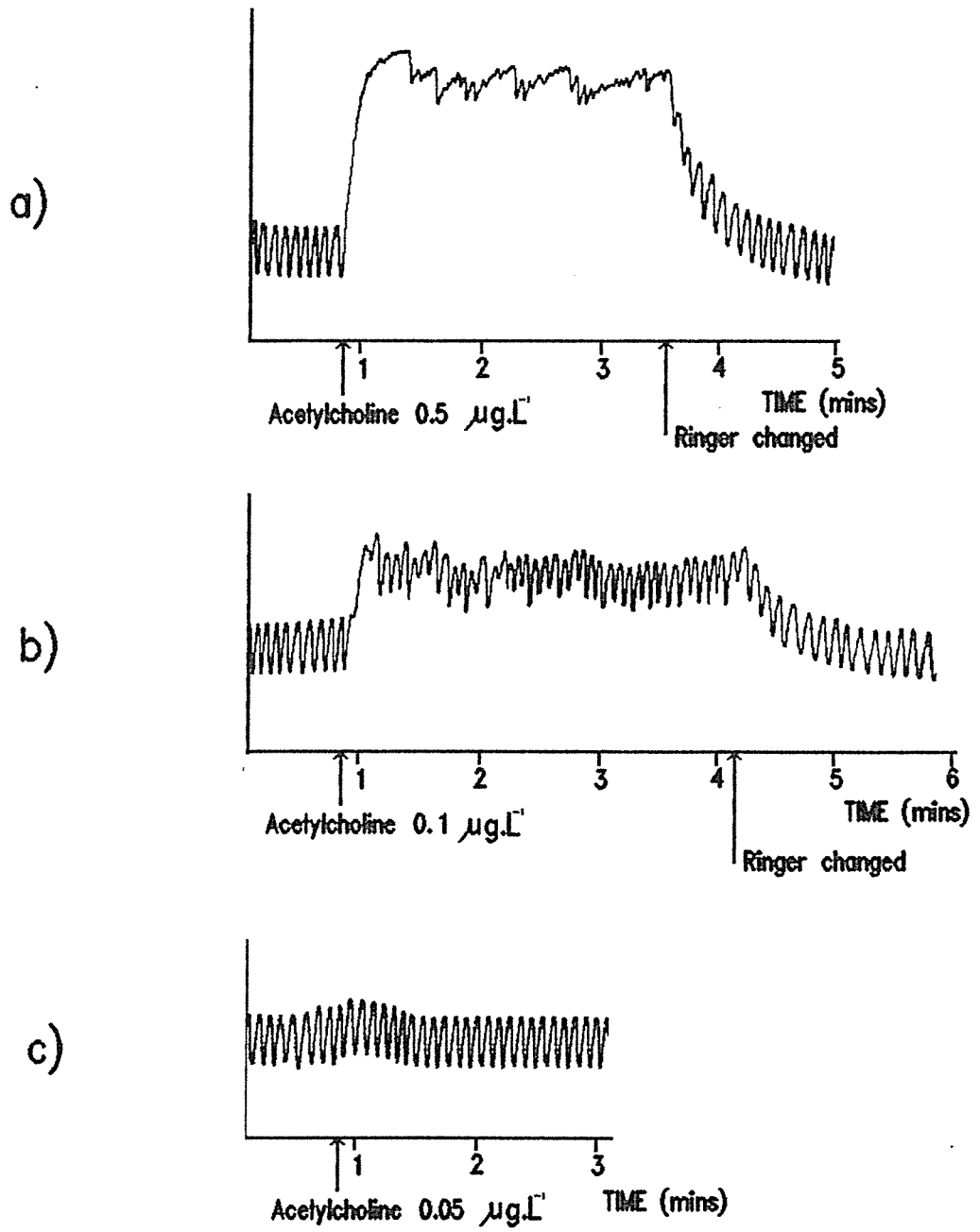


FIGURE 4.2 The effect of acetylcholine on circular muscle taken from the sheep ileocaecal junction (sheep 3).

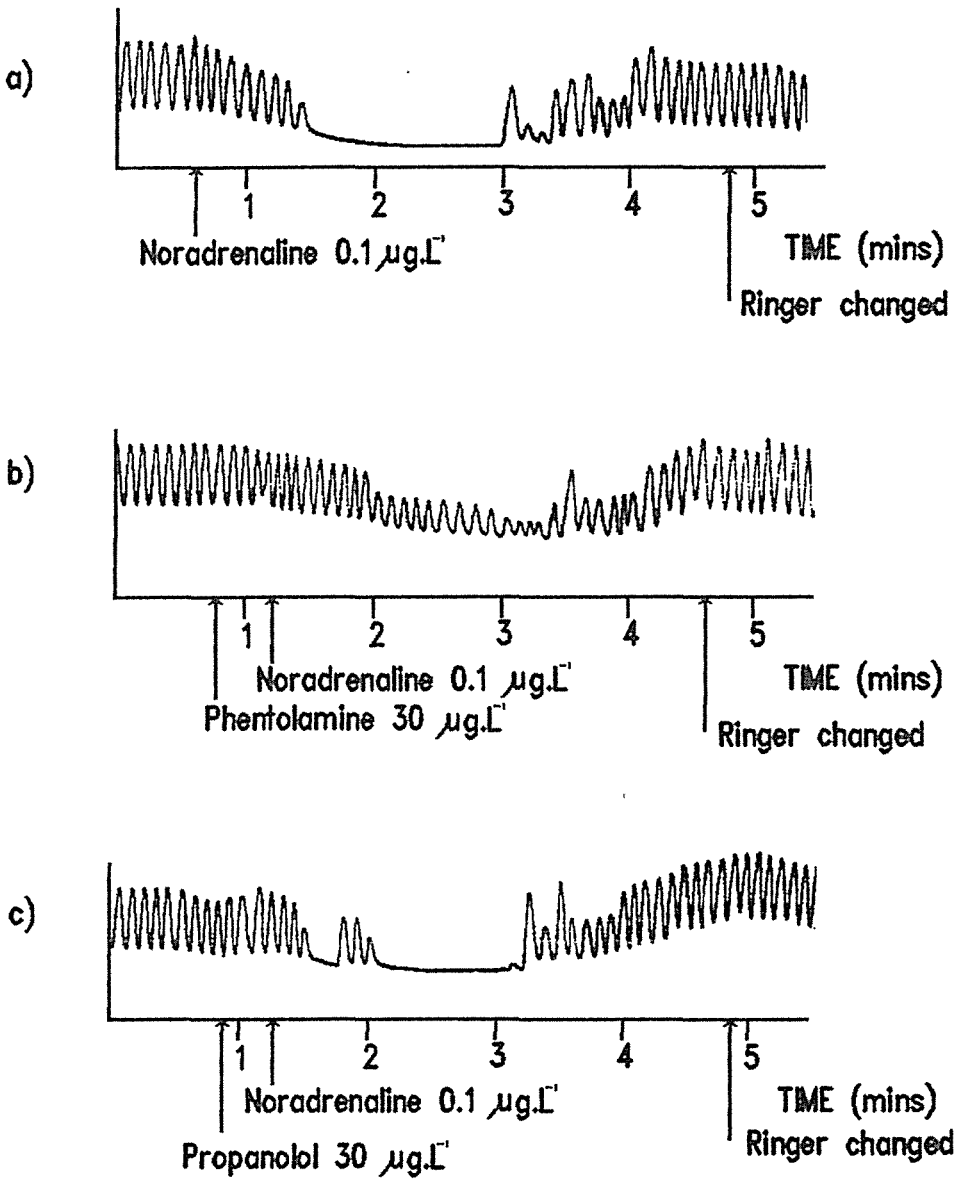


FIGURE 4.3 Inhibition of circular smooth muscle from the ileocaecal junction in response to noradrenaline (a). This effect was largely blocked by phentolamine (b), but not propanolol (c).

Isoprenaline - both responding muscle strips were inhibited by isoprenaline. A concentration of 0.05 μg ml^{-1} was sufficient to abolish contractile activity for approximately 2 - 3 minutes, while the application of 0.5 μg ml^{-1} isoprenaline caused a sustained inhibition lasting over 5 minutes, ending only after repeated flushing of the tissue bath.

Application of propanolol at 10 μg ml^{-1} immediately prior to 0.05 μg ml^{-1} isoprenaline did not prevent the isoprenaline from inhibiting the activity of the strip. However propanolol at concentrations of 20 μg ml^{-1} or more administered prior to the application of isoprenaline did block the inhibitory effect, and the contractile activity was sustained. (Figure 4.4).

4.3.2 In vivo pharmacology of the ICJ

The pentagastrin intravenous infusions had no observable effect on ICJ activity, as observed by EMG and pressure recordings. (Figure 4.5).

The response to adrenaline administered by bolus or infusion was quite consistent. A bolus of 2.4 μg kg^{-1} of adrenaline was sufficient to raise the blood pressure by up to 50 mmHg, and reduce EMG activity from all intestinal electrodes for over 3 minutes. Infusions of Adrenaline greater than 2 μg kg^{-1} min^{-1} also reduced EMG activity, and infusions of greater than 3.2 μg kg^{-1} min^{-1} inhibited all EMG and pressure activity as recorded from the terminal ileum. (See Figure 4.6)

After the administration of propanolol, subsequent infusion of catecholamines resulted in no observable intestinal inhibition with levels that had previously been effective.

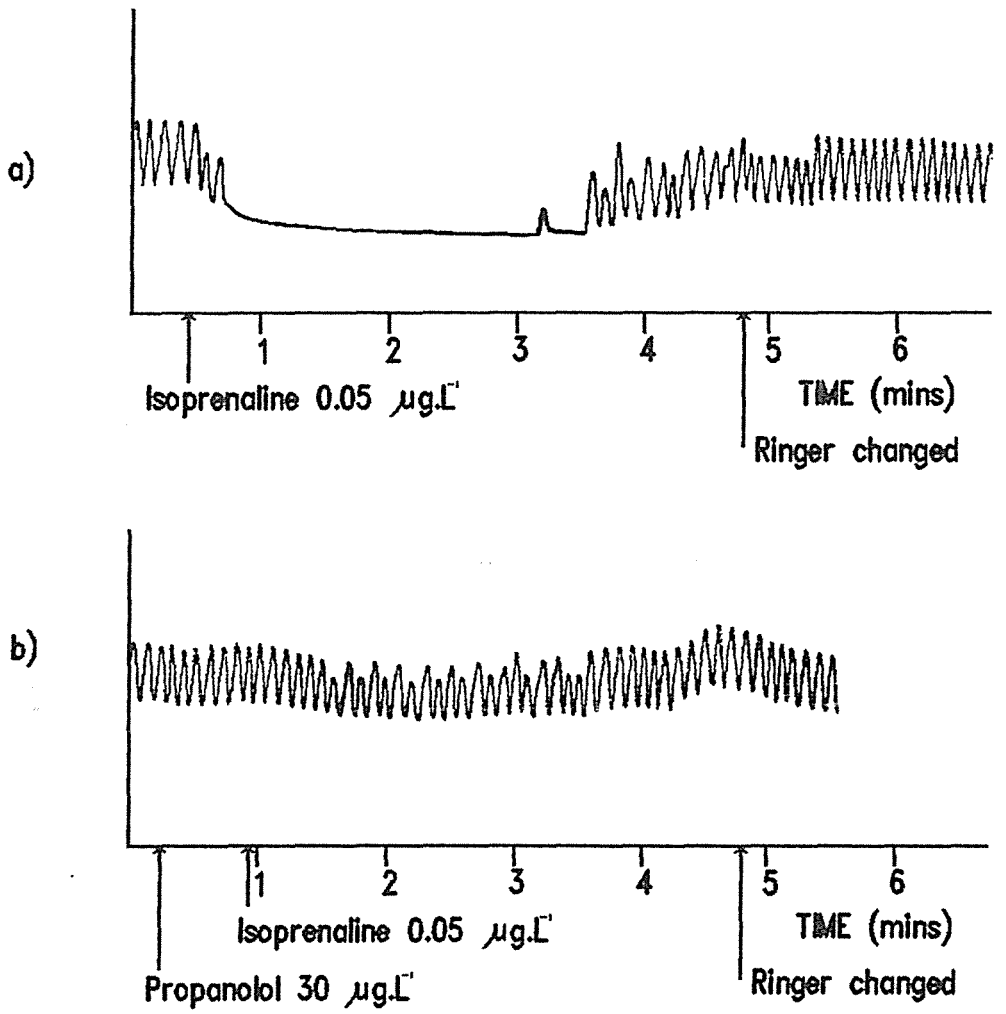


FIGURE 4.4 Inhibition of circular smooth muscle from the ileocaecal junction in response to isoprenaline (a). This effect was almost completely blocked by propanolol (b).

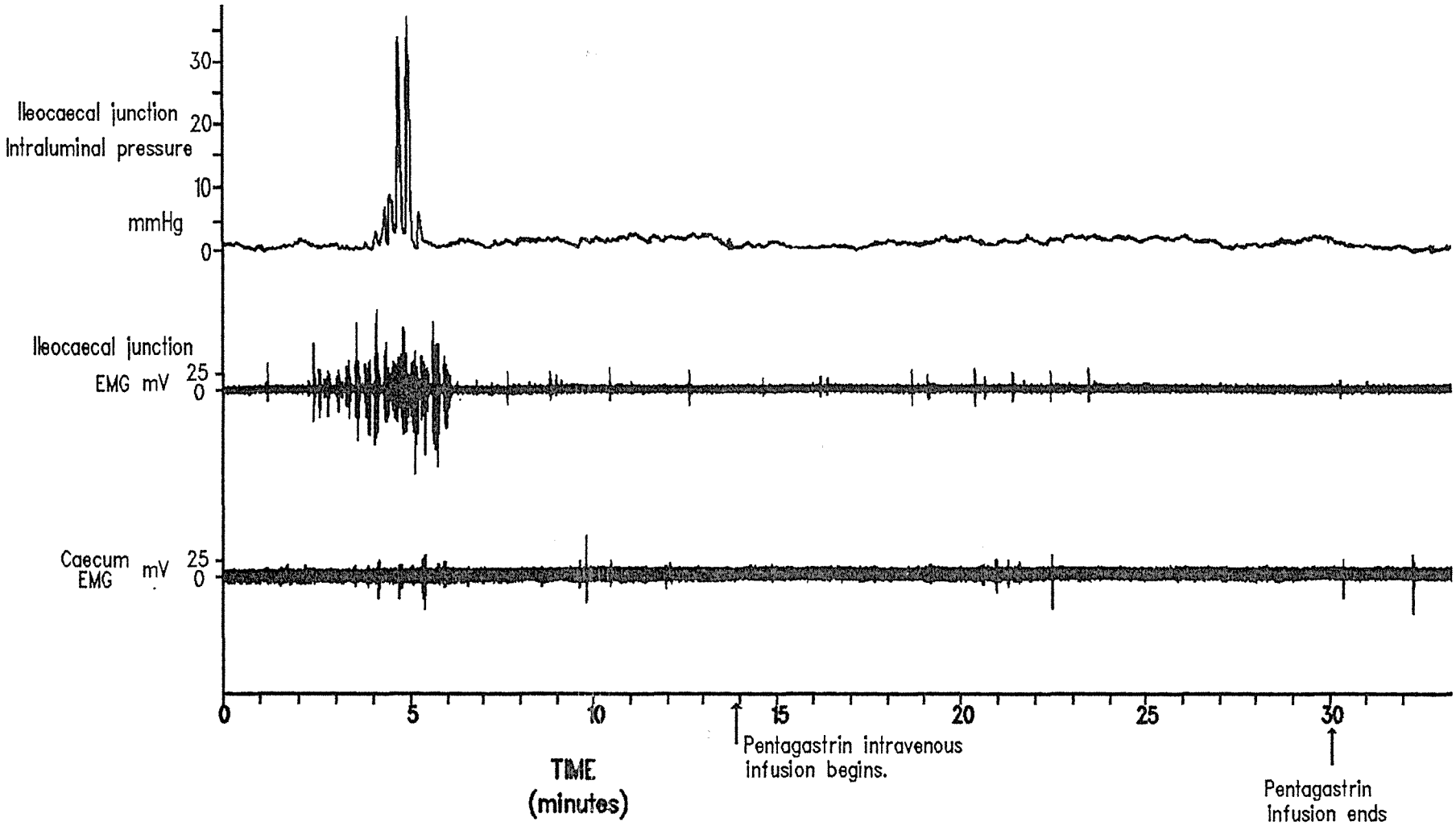


FIGURE 4.5 Pentagastrin intravenous infusion (0.05 ug.kg .min) had no noticeable effect on the activity of the ileocaecal junction (intraluminal pressure recording and upper EMG trace). Caecal activity appeared similarly unaffected. (Recordings from sheep 5).

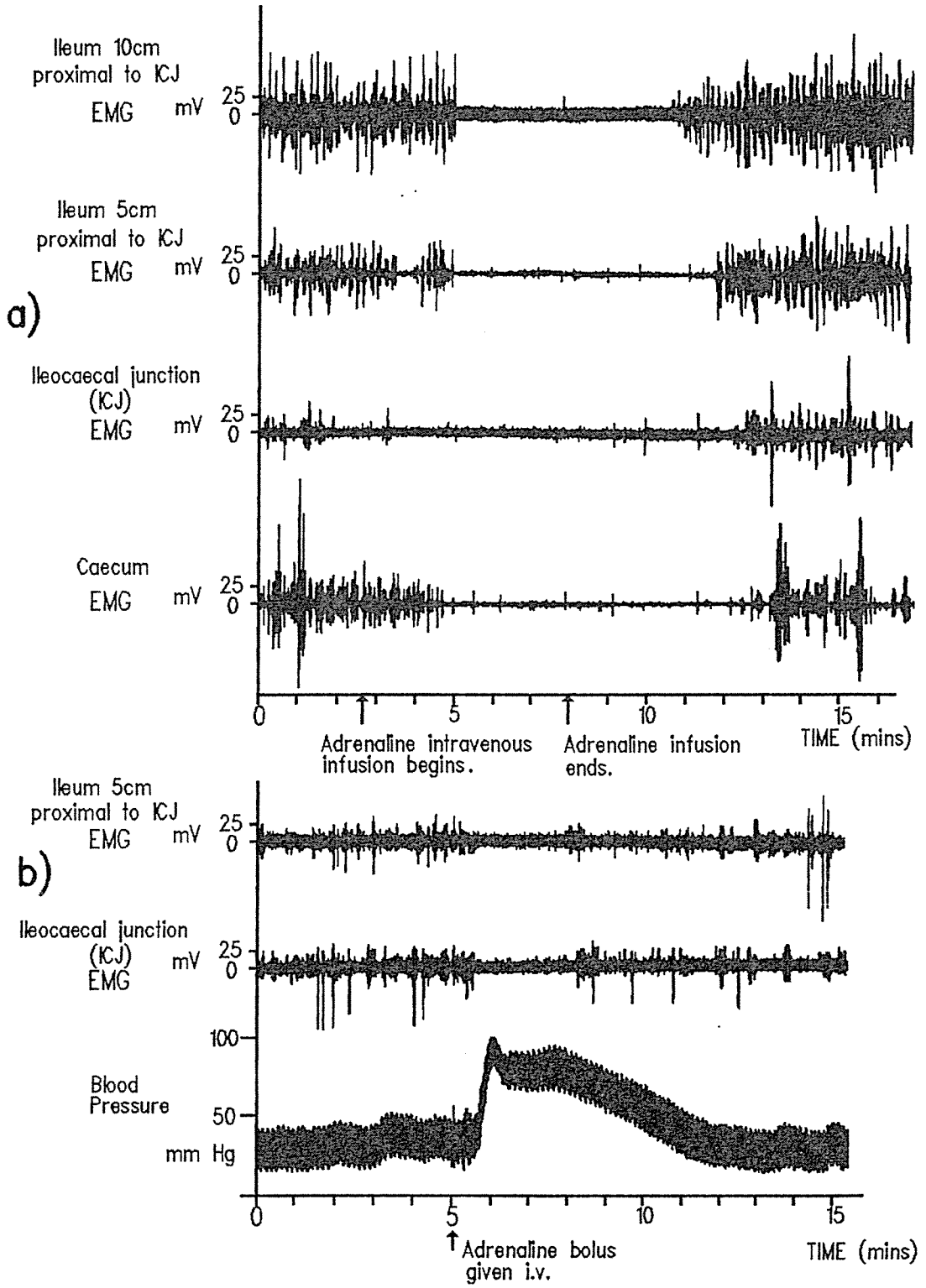


FIGURE 4.6 a) Adrenaline infusion (2.4 μg.kg^{-1}.min^{-1}) administered via a femoral arterial cannula, reduced all ileal and caecal activity as measured by EMG recordings. (Sheep 5)

b) Adrenaline bolus (2.4 μg.kg^{-1}) raised blood pressure and resulted in some intestinal inhibition.

Carbachol consistently resulted in a marked increase in activity of the entire region being observed. (See Figure 4.7)

4.4 Discussion

Ileus is a well defined state of no motor activity in small pieces of fresh intestine in vitro at 37°C (Wood, 1981). It is believed to be due to the unremitting activity of intrinsic inhibitory neurones, and would appear to be responsible for the low success rate in achieving regular contractions in the muscle strips in the in vitro part of this experiment.

The results from the two samples that did spontaneously contract and relax regularly showed no differences in their responses to the drugs used. Both showed characteristics of normal intestinal muscle, being stimulated by acetylcholine and inhibited by catecholamines. This inhibition was shown to occur in response to both alpha (noradrenaline) and beta (isoprenaline) agonists, and these effects were blocked by alpha and beta antagonists (phentolamine and propanolol) respectively.

If muscle with sphincteric properties similar to that described by Pahlin and Kerwenter (1975) does occur in the sheep ICJ, it would appear likely that it is restricted to the short length of muscularis within the ileal papilla, and not at the actual junction between the ileum and caecum.

The in vivo experimental results supported the conclusion drawn from the in vitro experiments, for at no time was an exitatory response of the ICJ to catecholamines observed. This is in contrast with Pahlin (1975) whose work in cats strongly supported the previous in vitro work, suggesting

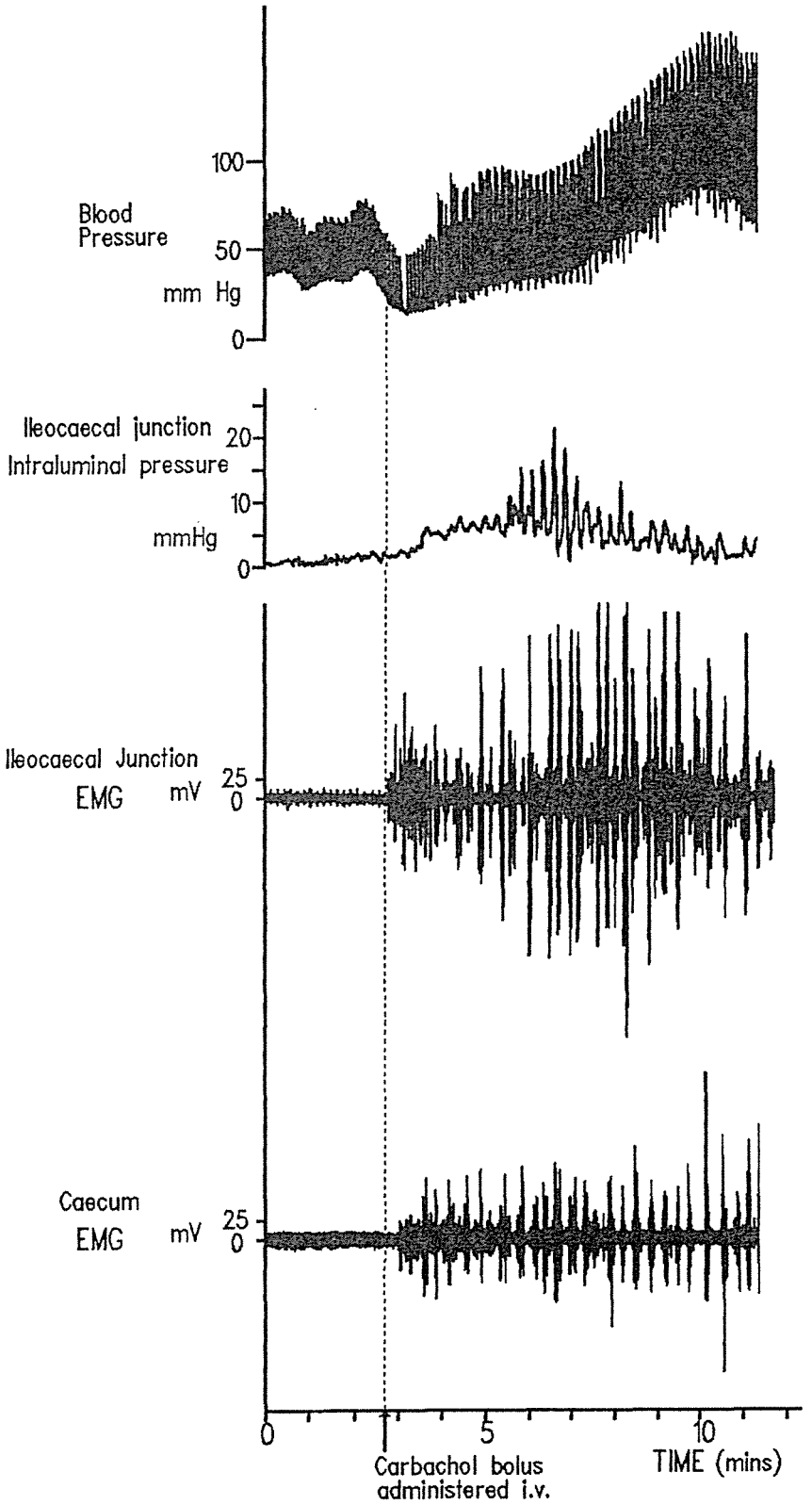


FIGURE 4.7 Carbachol administered intravenously as a bolus (5 μg.kg^{-1}) results in increased activity in the ileocaecal region, including the ileocaecal junction. (Sheep 2).

that alpha adrenergic receptors were responsible for a contractile response to catecholamines, and that beta receptors may have a tonic inhibitory effect. In the in vivo part of the experiment, propanolol infusions did not result in any observed increase in activity in the ICJ as would be expected if a tonic inhibition were removed. The results of this series of experiments suggest that the ICJ of the sheep does not exhibit an excitatory response to adrenergic agonists as reported for other species, either in vitro or in vivo. In addition this region of the sheep does not exhibit any sign of tonic inhibitory innervation mediated by ß adrenergic receptors. The response of the ICJ to the pharmacological agents tested was the same as found in the adjacent ileum.

The proposal by Bass, Ustach and Schuster (1970) that sphincteric tissue in the gut contracts in response to catecholamines is generally accepted (Weisbrodt, 1987). The apparent absence of this response in sheep is in direct contrast to the results in other species, and this is particularly surprising given the suggestion in chapter 2 of this thesis that pressure recordings from the sheep ICJ appear consistent with the existence of a sphincter. The results of this experiment are not conclusive, however. The ileus observed in most of the in vitro samples leaves insufficient data to validate any conclusion. In addition, the finding in Chapter 2 that any sphincteric effect only occurred over a very short length of intestine, probably within the ileal papilla, means that we cannot be confident that the muscle strips for the in vitro experiments were taken from the sphincteric region.

Further in vitro work specifically on the tissues within the ileal papilla could be illuminating in this regard, if the problem of ileus in the tissue samples could be overcome. The alternative is to concentrate on the intact

animal, though the short (less than 5mm) length of elevated pressure within the ICJ (where it was detected) poses practical problems in recording EMG and intraluminal pressures from the short zone within the ileal papilla. A side hole catheter was used to monitor intestinal intraluminal pressure, as this is believed to give a more accurate indication of actual physiological sphincter pressures due to minimal intestinal distention (Quigley, Phillips and Dent, 1984). However for monitoring responses to pharmacological agents in vivo, it may be more appropriate to use small balloons for recording, as they could be more easily placed with confidence across a very short sphincteric region and be capable of detecting responses from it. An alternative is the method described by Pahlin (1975), where saline is infused at a constant rate into the terminal ileum. The fluid pressure within the terminal ileum was monitored, as was the rate of saline flow through the ICJ, giving a good idea of sphincteric activity. The technique, however, is very invasive, with the terminal ileum being completely sectioned tranversely, and the caecum tightly tied off with a ligature. The effect of this treatment on the intramural innervation of the ICJ could be significant.

In conclusion, available results from this experiment suggest there is no difference between the adrenergic innervation of the ICJ and the adjacent small intestine in the sheep. This is in contrast with the published results for other species, but requires further work for confirmation.

CHAPTER 5

HISTOLOGY OF THE ILEOCAECAL REGION OF THE SHEEP.

5.1 Introduction

Very little information has been presented in the literature about the histology of the ileocaecal junction of any species. Conklin and Christensen (1975) reported a thickening in the muscularis at the level of the ileocaecal junction in the cat, but gave no details or data in support of this claim.

Recently there has been considerable interest in peptidergic innervation and control of the gut (Polak and Bloom, 1981; Costa, Furness, and Llewellyn-Smith (1987). The relatively recent advances in immunocytochemistry and radioimmunoassay have resulted in an increased awareness of the possible implications of the effects of these substances on gut control and motility.

This study was made first to determine if a zone of muscular thickening occurs at the ICJ in the sheep and second to determine if there is an observable difference between the ICJ and the more proximal ileum, using histological and immunocytochemical methods.

5.2 Materials and Methods.

5.2.1 Initial Sample processing.

Six adult sheep were slaughtered by captive bolt and subsequent exsanguination. Immediately following death, the abdominal viscera were exposed and the ileocaecal junction identified. The distal 120mm of the small intestine was then removed, including the ileal papilla with a small amount of surrounding caecal tissue. Each

sample was then rinsed in 0.9% saline, and trimmed to provide samples for further processing. In three sheep (designated A, B, and C) 10 mm long sections of the intestine were taken, one from the ICJ, and the midpoint of the other being from a site 100mm proximal to this (Figure 5.1). These were samples for later transverse sectioning (T/S) of the intestine wall. In sheep D, E and F the distal 40mm of the ileum was taken, to be used for longitudinal sectioning (L/S). The tissue samples thus obtained were immersed in Bouin's fluid (see Appendix C) for 1 day (tissues from sheep A, B and C) or for 2 days (tissues from sheep D, E and F) for fixation, following which the tissues were stored in 70% ethanol until processed.

After fixation the samples were further trimmed to 5mm long (samples from sheep A, B and C) or 30mm long (samples from sheep D,E and F) and then dehydrated, cleared and impregnated with paraffin wax using an automatic tissue processor (Shandon Elliot Scientific Co., England) according to the schedule shown in Table VI.

The tissue samples were then embedded in paraffin wax blocks and sectioned to 5 µm using a sliding microtome (Reichert, Austria). The sections were floated on warm (45°C) water to allow them to flatten, and then were transferred to a 3"x1" glass slide that had been lightly smeared with PVA glue (Pettersen Chemicals, NZ) to improve adhesion of the section to the glass. The sections were then air dried overnight at 60°C.

5.2.2 Section rehydration and staining.

Before staining, all sections were dewaxed by immersing in two xylene baths (5 minutes in each), and then rehydrated by rinsing briefly in absolute ethanol (twice), 70% ethanol and then tap water.

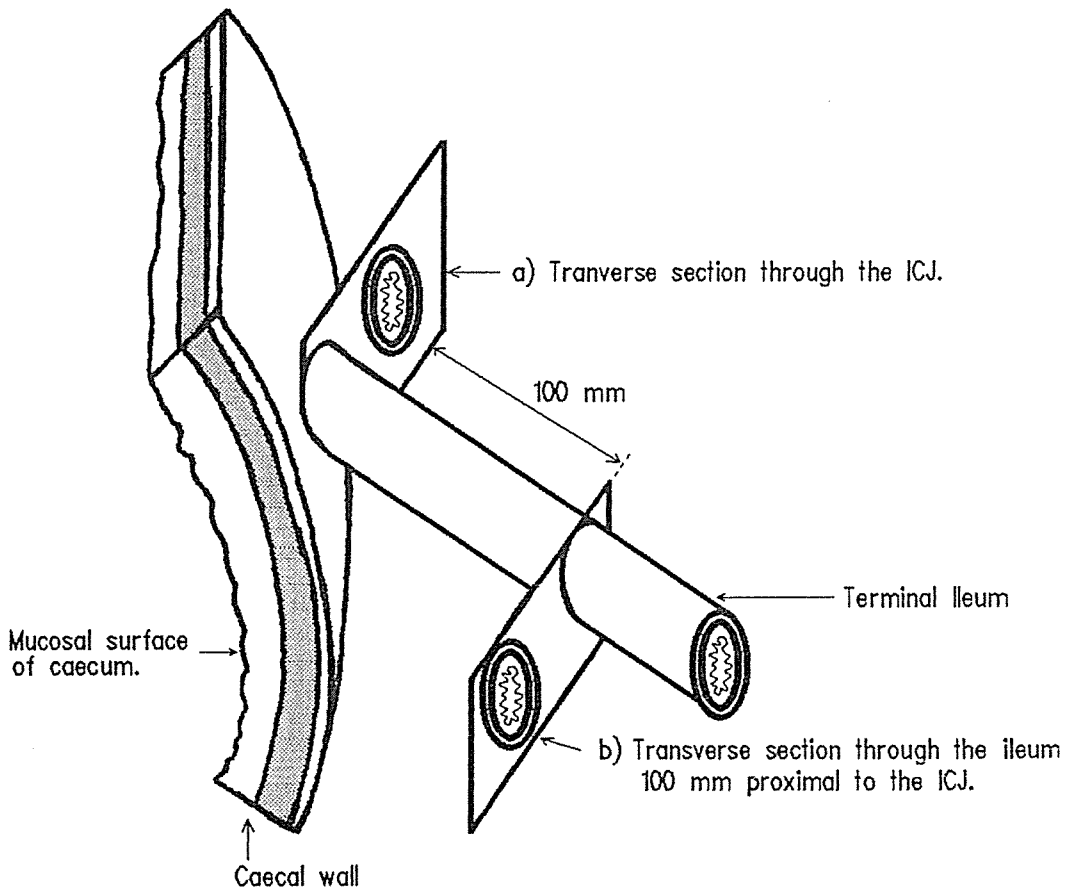


FIGURE 5.1 Schematic diagram of the the ileocaecal junction showing the orientation of the tranverse sections taken for histology. a) Through the ileocaecal junction (ICJ) and b) through the terminal ileum 100mm proximal to the ICJ.

Process	Reagent	Length of immersion
Dehydration	70% ethanol	1 hour
	95% ethanol	1 hour
	100% ethanol	1 hour
	100% ethanol	1 hour
	100% ethanol	1 hour
	100% ethanol	2 hours
	chloroform	1 hour
Clearing	Xylene	1 hour
	Xylene	1 hour
Wax impregnation	Paraffin wax at 56°C	2 hours
	Paraffin wax at 56°C	2 hours

Table VI
Processing schedule for tissue samples using the Shandon Elliott automatic tissue processor.

Step	Procedure
1	Stained for 2 minutes with Mayer's Haemalum (BDH, England)
2	Rinsed in tap water
3	`Blued' in Scott's tap water
4	Rinsed in tap water
5	Stained for 1 minute 30 seconds in 1% Eosin (BDH, England)
6	Rinsed in tap water
7	Rinsed in 70% ethanol
8	Rinsed in 100% ethanol
9	Rinsed in 100% ethanol
10	Rinsed in xylene
11	Rinsed in xylene
12	Mounted in DPX (BDH, England)

Table VII
Haematoxylin and Eosin staining method
(slide sections in aqueous phase)

5.2.2.1 Haematoxylin and Eosin stained sections.

The rehydrated sections from all 6 sheep were stained with haematoxylin and eosin (BDH Chemicals Ltd., England) according to the schedule shown in Table VII. Once mounted, these sections were viewed under an Olympus CH microscope (Olympus Optical Company, Japan) at 100x magnification. The thickness of the muscle layers within the muscularis was measured using a graduated eyepiece (Olympus, Japan) calibrated with an Olympus objective micrometer. When measuring transverse sections measurements of muscularis thickness were made at 8 equidistant points around the section. This provided measurements of relative thickness of the muscularis of the ileum at the ICJ and 100 mm proximal to this in each sheep (sheep A, B and C). Measurements of the L/S from sheep D,E and F were made at varying points along the sections (Figures 5.2 - 5.4).

5.2.2.2 Immunocytochemically stained sections.

Transverse sections from sheep A, B and C were immunocytochemically stained using 6 commercially prepared antisera: NSE (Neuron Specific Enolase), VIP (Vasoactive Intestinal Polypeptide), Substance P, Somatostatin, and DßH (Dopamine Beta Hydroxylase). These antisera were used in the indirect peroxidase-conjugate method of Taylor (1978) with modifications (Gurnsey, 1985) as outlined in Table VIII, at the dilutions given in Table IX.

The enzyme DßH converts Dopamine into Noradrenaline (DßH), and therefore will be present in catecholaminergic neurones (Ganong, 1989).

As each group of 6 slides was being processed using a particular primary antibody, a negative control slide (a duplicate of one of the other 6) was simultaneously

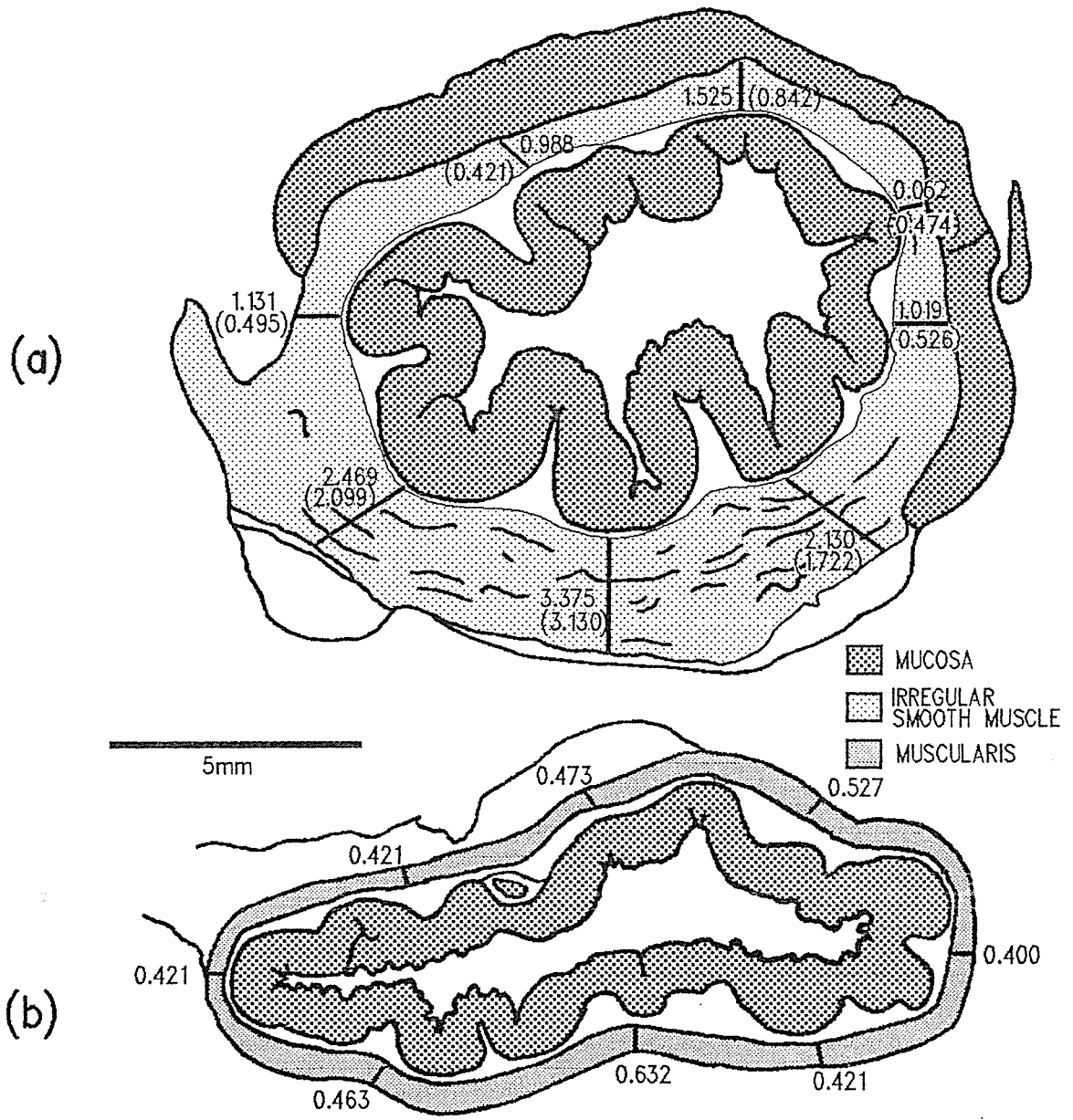


FIGURE 5.2 Sheep A: Outline diagrams of transverse sections through (a) the ileocaecal junction and (b) terminal ileum 10cm proximal to (a). Figures are given in mm. In (a) the muscle tissue is interspersed with loose connective tissue and blood vessels. The sectional width of this area is given, with the actual width of the smooth muscle tissue given in brackets.

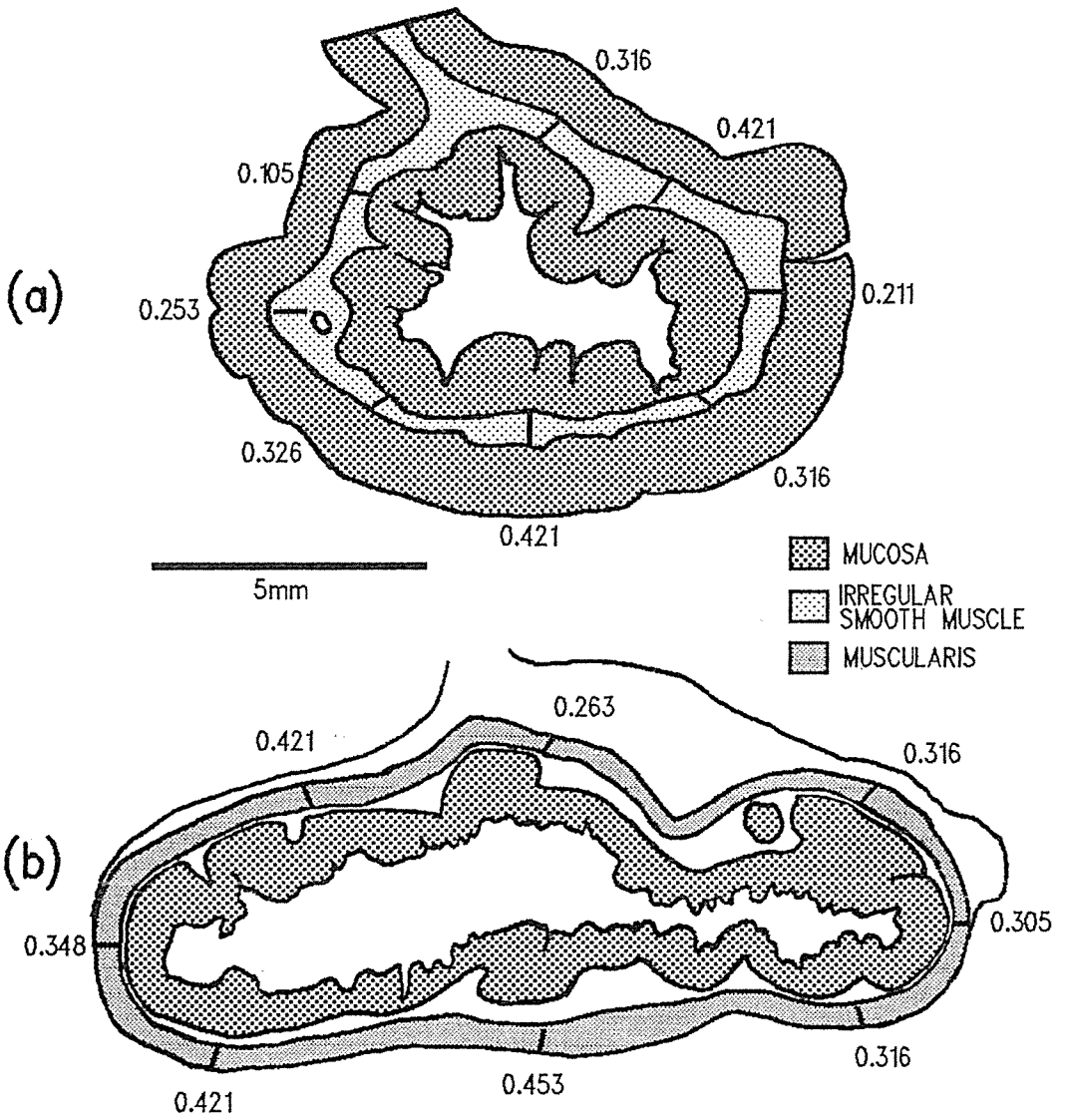


FIGURE 5.3 Sheep B : outline diagrams of transverse sections through (a) the ileocaecal junction and (b) terminal ileum 10cm proximal to (a). The figures given represent the thickness of the smooth muscle tissue (in mm) at the point shown in the section. In (a) the muscle tissue is irregular and interspersed with loose connective tissue and blood vessels.

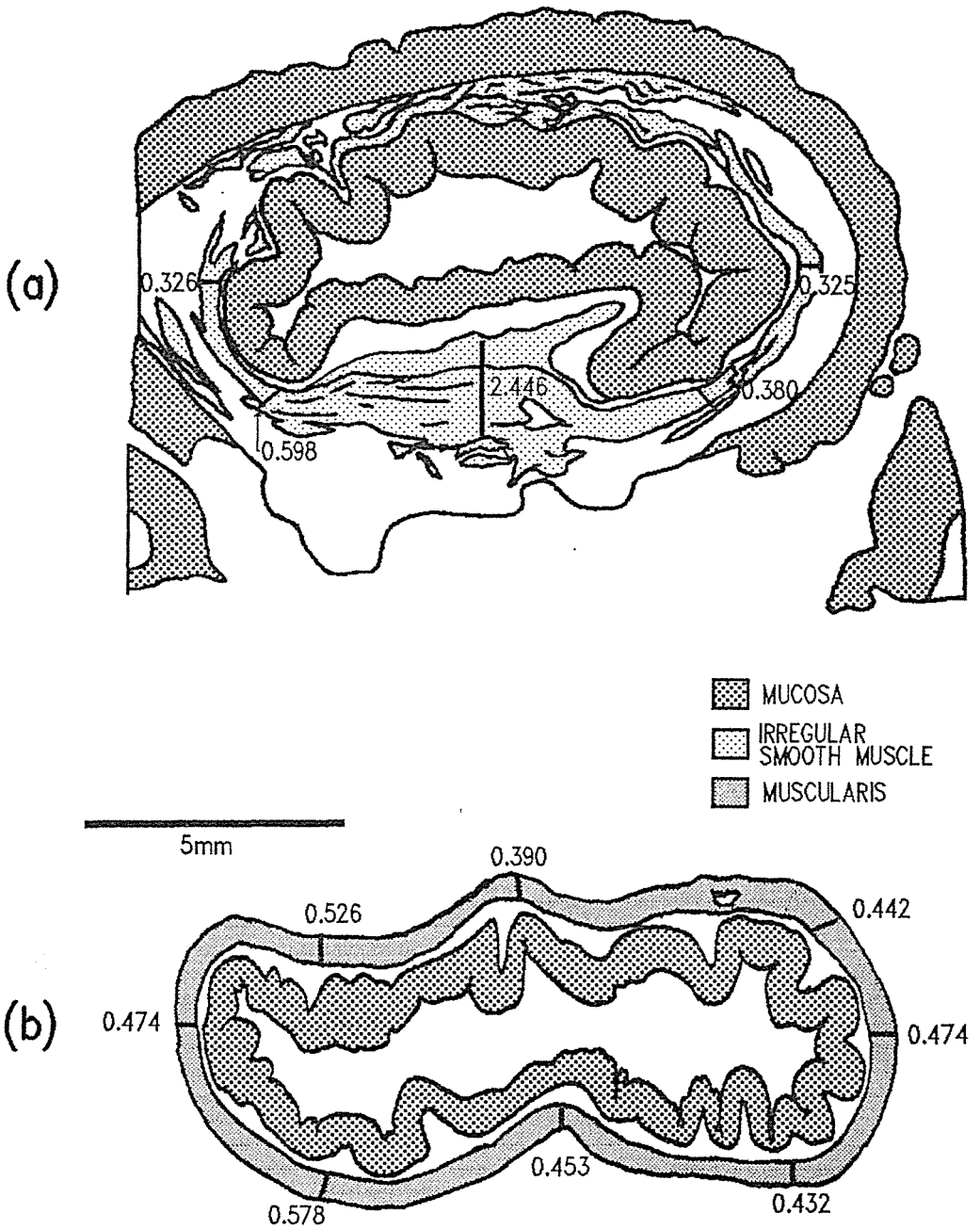


FIGURE 5.4 Sheep C: Outline diagrams of transverse sections through (a) the ileocaecal junction and (b) terminal ileum 10cm proximal to (a). Figures are given in mm. In (a) the muscle tissue is interspersed with loose connective tissue and blood vessels. Where possible the actual width of smooth muscle has been measured.

Step	Procedure
1	Equilibrate in PBS* for at least 5 minutes.
2	Pretreat in 1% BSAΔ in PBS for at least 5 minutes.
3	Drain and blot to remove excess buffer without allowing the section to dry ("Drain and Blot")
4	Incubate with primary antiserum (see Table IX) for 1 hour.
5	Wash in three changes of PBS for 1 minute each
6	Drain and Blot.
7	Incubate for 30 minutes with anti-rabbit biotinylated IgG diluted 1:200 with 1% BSA in PBS.
8	Wash in three changes of PBS for 1 minute each
9	Drain and Blot.
10	Incubate for 20 minutes with streptavidin-biotinylated horseradish peroxidase complex diluted 1:200 with 1% BSA in PBS.
11	Wash in three changes of PBS for 1 minute each
12	Stain peroxidase using freshly made DAB-hydrogen peroxide▲ for 3-4 minutes.
13	Wash in PBS.
14	Counterstain in Mayer's Haemalum for 1 minute.
15	Rinse in tap water.
16	"Blue" in Scott's tap water for 1 minute.
17	Dehydrate by rinsing in 70% and 100% ethanol.
18	Clear in Xylene.
19	Mount in DPX (BDH, England)

Table VIII
Immunocytochemical staining procedure (All steps were carried out at room temperature.)

* PBS: Phosphate Buffered Saline; pH = 7.2
36 ml 0.2 M Na_2HPO_4; 14 ml 0.2 M NaH_2PO_4; 50 ml d.w.

Δ BSA: Bovine serum albumin

▲ DAB-Hydrogen Peroxide:
Diaminobenzidine tetrahydrochloride
4mg in 8ml PBS to which is added 8µl hydrogen peroxide.

Steps 2,4,7 and 10 were carried out in a moist chamber (polypropylene tray lined with paper towels moistened by PBS and covered by a perspex sheet).

Reagents for steps 7,10 and 12 were obtained from Amersham International Inc., (UK).

Primary Antisera	(Source)	Diln. in PBS 1%BSA
NSE	(Dako Corporation, USA)	1:100
VIP	(Dako Corporation, USA)	1:1000
Substance P	(Dako Corporation, USA)	1:500
Serotonin	(Dako Corporation, USA)	1:1000
DβH	(Eugene Tech. Intnl., UK)	1:250

TABLE IX
Primary antibodies used in immunocytochemistry.

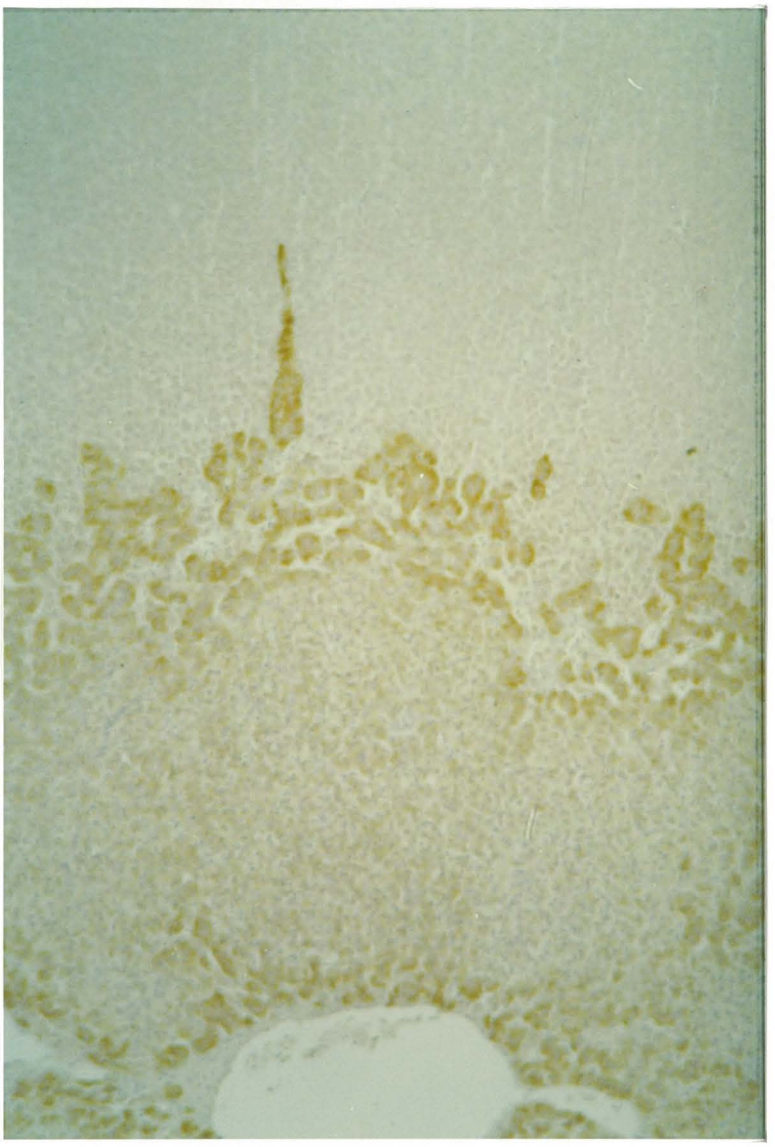
processed with the primary antibody incubation step (4) omitted to enable a visual evaluation of any non-specific staining that may have occurred as a result of other steps in the procedure.

When using the DβH primary antiserum, a section of sheep adrenal gland processed in the same way as described for the gut sections in Sections 5.2.1 and 5.2.2, was included as a positive control (Plate 5.1).

Positive control slides were not included for NSE, VIP, Substance P or Somatostatin as the specificity of the antisera at the dilutions used in this experiment have already been demonstrated in this laboratory (Gurnsey, 1985; Ball, 1987).

Once mounted, the sections were viewed under the microscope and the stained areas visually compared for immunoreactivity. The sections were then photographed. Very little immunoreactivity was observed in the intestinal sections in response to the DβH antiserum, despite a good response being observed in the adrenal tissue. This was suggestive of a masking of the antigenic sites on these enzymes, as others workers have demonstrated such fibres in the gut of other species (Gershon and Sherman, 1987; Gabella, 1987). In an effort to `unmask' the enzymes and expose them to the primary antibodies, further sections (and controls) were briefly digested in a trypsin solution (Norbert, Wittkuhn and McCaughey, 1980). A solution of 0.1% Trypsin in PBS was used to treat the slides for periods of 5 and 10 minutes at room temperature, before being treated as described in Table VIII using the DβH primary antibody.

PLATE 5.1
Adrenal gland, stained with antiserum to Dopamine ß Hydroxylase (DßH), and used as a positive control for the ileal sections.
(10x Magnification).



5.3 Results

5.3.1 Muscularis thickness at the ICJ.

Visual examination of the sections taken 100mm proximal to the ICJ from sheep A,B and C and the longitudinal sections from sheep D,E and F showed the distal ileum of the sheep to have a typical intestinal muscularis, divided into m. interna and m. externa (Gurnsey, 1985). Muscle thickness in the transverse sections is shown in Table X, with an average thickness of 0.432 mm.

At the ICJ the muscularis of the ileum is seen to merge with that of the caecum, resulting in a thickening of the muscularis. At the point of junction between ileum and caecum the muscle fibre orientation is essentially circular, with the band of thickened muscle forming an elliptical ring around the base of the ileal papilla as the ileum penetrates the caecal wall at an angle. Transverse sections through the ICJ thus tend to show a thickened band of muscle on one side only. This can be seen in sections from sheep A and sheep C (Figures 5.2 and 5.4). A similar section from sheep B appears slightly more distal in its location, and misses the thickened zone. The thicker muscle at the ICJ is of the order of 1 - 2 mm or more thick (2 to 3 times thicker than the muscularis in the ileal sections 100mm proximal to the ICJ).

Within the ileal papilla smooth muscle fibres were seen to be irregularly arranged, particularly near the tip of the papilla. The muscularis breaks up into small bundles of muscle fibres interspersed with the connective tissues of the submucosa. The thickness of the muscle in this part of the ileal papilla cannot easily be compared with the thickness of the muscularis in more proximal areas of the ileum, and measurements of muscle thickness that were made in these areas are the sum of the thicknesses of the small

Sheep	ICJ	100mm proximal to ICJ		
		circular	longitudinal	Total
A	0.842	0.305	0.168	0.473
	0.474	0.316	0.211	0.527
	0.526	0.274	0.126	0.400
	1.722*	0.295	0.126	0.421
	3.130*	0.421	0.211	0.632
	2.099*	0.316	0.147	0.463
	0.495	0.295	0.126	0.421
	0.421	0.301	0.120	0.421
Mean:	1.214	0.315	0.154	0.470
SEM :	±0.694	±0.031	±0.026	±0.053
B	0.316	0.189	0.074	0.263
	0.421	0.211	0.105	0.316
	0.211	0.200	0.105	0.305
	0.316	0.206	0.110	0.316
	0.421	0.379	0.074	0.453
	0.326	0.274	0.147	0.421
	0.253	0.221	0.127	0.348
	0.105	0.280	0.141	0.421
Mean:	0.296	0.245	0.110	0.355
SEM :	±0.073	±0.044	±0.027	±0.047
C	0.325	0.212	0.178	0.390
	0.380	0.264	0.178	0.442
	2.446*	0.327	0.147	0.474
	0.598	0.264	0.168	0.432
	0.326	0.296	0.157	0.453
		0.421	0.157	0.578
		0.327	0.147	0.474
		0.442	0.084	0.526
Mean:	0.815	0.319	0.152	0.471
SEM :	±0.806	±0.034	±0.021	±0.040

Table X
Thickness of the muscularis of the terminal ileum at equidistant points around the circumference of transverse sections.
(All values in mm, see Figures 5.2 - 5.4)
Muscle at the ICJ is predominantly circular in orientation, but did not form a cohesive layer.
* Measurements across combined ileal/caecal muscularis.

bundles of the smooth muscle in the sections.
One notable feature of the sections through the ileal papilla in this experiment was the vascular nature of the tip of the ileal papilla (Plate 5.2).

5.3.2 Distribution of NSE, VIP, Substance P, Serotonin and DβH in the distal ileum.

Immunoreactivity to NSE, VIP, Substance P, Serotonin and DβH in the distal ileum is summarised in Table XI, and illustrated in Plates 5.3 - 5.12.

Immunoreactivity against NSE showed a rich innervation in all sections. In the ileal sections taken 10 cm from the ICJ, both submucosal and myenteric plexuses containing ganglia were readily identified (Plate 5.4). Where the submucosa and muscularis merge in the ileal papilla, the plexuses also appear to merge to form an area rich in nerve fibres (Plate 5.3).

Immunoreactivity to VIP (Plates 5.5, 5.6) and Substance P (Plates 5.7, 5.8) showed that the innervation of the distal ileum has a significant number of nerve cells and fibres containing these neurotransmitters, though when comparing the sections from the ICJ with the sections taken 100mm proximally, there was no apparent overall difference in the proportion of nerve fibres of these two types.

Only slight immunoreactivity was observed in these ileal sections to Serotonin, and again, this did not appear to vary in intensity between the ICJ and 100mm ileum sections (Plates 5.9, 5.10).

Immunoreactivity to DβH clearly showed catecholaminergic cells in the adrenal sections used as controls (Plate 5.1), but failed to show any significant staining in the

PLATE 5.2
Longitudinal section through ileal papilla (Sheep F) stained with haematoxylin and eosin. The submucosal area at the tip of the ileal papilla is notably vascular. A thickening of the of the muscularis can be seen where the muscle layers of the caecum and ileum merge. (See also Figure 5.7).
(1.5x magnification).

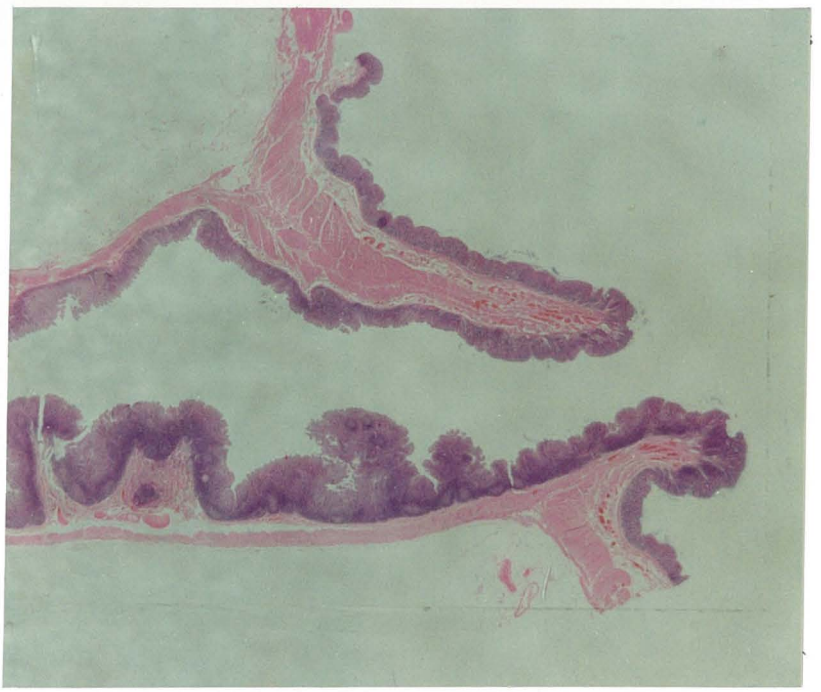
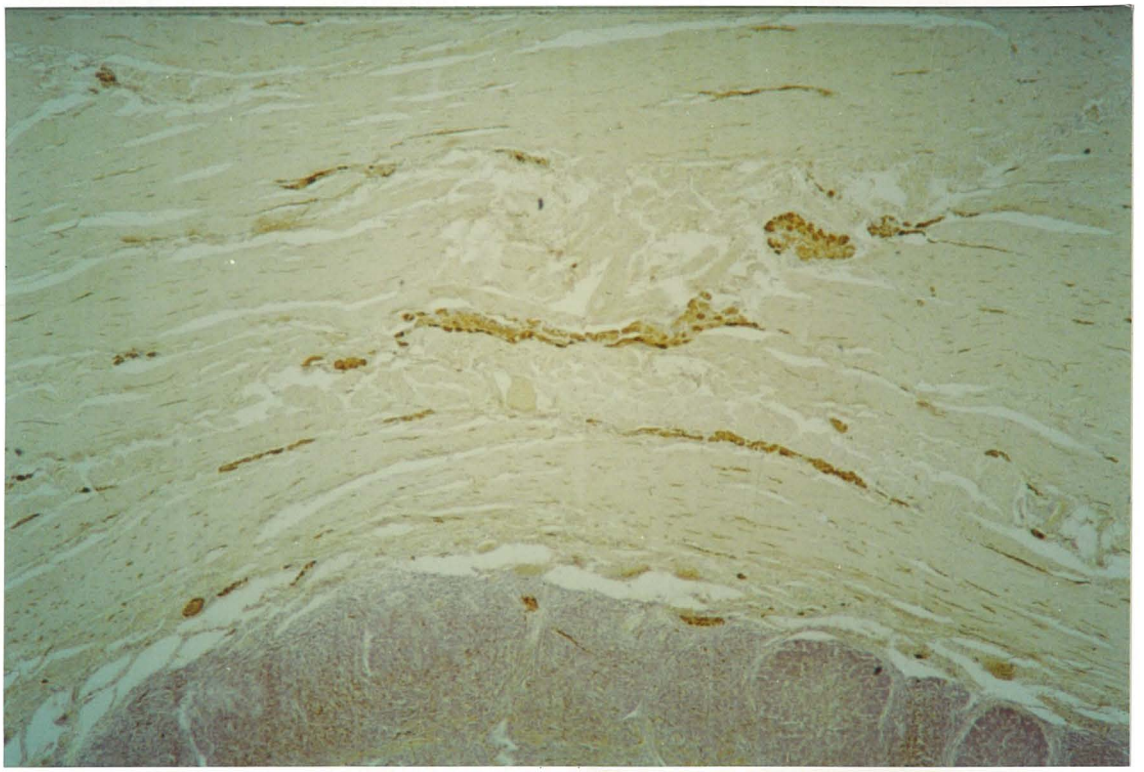


PLATE 5.3
Sheep C, ICJ transverse section stained with antiserum to Neuron Specific Enolase (NSE). In this region the muscularis loses it's distinct circular and longitudinal sublayers, and while no distinct submucosal and myenteric plexus' can be observed, there is a rich enteric nerve content in the section. Many ganglia were observed (large stained masses) as well as fine nerves and axons within the muscle bundles (showing as fine lines and spots of reactivity in the muscle tissue).
(4x magnification).

PLATE 5.4
Sheep C, Ileum 10 cm proximal to the ICJ, transverse section stained with antiserum to NSE. This section shows a clear division of the submucosal and myenteric plexus. The ganglia and nerves of the submucosal plexus are scattered through the submucosal region; the ganglia and nerves of the myenteric plexus lie mainly in a band between the inner circular and outer longitudinal sublayers of the muscularis. Small nerves and axons can be seen as fine points and lines of reactivity in the muscle tissue.
(4x magnification).



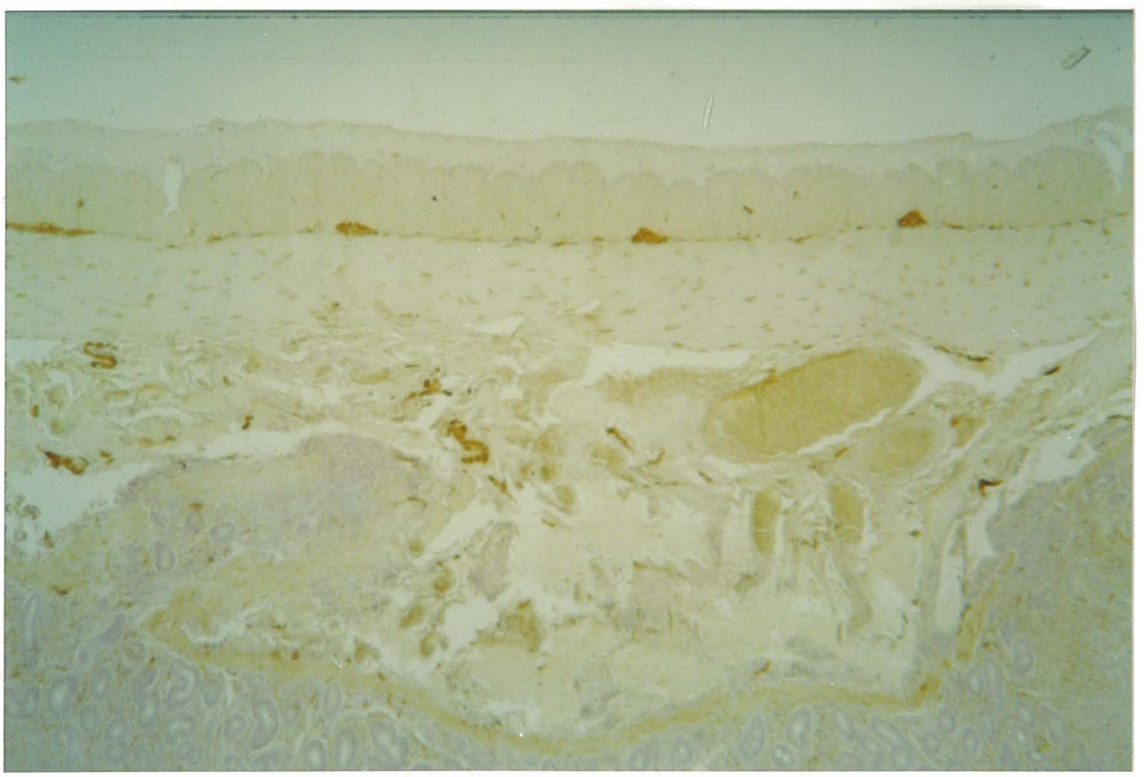
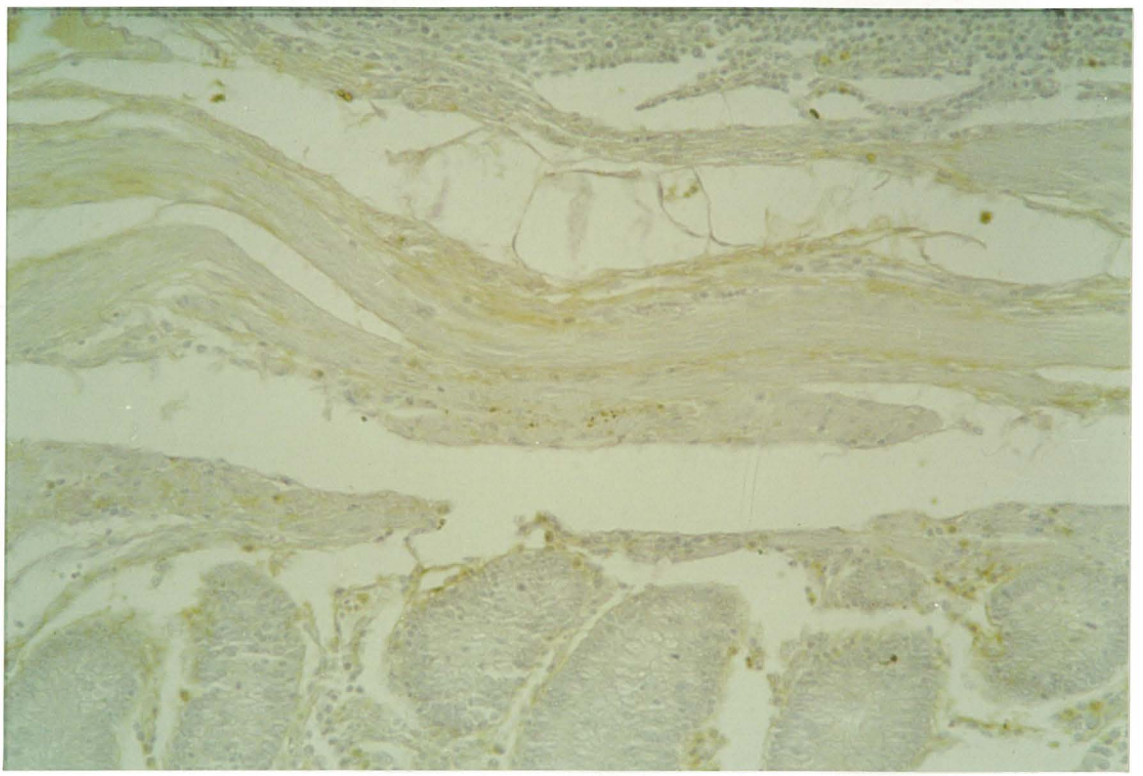


PLATE 5.5
Sheep B, ICJ transverse section stained with antiserum to Vasoactive Intestinal Polypeptide (VIP). Some reactivity can be observed in fine nerve fibres in the centre of this view. Small sites of reactivity such as this were scattered throughout the merged submucosa/ muscularis in the ICJ.
(25x magnification).

PLATE 5.6
Sheep A, Ileum 10 cm proximal to the ICJ, transverse section stained with antiserum to VIP. An area of muscularis has been magnified, with the myenteric plexus in the middle (longitudinal muscle below, circular muscle above). Some reactivity can be observed in cell bodies and fibres of the myenteric plexus.
(40x magnification).



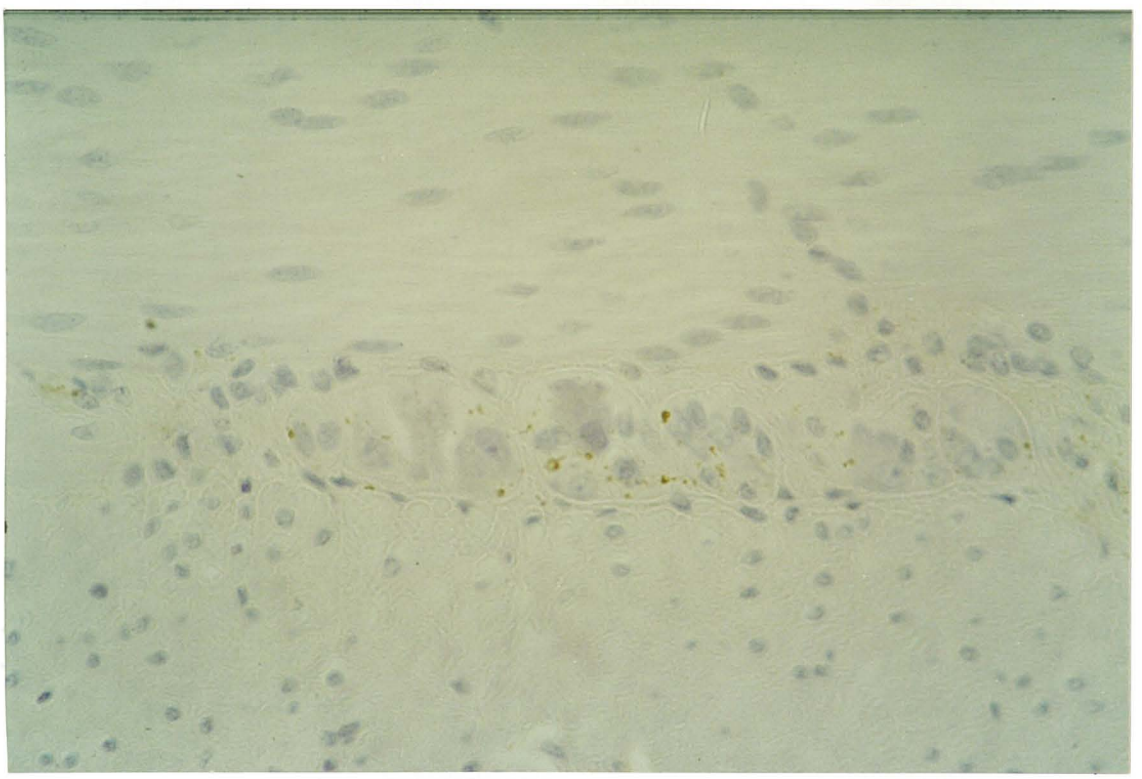


PLATE 5.7
Sheep C, ICJ transverse section stained with antiserum to Substance P. Reactivity can be observed in neurone cell bodies and fibres within the muscle tissue of the ICJ. The degree of response varied between sections.
(20x magnification).

PLATE 5.8
Sheep B, ileum 10 cm proximal to ICJ, transverse section stained with antiserum to Substance P. Neurone cell bodies and fibres can be seen within the myenteric plexus.
(25x magnification).

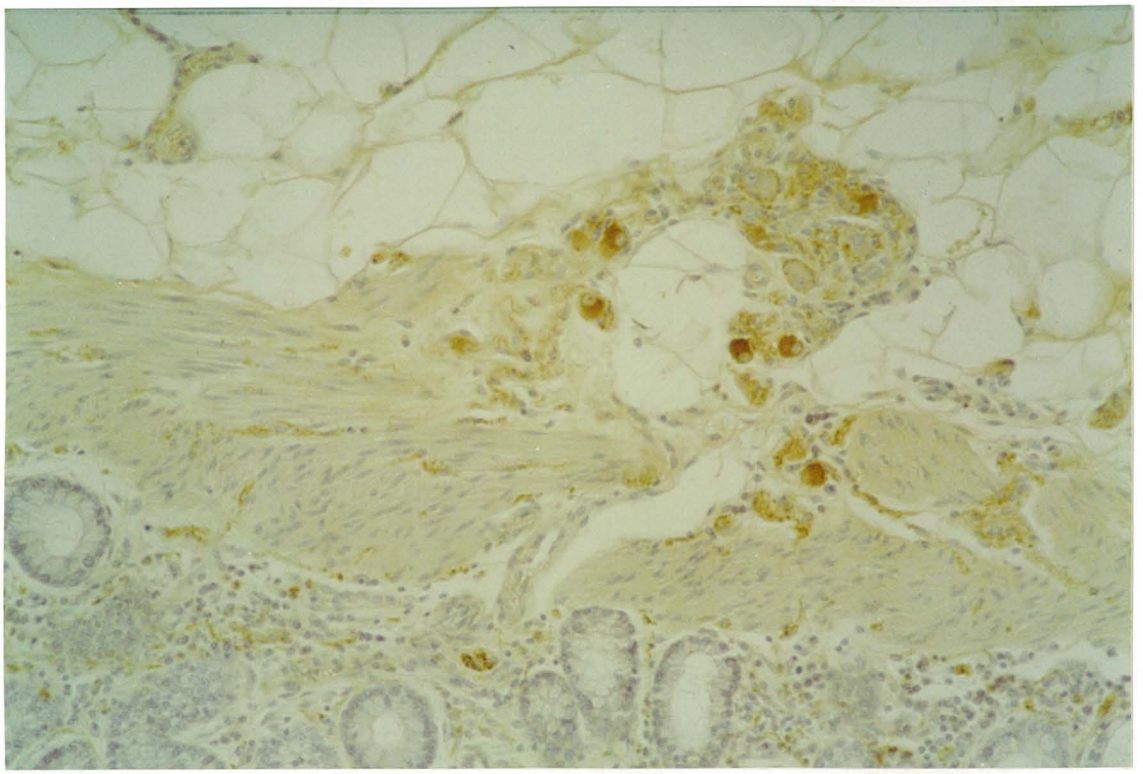
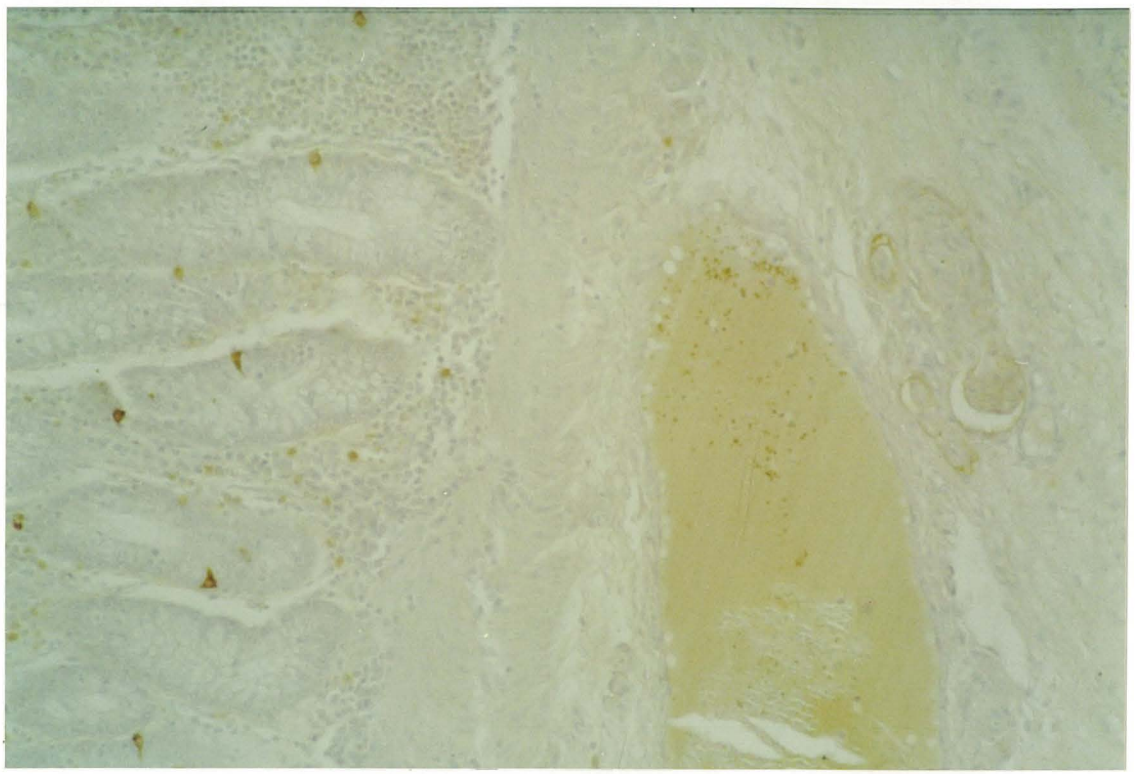


PLATE 5.9
Sheep A, transverse section through ICJ stained with antiserum to serotonin. Reactivity can be seen in the serotonergic endocrine cells within the mucosal epithelium, and some small fibres within the mucosal lamina propria and submucosal areas. There is some nonspecific staining inside the lumen of the blood vessel, presumably due to reactivity against plasma antibodies.
(10x magnification)

PLATE 5.10
Sheep B, ileum 10cm proximal to the ICJ stained with antiserum to serotonin. A small amount of reactivity in nerve fibres can be observed in this submucosal region.
(40x magnification)



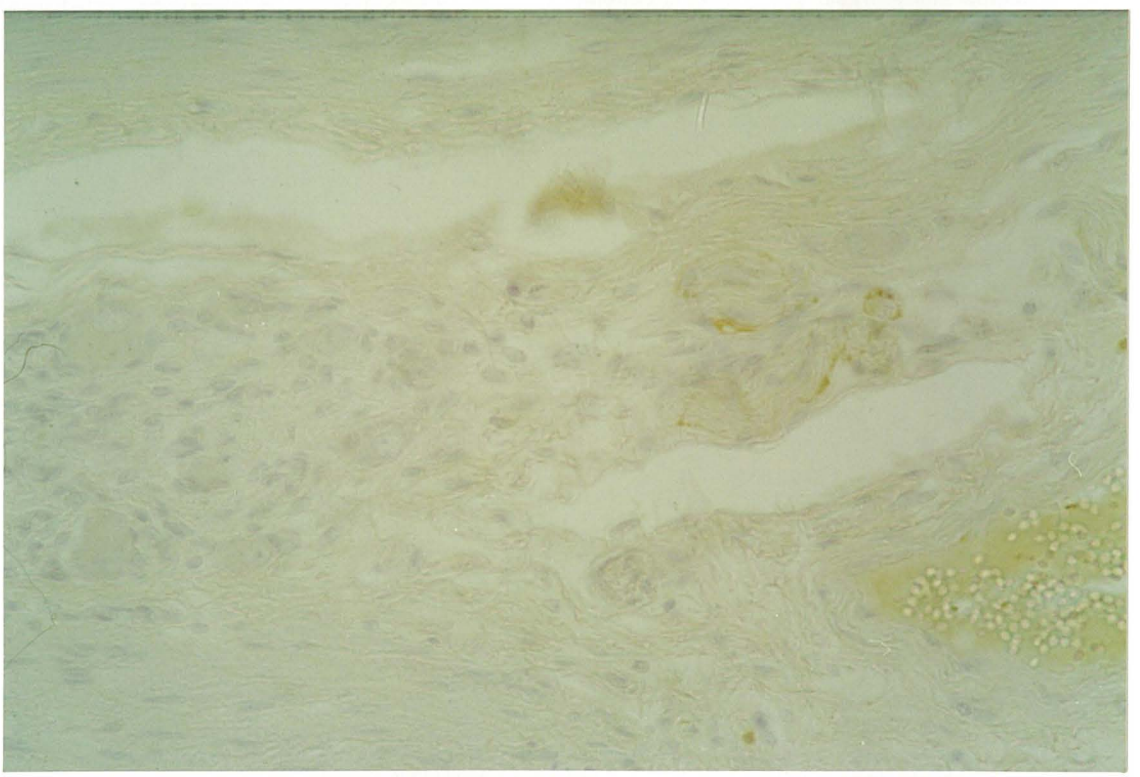
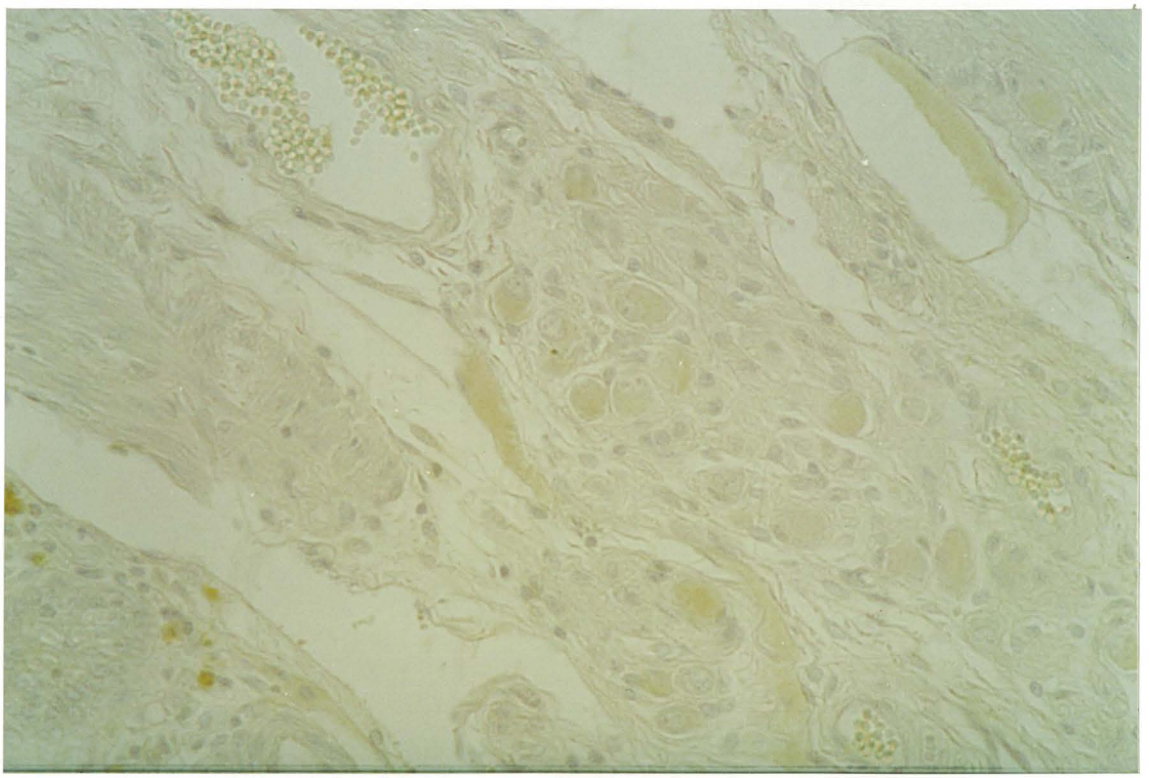
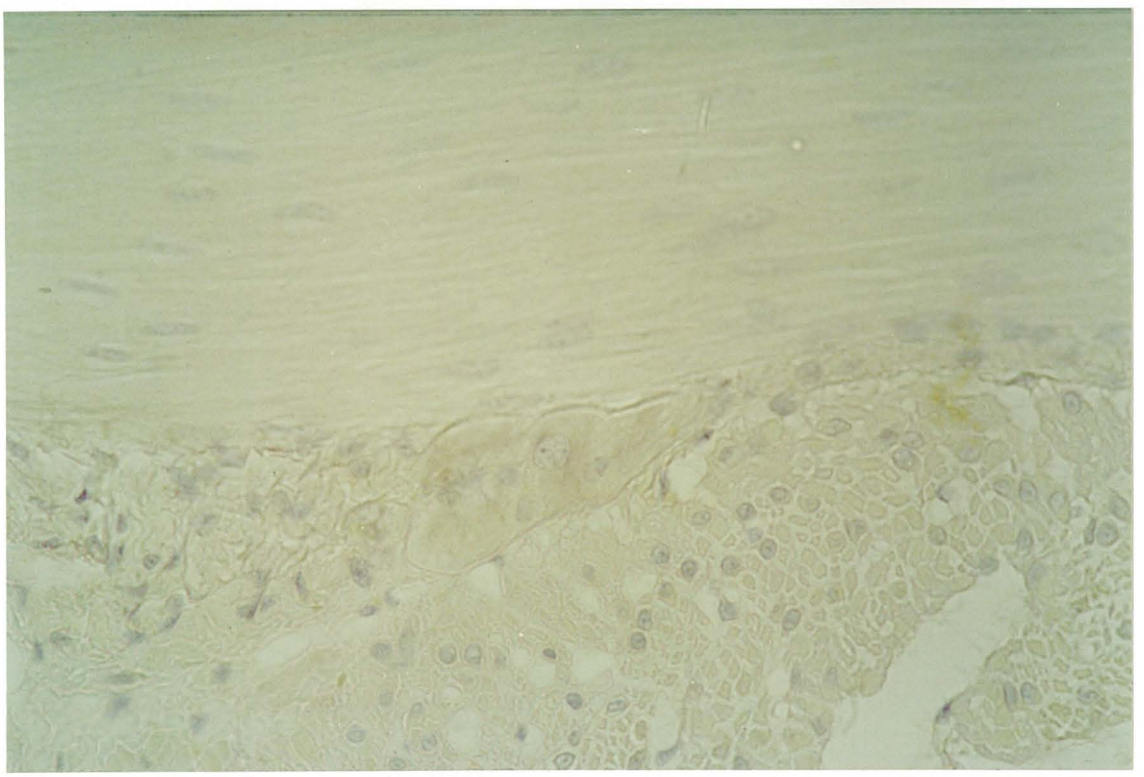


PLATE 5.11
Sheep B, transverse section through ICJ stained with antiserum to DβH, an enzyme found in catecholaminergic neurones. Very little consistent reactivity was observed. The small amount of reactivity observed in this section could have been nonspecific, and was not observed in the other two ICJ sections from sheep A and C.
(40x magnification).

PLATE 5.12
Sheep C, ileum 10cm proximal to the ICJ, transverse section stained with antiserum to DβH. These sections were indistinguishable from the negative control sections, with only very minor nonspecific background staining visible, even after pretreatment with trypsin. No significant reactivity was observed.
(40x magnification)





Transmitter	Response Intensity				
	Neurone cell bodies		Nerves		Nerve axons in muscle
ICJ					
NSE	+++++		+++++		+++++
VIP	+		+		+
Substance P	+ +		+ +		+
Serotonin	+		+		0
DßH	0		0		+?
	Submucosal Plexus		Myenteric Plexus		Nerve axons in muscle
	Nerves	Cell Bodies	Nerves	Cell Bodies	
Ileum 100mm proximal to ICJ					
NSE	+++++	+++++	+++++	+++++	+++++
VIP	+	+	+ +	+ +	+
Substance P	+ +	+	+ +	+ +	+
Serotonin	+	0	0	0	0
DßH	0	0	0	0	0

Table XI
Relative immunoreactivities in the sections from the ICJ and ileum 10 cm proximal to the ICJ. Sections were examined for immunoreactivity to the listed antibodies both within the nerve plexuses (nerves and ganglia). The muscle tissue of the muscularis externa was also examined for evidence of immunoreactive axons.

ileal sections (Plates 5.11, 5.12). The sections from sheep B (one of which is shown in plate 5.11) possibly show some sign of staining in response to DβH in very fine fibres within the submucosal plexus. These results were inconclusive.

5.4 Discussion

5.4.1 Histology of the ICJ.

The measurement of muscularis thickness at different points along the ileum can only be regarded as providing a means of comparison of the muscularis thickness, and cannot be taken as a definitive measurement of actual muscularis thickness. Visual observations made during this experiment suggested the smooth muscle in the tissue samples contracted in response to the fixative used, and the degree of contraction and resultant thickening of the muscularis externa cannot be estimated. Thus the figures quoted have a built in artifact and do not represent an estimate of the actual in vivo thickness of the ileal muscularis externa.

The results obtained by measuring the thickness of the muscularis externa of the ileum at different distances from the distal tip of the ileal papilla suggests that there is a thickening of the muscularis externa in the region of the ICJ of the sheep. This area of thicker muscle occurs at the base of the ileal papilla, and is elliptical in shape due to the angle at which the ileum penetrates the caecal wall. The thickening of the muscularis appears due to the merging of the caecal muscularis with that of the ileum, and is generally no more than 2-3 mm long. This is illustrated in the longitudinal sections from sheep D, E and F (Figures 5.5-5.7). In addition, a tranverse section square to the ileum will section the wall of the caecum obliquely, thus

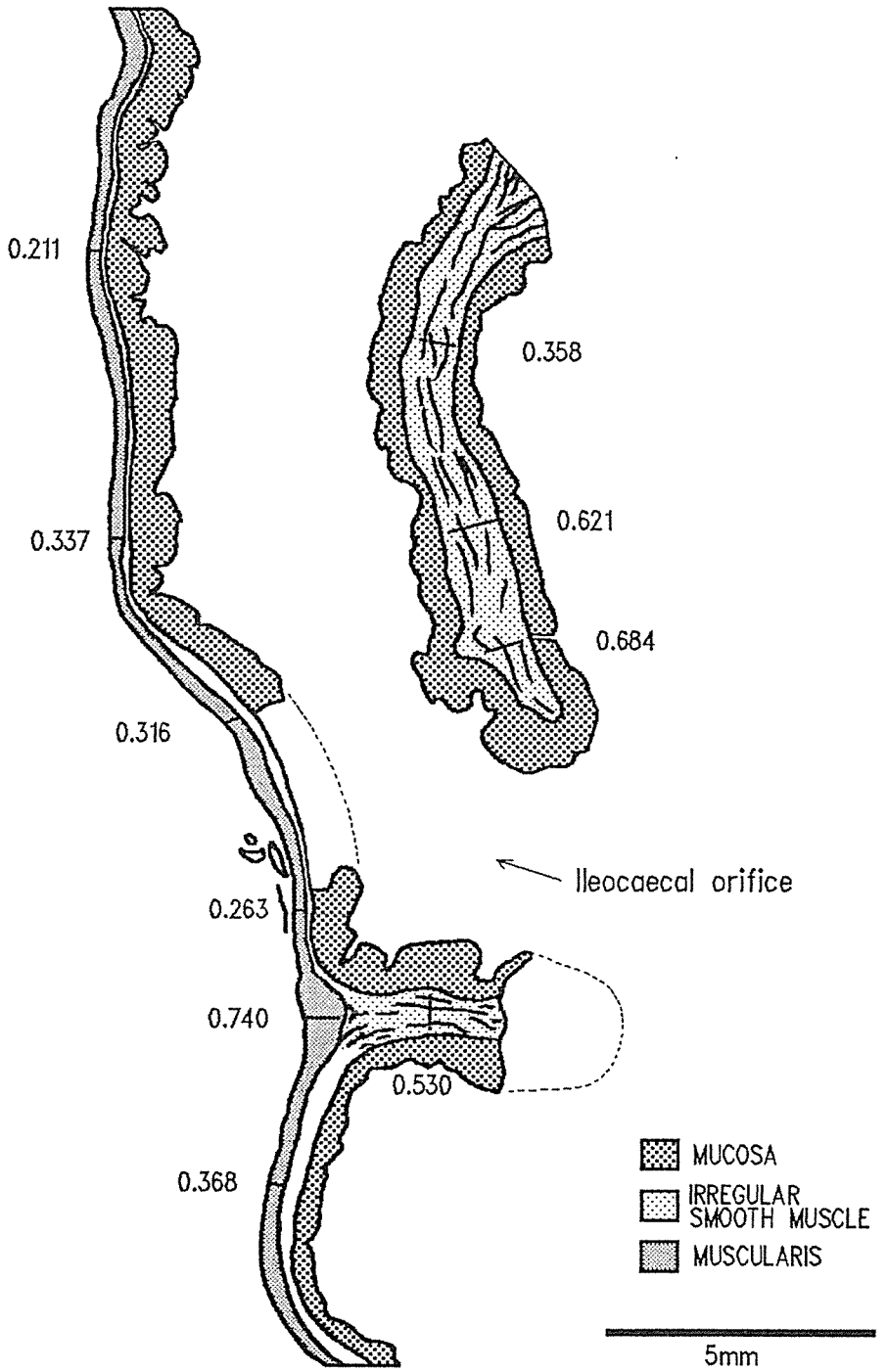


FIGURE 5.5 Sheep D: Outline diagram of a longitudinal section through the ileocaecal junction. The thickness of muscle tissue at various points is given in mm. Where the muscle tissue is is interspersed with loose connective tissue, only the muscle tissue width has been measured.

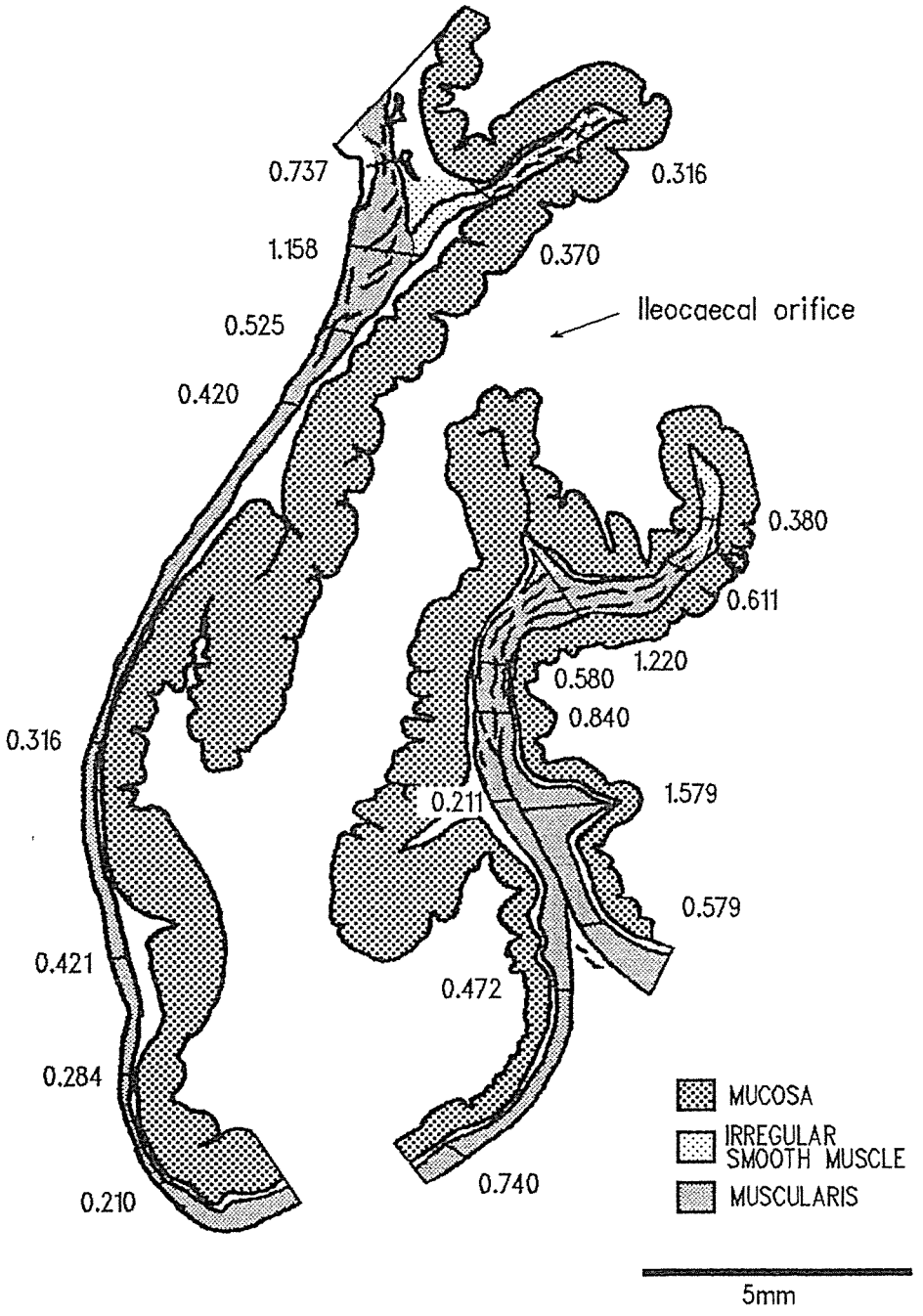


FIGURE 5.6 Sheep E: Outline diagram of a longitudinal section through the ileocaecal junction. The thickness of muscle tissue at various points is given in mm. Where the muscle tissue is is interspersed with loose connective tissue, only the muscle tissue width has been measured.

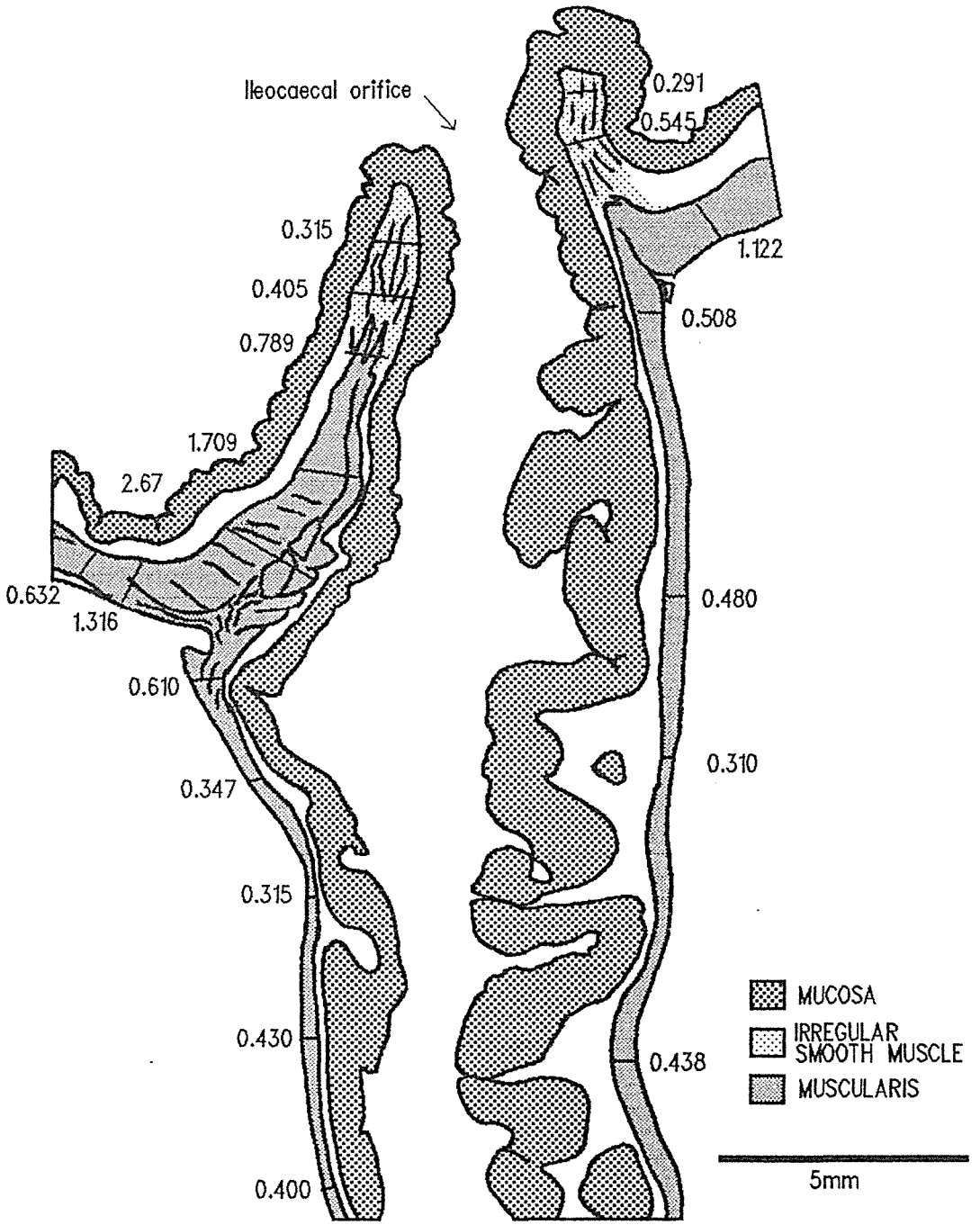


FIGURE 5.7 Sheep F: Outline diagram of a longitudinal section through the ileocaecal junction. The thickness of muscle tissue at various points is given in mm. Where the muscle tissue is is interspersed with loose connective tissue, only the muscle tissue width has been measured.

possibly exaggerating the thickness of the muscularis at that point.

It was noted during examination of the sections that the submucosa within the tip of the ileal papilla was very vascular, while the smooth muscle lacked the structural organisation typical elsewhere in the intestine. This structure suggests a possibility that does not seem to have been considered before - that there may be a hydrostatic mechanism within the tip of the protuberant ileum, capable of increasing the pressure within the ileal papilla lumen by venous engorgement of the papilla wall. This, rather than muscular sphincter action, could be responsible for the slight tonic pressure recorded from within the ileal papilla in some sheep in the experiment reported in Chapter 2. If so, then control of ICJ pressure would be through vascular mechanisms rather than by direct control of intestinal smooth muscle. The similarity in the reported anatomical structure of the ICJ in sheep and other species (Fioramonti, 1981) suggests other species may also have a similar vascularity within the ileal papilla also - a possible avenue for further research.

5.4.2 Immunocytochemistry

Staining with anti-NSE shows clearly the rich innervation of the distal ileum, including cells and fibres, both in the submucosal and myenteric plexuses (Plate 5.4). In addition, nerve fibres within the muscularis were clearly demonstrated. Within the ileal papilla, numerous bundles of nerve fibres were observed among the bundles of muscle fibres (Plate 5.3), indicating that the ileal papilla is a densely innervated region of the sheep small intestine.

VIP is an inhibitory peptide neurotransmitter, causing the relaxation of cat intestinal smooth muscle (Sjöqvist and

Fahrenkrug, 1987). VIP immunoreactivity was most noticeable within the plexuses, especially the ganglia, as well as within the muscularis of most sections (see Table XI, Plates 5.5 and 5.6). VIP has been suggested as being the mediator of the descending inhibition occurring during peristalsis (Weisbrodt, 1987), and the presence of VIP immunoreactive neurones in the sheep small intestine wall indicate these neurones play a significant role in the control of contractile activity in the sheep gut. There did not appear to be any great difference in the density of VIP immunoreactive cells or fibres when comparing ICJ sections with those taken from the intestine 100mm proximal to the ICJ, suggesting that VIP innervation of the ICJ is not significantly different to other parts of the ileum.

Substance P appeared well distributed throughout the plexuses, particularly the submucosal plexus, though reactivity in the muscularis was only weakly present in some sections. (Table XI, Plates 5.7 and 5.8). Substance P is believed to be involved in stimulating smooth muscle activity in other species, but is reportedly concentrated in the ganglia in interneurones and localised motor neurones in those species (Bornstein et al, 1984). In the sheep intestine some Substance P immunoreactivity was observed in the nerve plexuses between the ganglia, suggesting Substance P may have a wider role in sheep than in the other species studied.

Erde et al (1985) suggested that Serotonin, another neurotransmitter with excitatory effects on smooth muscle, is responsible for excitation over longer distances in the guinea pig intestine. In this study, however, the slight serotonergic immunoreactivity that was observed was localised not within the myenteric plexus, but more within the submucosal plexus and mucosa (Table XI, Plates 5.9, 5.10). This distribution would suggest that the role of

serotonin in the sheep intestine is either as a neurotransmitter associated with the submucosal plexus (possibly in a sensory pathway), or as an excitatory agent on mucosal endocrine cells. The reduced distribution of serotonin in the sheep compared to other species (Erde et al, 1985), and the presence of Substance P in the nerves between ganglia in addition to its described location in myenteric ganglia in other species (Bornstein et al, 1984) could indicate Substance P has a greater role in controlling muscle activity than serotonin has in the sheep.

There was an apparent lack of immunoreactivity to DßH in the intestinal sections (Plates 5.11 and 5.12), even after the sections were treated with trypsin before immunocytochemical staining. The positive result obtained with the control slides (both with and without the trypsin pretreatment) suggests that any catecholaminergic innervation of the sheep intestine is sparse, and beyond the sensitivity of the method used without further modification. Such modifications of the technique used was beyond the scope of the present study, but further investigation of adrenergic innervation of the sheep small intestine could be worth further study in the light of the report by Wood and Meyer (1979) that adrenergic stimulation results in inhibition of serotonin release in the guinea pig intestine.

The ICJ appears to have a rich innervation, but there does not appear to be any difference in the proportion of nerve cells and fibres containing Substance P, VIP or Serotonin, and possibly catecholamines, in the ICJ compared to the more proximal ileum.

SUMMARY AND CONCLUSIONS

In this study evidence has been presented which demonstrates the existence of sphincterlike properties at the sheep ileocaecal junction, fulfilling the criteria identified by Fisher and Cohen (1973). First, an intraluminal resting pressure was measured within the ICJ (2 - 5 mm Hg). Second, the ICJ intraluminal pressure was shown to increase when the caecum is distended, and third, there was some evidence that ileal distention resulted in a decrease in intraluminal pressure within the ICJ. These results confirm that the ICJ in sheep functions as a sphincter, and suggest a similarity to the sphincter at the human ileocaecal junction as described by Quigley, Borody et al (1984). This short (less than 5mm) zone of elevated pressure in the sheep would appear to work in conjunction with a valvular action as described by Kuman and Phillips for the human ICJ, preventing retrograde digesta flow from the caecum to the ileum.

Normal digesta movement through the small intestine across the ICJ into the caecum of the sheep is due to the regular activity of the MMC. Digesta is progressively propelled through the small intestine by Phase 2 and Phase 3 activity (Fioramonti, 1981). In this study, EMG recordings of the ileocaecal region in the conscious sheep showed approximately 70-80% of the MMC phase 3 activity that reaches the distal ileum progresses as far as the ICJ. This appears to be the main motility pattern present in this region in the conscious sheep, and very little coordination was observed between patterns of caecal and ileal activity. This is in contrast with the report by Fioramonti (1981), who suggested that the caecum typically empties prior to the arrival of MMC phase 3 activity at the ICJ.

Feeding was found to decrease the amount of MMC activity

in the distal ileum by lengthening the interval between successive MMC phase 3 patterns of motility. This was particularly so if the animal showed an increase in serum gastrin after eating began (a "feeding response"). This effect of feeding on MMC activity appears not to be due to the levels of circulating gastrin, however, since the administration of gastrin had no observed effect on MMC activity, and in the nematode infected animal with very high serum gastrin levels, the interval between phase 3 MMC activity markedly decreased. Clearly the relationship between feeding and MMC activity (and consequently ICJ activity) is due to something other than the release of gastrin.

The reduced activity could be due to a reflex inhibition such as the mechanism reported by Jansson and Lisander (1969) in cats, where intestinal distention (such as could occur following feeding) resulted in increased sympathetic activity, causing presynaptic noradrenergic inhibition of enteric cholinergic neurons. Intestino-intestinal reflexes such as this have been described for monogastric species, and though none have yet been described for ruminants it seems probable that they exist (Cottrell and Gregory, 1991). Interestingly, immunocytochemical studies performed as a part of this investigation found no evidence of catecholaminergic neurones within the distal small intestine, though this doesn't mean that such neurones are not present. In vitro and in vivo adminstration of catecholamines clearly demonstrated the inhibitory effect of these chemicals on the activity of the small intestine and caecum, so receptors to these compounds are present in this area, and are suggestive of an inhibitory adrenergic innervation.

The effect of feeding on intestinal activity in the sheep could be also be due in part to humoral mechanisms (other than gastrin), as Gregory and Miller (1989) report

infusions of fat or oleic acid disrupt MMC activity in sheep duodenum and jejunum. In monogastric species such changes in motility in the small intestine are associated with the release of a variety of gut-active hormones, including cholecystekinin (Walsh, 1987). Cholecystekinin is reported to disrupt MMC activity in the sheep (Cottrell and Gregory, 1991).

VIP may also be involved in controlling motility of the small intestine. It is recognised as having inhibitory effects on the reticulo-omasal orifice and abomasum in sheep, and has been shown to be released into gastric venous effluent (Reid, Post and Titchen, 1991). In this investigation VIP containing neurones were found in the ileum, particularly in the myenteric plexus, suggesting some role for this substance in controlling ileal motility.

Further investigation into the effects of cholecystekinin, VIP and other humoral agents found in the small intestine such as Substance P (also present in ileal enteric neurones) will undoubtedly reveal much about the control of the MMC and by extension, the activity of the ICJ.

Perhaps one of the most significant features of this study was the observation that the ICJ appeared more like a section of the ileum than a distinctly separate sphincter region. Its motility patterns are integrated with the rest of the ileum, being mainly coordinated with MMC activity. Catecholamines inhibit the ICJ in the same manner as they do the ileum, whereas gastrointestinal sphincters are generally exited by adrenergic stimulation (Bass et al, 1970). In this aspect the ICJ does not appear to be a typical gastrointestinal sphincter.

In addition, immunocytochemical investigations for NSE, VIP, Substance P, Serotonin and DβH did not demonstrate

any differences in the type or density of the intramural innervation of the ICJ compared to the more proximal ileum. Examination of the histological sections did reveal that the muscularis within the ileal papilla breaks up into irregular fragments of muscle tissue interspersed with a very vascular loose connective tissue. It is suggested that the slightly elevated intraluminal resting pressure within the ICJ could be due to venous engorgement rather than conventional tonic sphincter muscle action. This mechanism could easily provide the few mm Hg pressure recorded, and assist the valvular action of the ICJ to prevent retrograde flow of digesta. It would also conform with the observations by Fioramonti (1981) and Reid and Dellow (1972) of digesta being squirted in a pulsatile manner through the orifice of the ileal papilla into the caecum, for an ileal papilla engorged with blood would impede the flow of digesta into the caecum until pressure built up in the distal ileum during contractile activity, forcing the digesta through the orifice.

The control of the motility of the ruminant small intestine is as yet poorly understood. Some simple reflexes have been demonstrated, but the neural pathways (enteric, peripheral ganglionic and spinal) have only been broadly outlined. The role of many neurotransmitters and/or hormones that have been shown to be present in the intestine has not yet been defined. Much further investigation is required in this area in order to enable us to understand the factors controlling the passage of digesta through the small intestine to the large intestine.

Appendix A

Gastrin Assay Pools

Assay No.	Pool 5	Pool 6	Pool 7	% binding
	20.6	38.8	71.2	
1	17.0	40.6	79.7	44
	23.2	48.5	76.6	
	T= 60.8	127.9	227.5	
	19.8	42.8	65.8	
2	17.9	44.1	68.5	39
	23.3	46.6	66.9	
	T= 61.0	133.5	201.2	
	18.2	38.4	64.3	
3	18.3	36.5	63.8	39
	17.7	38.0	69.8	
	T= 54.2	112.9	197.9	
	17.0	36.6	51.7	
4	15.4	37.7	58.5	34
	16.6	34.3	65.7	
	T= 49.0	108.6	175.9	
	17.5	37.2	57.4	
5	15.5	36.4	57.3	31
	15.1	35.8	55.6	
	T= 48.1	109.4	170.3	
	18.8	35.1	60.4	
6	18.8	38.5	63.6	37
	18.7	35.5	62.7	
	T= 56.3	94.1	186.7	
	18.7	35.3	71.1	
7	19.4	36.4	63.3	41
	20.2	35.0	59.8	
	T= 58.3	109.1	194.2	

Pool statistics:

	Pool 5	Pool 6	Pool 7
n =	21	21	21
nr =	3	3	3
$\bar{X}$ =	18.5	38.5	64.5
ΣX =	388	808	1353
ΣX^2 =	7253	31403	88207
SD =	2.2	3.9	6.9
DF (between) = (n/nr-1)	6	6	6
DF (within) = (n/nr)	7	7	7
V (between) =	16%	16%	17%
V (within) =	13%	9%	9%

FORMULAE

sum of squares between assays:

$$ss(\text{between}) = \frac{\Sigma(T^2)}{3} - \frac{(\Sigma X)^2}{n}$$

$$ss(\text{within}) = \Sigma X^2 - \frac{(\Sigma X)^2}{n} - ss(\text{between})$$

Mean of squares:

$$\text{between assays} = \frac{ss(\text{between})}{DF(\text{between})}$$

$$\text{within assays} = \frac{ss(\text{within})}{DF(\text{within})}$$

Variation:

$$V = \frac{\sqrt{(\text{Mean of squares})}}{\bar{X}}$$

Variation expressed as a percentage = V x 100

APPENDIX B

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
99	23	0	1	0	1	0	1	0	2
114	32	17	2	22	2	39	2	74	3
129	28	65	3	67	3	70	3	81	1
144	36	71	1	75	1	80	1	92	2
159	29	94	2	100	2	127	2	121	4
174	33	107	1	102	1	233	3	138	2
189	33	117	2	118	2	237	1	216	3
204	30	228	3	232	3	267	2	228	4
219	27	236	1	237	1	270	1	239	2
234	36	279	2	260	2	287	2		
249	39			269	1				
264	39			287	2				
279	40								
294	41								
309	38								

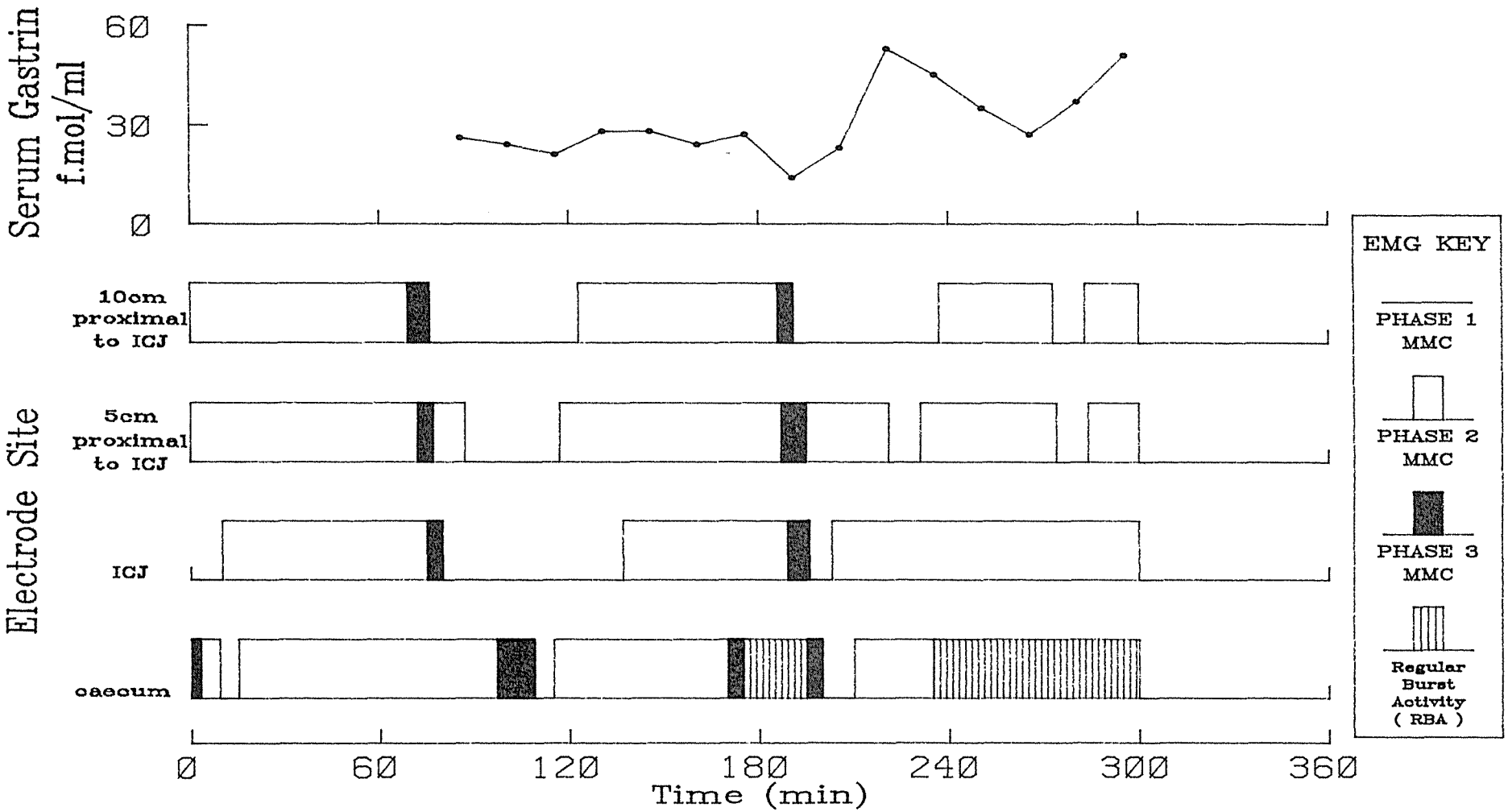
EMG recording ended at T = 310 minutes

TABLE XII
Data for Sheep 1 Control Experiment 1

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
86	26	0	2	0	2	0	1	0	3
101	24	69	3	72	3	10	2	3	2
116	21	76	1	77	2	75	3	9	1
131	28	123	2	87	1	80	1	15	2
146	28	186	3	117	2	137	2	97	3
161	24	191	1	187	3	189	3	109	1
176	27	237	2	195	2	196	1	115	2
191	14	273	1	221	1	203	2	170	3
206	23	283	2	231	2			175	4
221	53			274	1			195	3
236	45			284	2			200	1
251	35							210	2
266	27							235	4
281	37								
296	51								

EMG recording ended at T = 300 minutes

TABLE XIII
Data for Sheep 1 Control Experiment 2

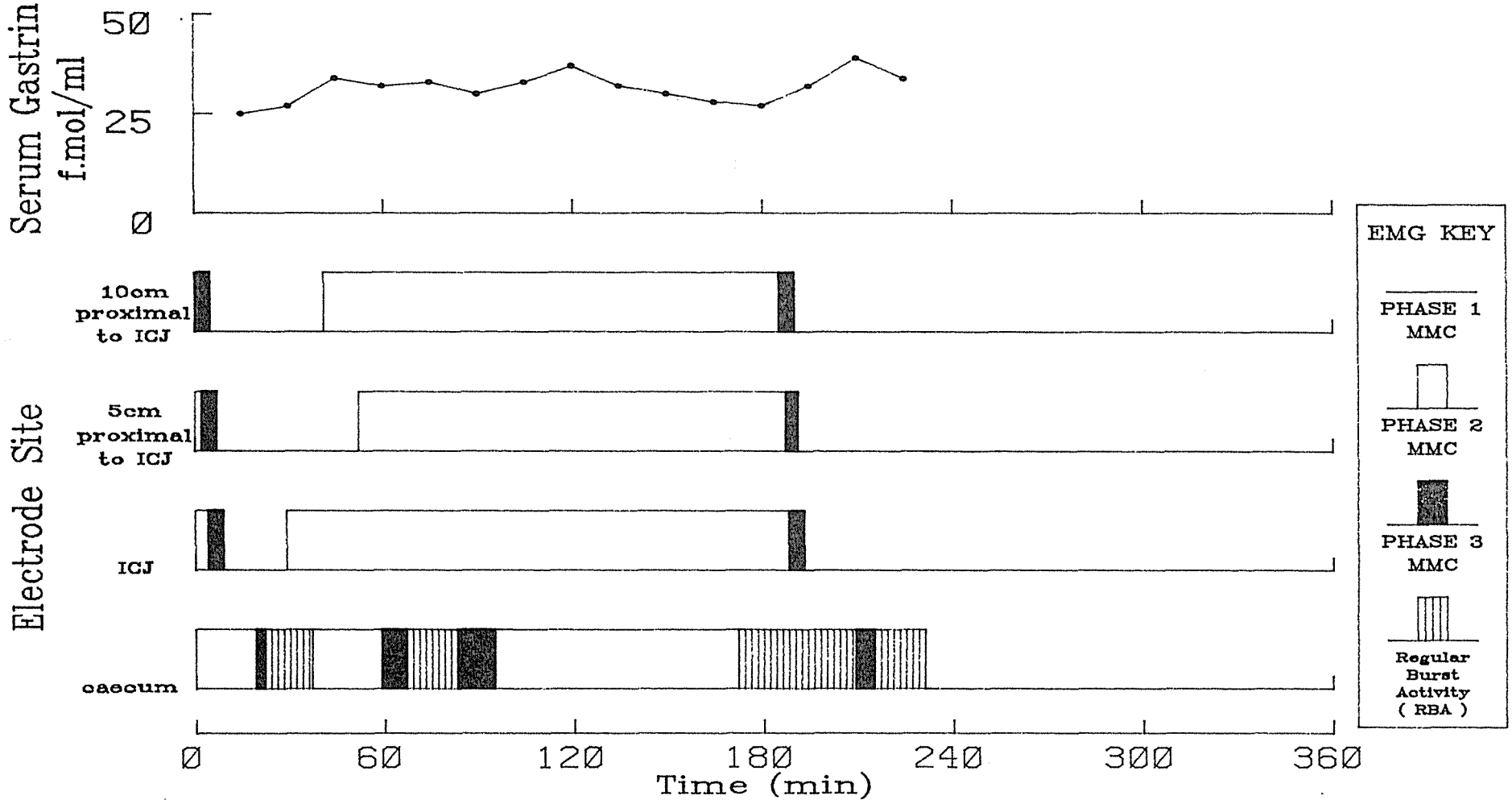


APPENDIX B FIGURE 1 Sheep 1, second control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 300 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
15	25	0	3	0	2	0	2	0	2
30	27	5	1	2	3	4	3	19	3
45	34	41	2	7	1	9	1	22	4
60	32	185	3	52	2	29	2	37	2
75	33	190	1	187	3	188	3	59	3
90	30			191	1	193	1	67	4
105	33							83	3
120	37							95	2
135	32							172	4
150	30							209	3
165	28							215	4
180	27								
195	32								
210	39								
225	34								

EMG recording ended at T = 231 minutes

TABLE XIV
Data for Sheep 1 Control Experiment 3

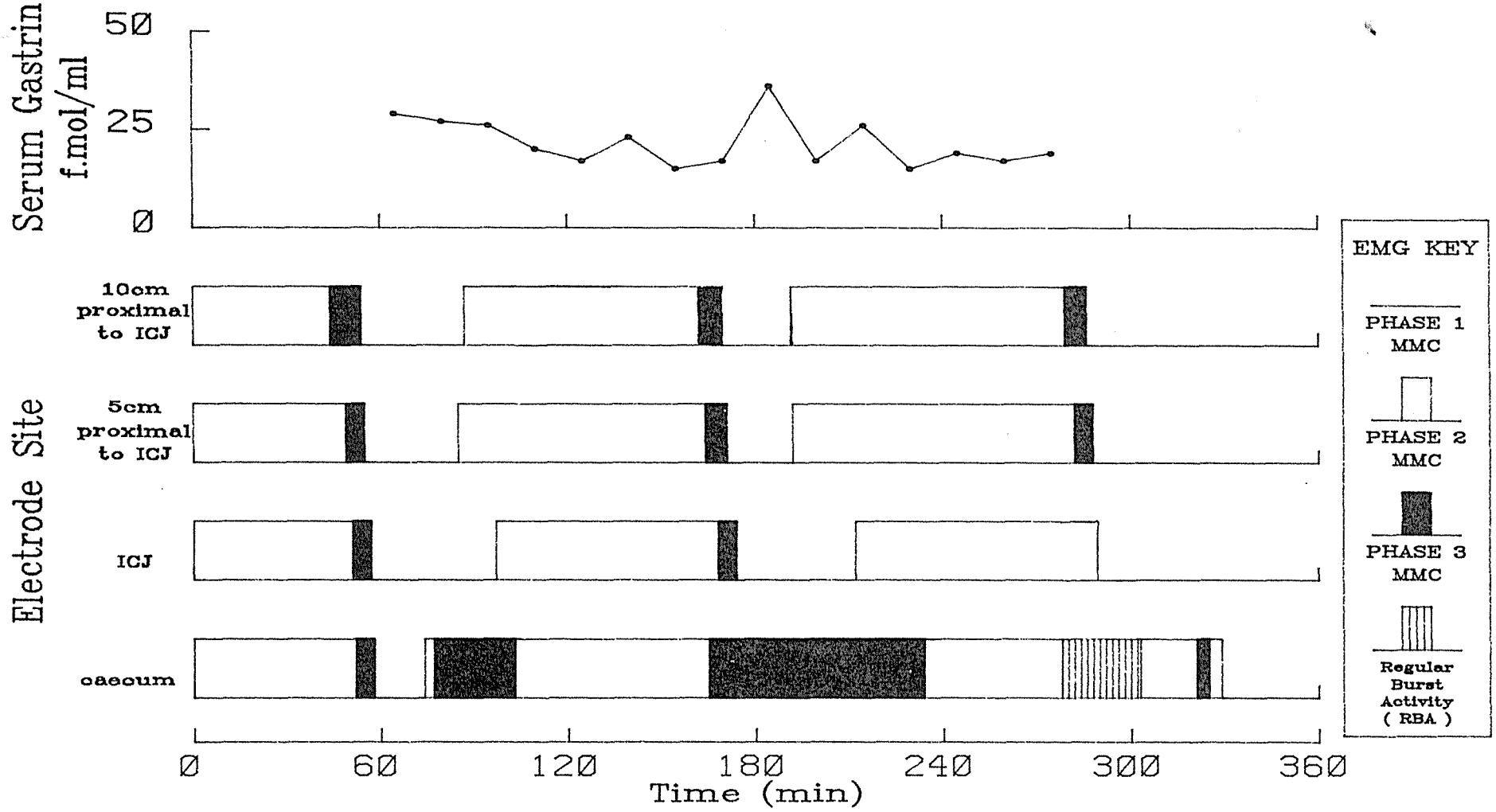


APPENDIX B FIGURE 2 Sheep 1, third control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 231 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
65	29	0	2	0	2	0	2	0	2
80	27	44	3	49	3	51	3	52	3
95	26	54	1	55	1	57	1	58	1
110	20	87	2	85	2	97	2	74	2
125	17	163	3	164	3	168	3	77	3
140	23	168	1	171	1	174	1	103	2
155	15	191	2	192	2	212	2	165	3
170	17	279	3	282	3	281	3	234	2
185	36	286	1	288	1	287	1	278	4
200	17							303	2
215	26							321	3
230	15							325	2
245	19							329	1
260	17								
275	19								

EMG recording ended at T = 360 minutes

TABLE XV
Data for Sheep 1 Control Experiment 4

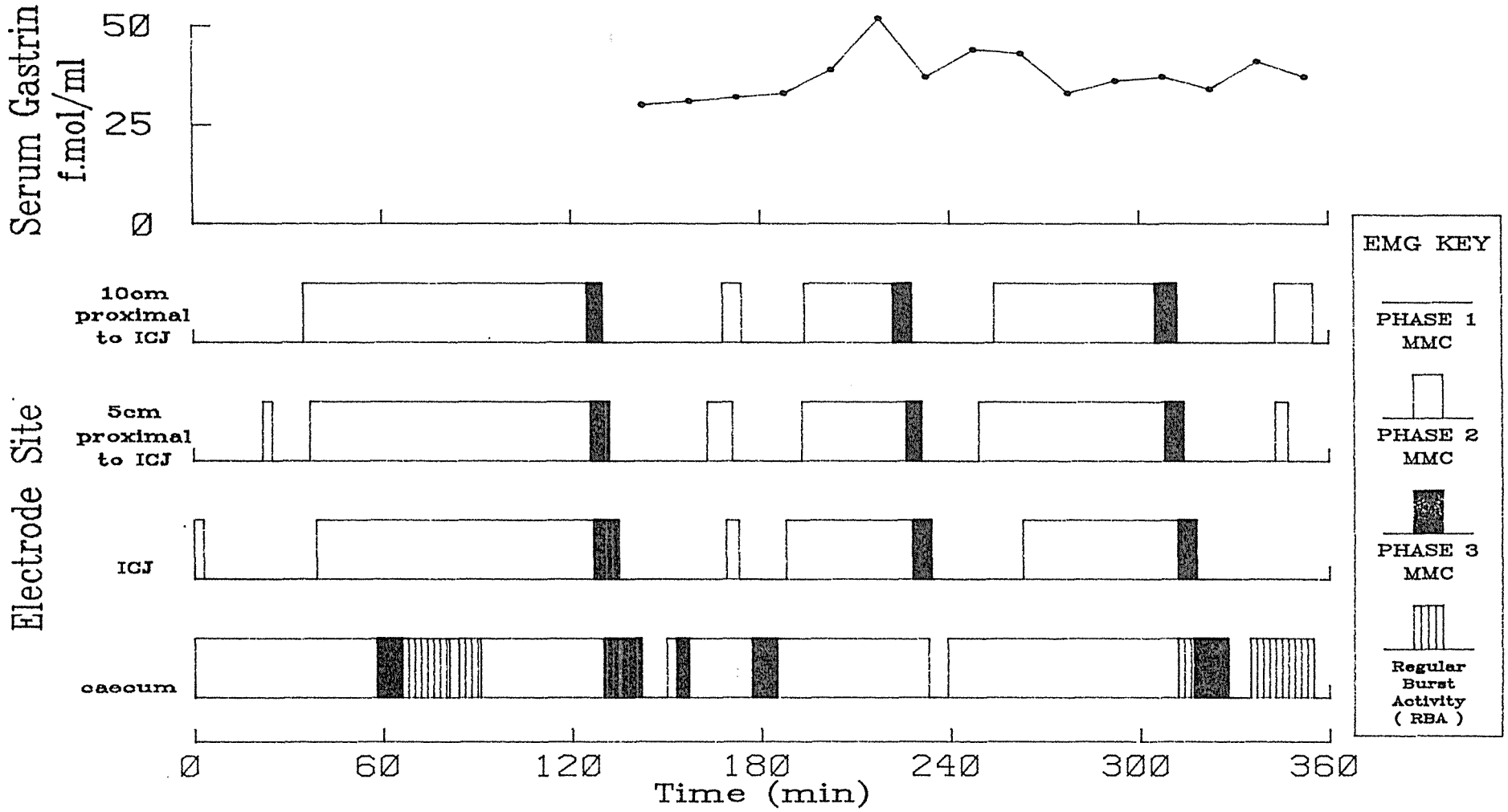


APPENDIX B FIGURE 3 Sheep 1, fourth control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 360 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
143	30	0	1	0	1	0	2	0	2
158	31	35	2	22	2	3	1	58	3
173	32	125	3	25	1	39	2	66	4
188	33	130	1	37	3	127	3	81	2
203	39	168	2	126	1	135	1	84	4
218	52	174	1	132	2	169	2	91	2
233	37	194	2	163	2	173	1	130	3
248	44	222	3	171	1	188	2	142	1
263	43	228	1	193	2	228	3	150	2
278	33	254	2	226	3	234	1	153	3
293	36	305	3	231	1	263	2	157	2
308	37	312	1	249	2	312	3	177	3
323	34	343	2	308	3	318	1	185	2
338	41			314	1			233	1
353	37			343	2			239	2
				347	1			312	4
								317	3
								328	1
								335	4

EMG recording ended at T = 355 minutes

TABLE XVI
Data for Sheep 1 Control Experiment 5

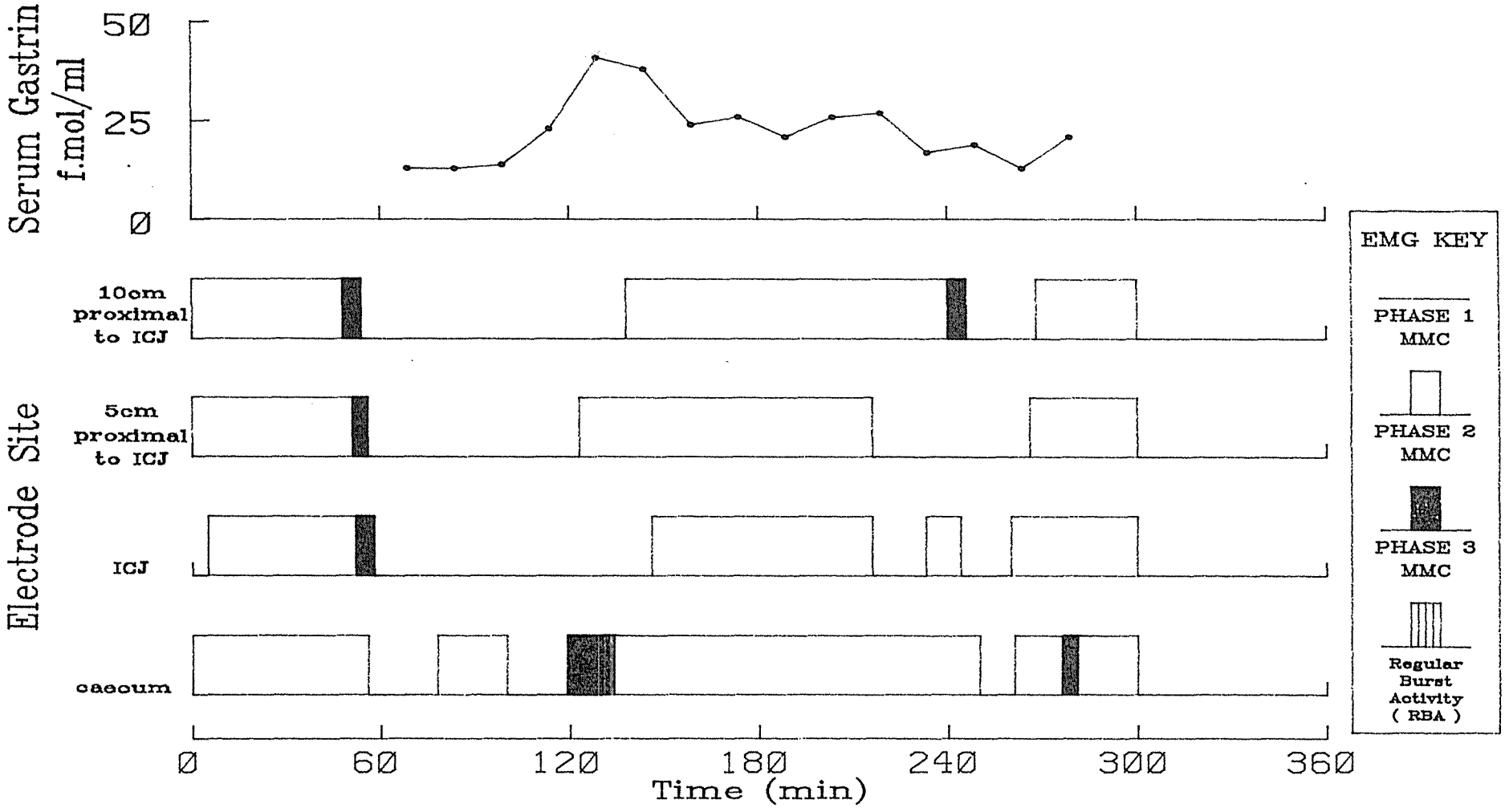


APPENDIX B FIGURE 4 Sheep 1, fifth control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 355 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
69	13	0	2	0	2	0	1	0	2
84	13	48	3	51	3	5	2	56	1
99	14	54	1	56	1	52	3	78	2
114	23	138	2	123	2	58	1	100	1
129	41	240	3	216	1	146	2	119	3
144	38	246	1	266	2	216	1	135	2
159	24	268	2			233	2	250	1
174	26					244	1	261	2
189	21					260	2	276	3
204	26							281	2
219	27								
234	17								
249	19								
264	13								
279	21								

Feeding commenced at T = 100 minutes
EMG recording ended at T = 300 minutes

TABLE XVII
Data for Sheep 1 Feeding Experiment 1

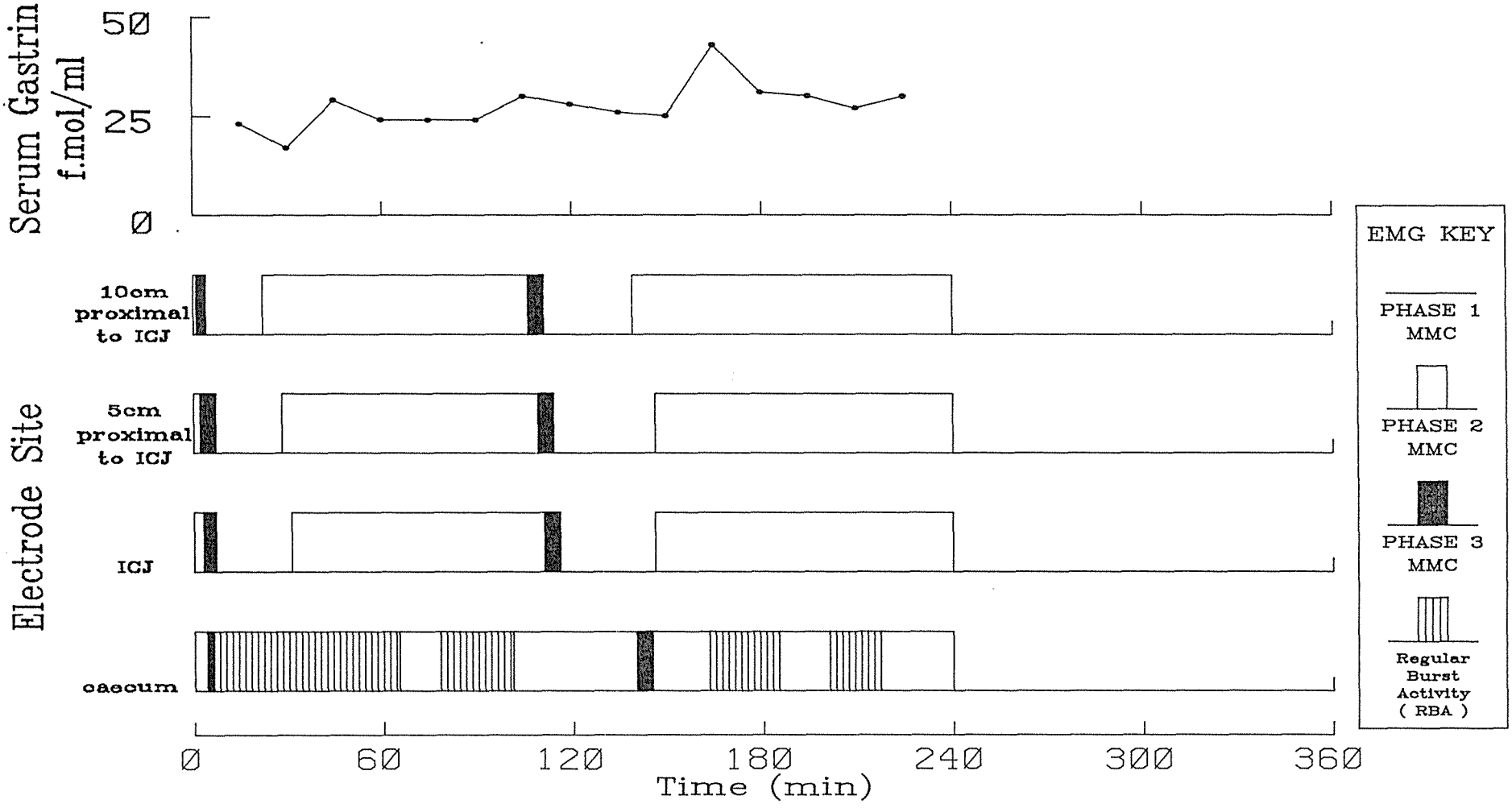


APPENDIX B FIGURE 5 Sheep 1, first feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 100 minutes. An increase in serum gastrin was observed following feeding. EMG recording ended at T = 300 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
15	23	0	2	0	2	0	22	0	2
30	18	1	3	2	3	3	3	4	3
45	29	4	1	7	1	7	1	6	4
60	24	22	2	28	2	31	2	65	2
75	24	106	3	109	3	111	3	78	4
90	24	111	1	114	1	116	1	101	
105	30	139	2	146	2	146	2	140	3
120	28							145	2
135	26							163	4
150	25							185	2
165	43							201	4
180	31							217	2
195	30								
210	27								
225	30								

Feeding commenced at T = 46 minutes
EMG recording ended at T = 240 minutes

TABLE XVIII
Data for Sheep 1 Feeding Experiment 2

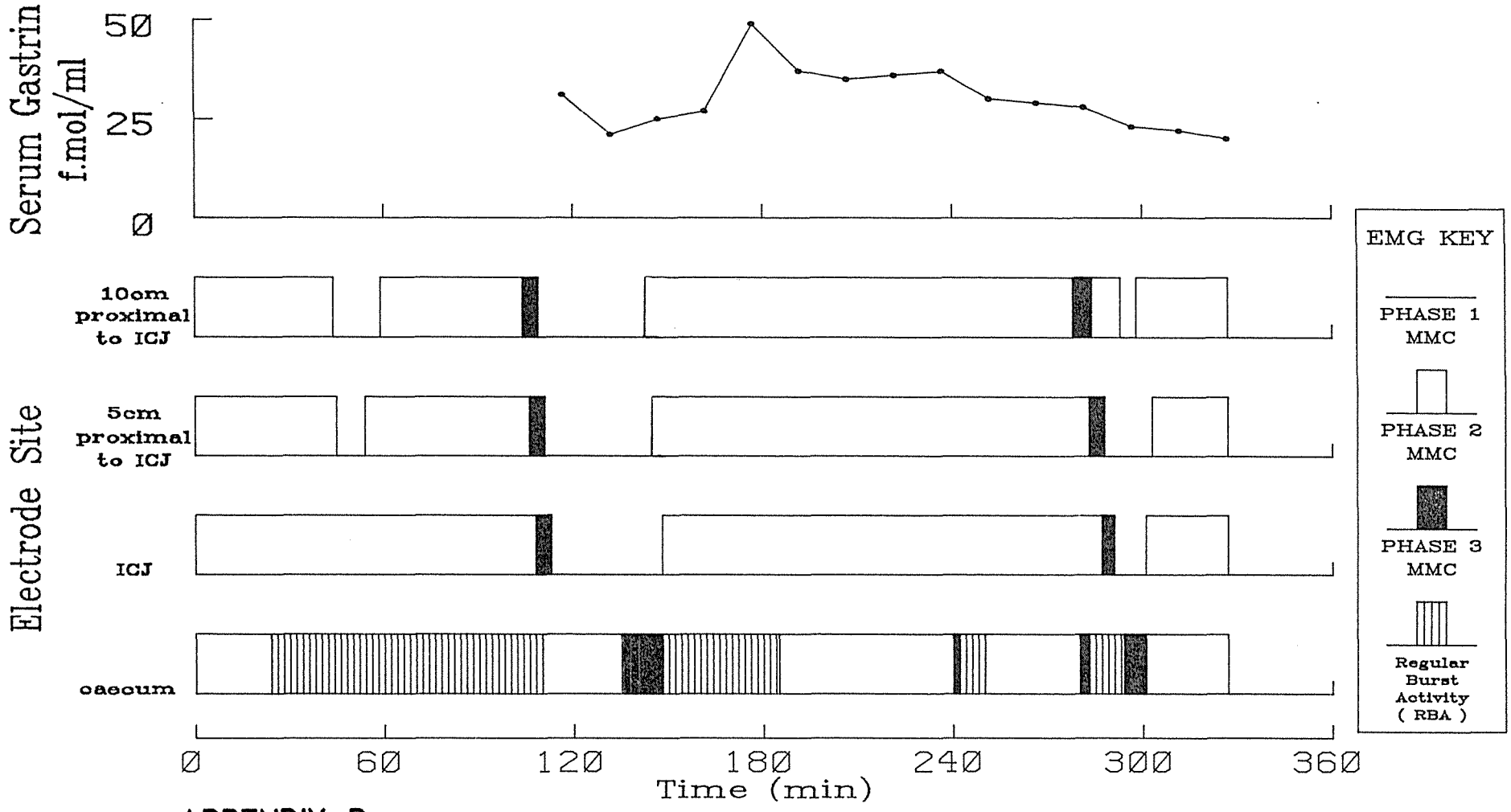


APPENDIX B FIGURE 6 Sheep 1, second feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 46 minutes. EMG recording ended at T = 240 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
117	31	0	2	0	2	0	2	0	2
132	21	44	1	45	1	108	3	24	4
147	25	59	2	54	2	113	1	110	2
162	27	104	3	106	3	148	2	135	3
177	49	109	1	111	1	287	3	148	4
192	37	143	2	145	2	291	1	185	2
207	35	278	3	283	3	301	2	240	3
222	36	284	2	288	1			242	4
237	37	293	1	303	2			250	2
252	30	298	2					280	3
267	29							283	4
282	28							294	3
297	23							301	2
312	22								
327	20								

Feeding commenced at T = 148 minutes
EMG recording ended at T = 327 minutes

TABLE XIX
Data for Sheep 1 Feeding Experiment 3

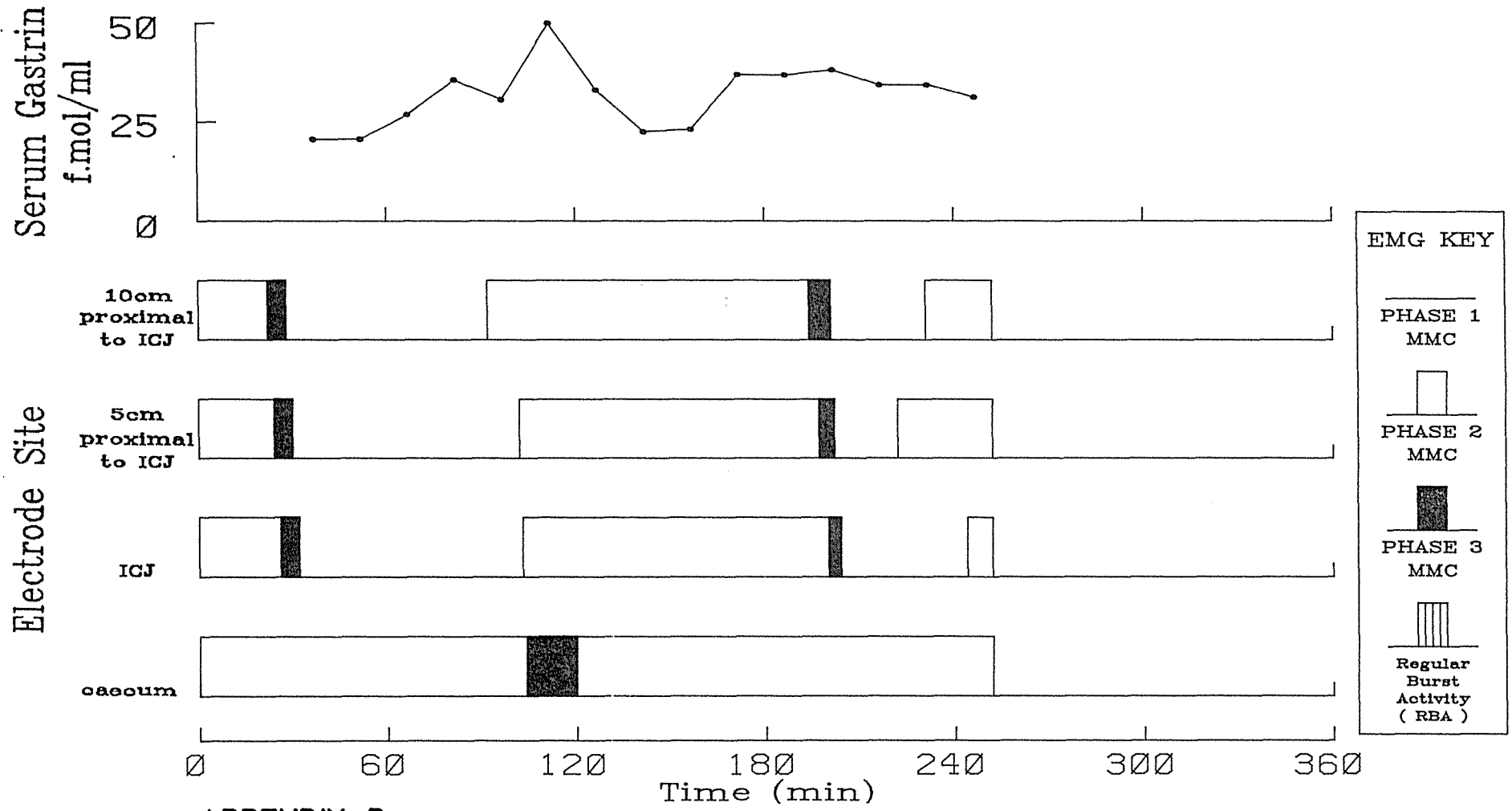


APPENDIX B FIGURE 7 Sheep 1, third feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 148 minutes. An increase in serum gastrin was observed following feeding. EMG recording ended at T = 327 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
37	33	0	2	0	2	0	2	0	2
52	33	22	3	24	3	26	3	104	3
67	43	28	1	30	1	32	1	120	2
82	57	92	2	102	2	103	2		
97	49	194	3	197	3	200	3		
112	80	201	1	202	1	204	1		
127	53	231	2	222	2	244	2		
142	36								
157	37								
172	59								
187	59								
202	61								
217	55								
232	55								
247	50								

Feeding commenced at T = 68 minutes
EMG recording ended at T = 252 minutes

TABLE XX
Data for Sheep 1 Feeding Experiment 4

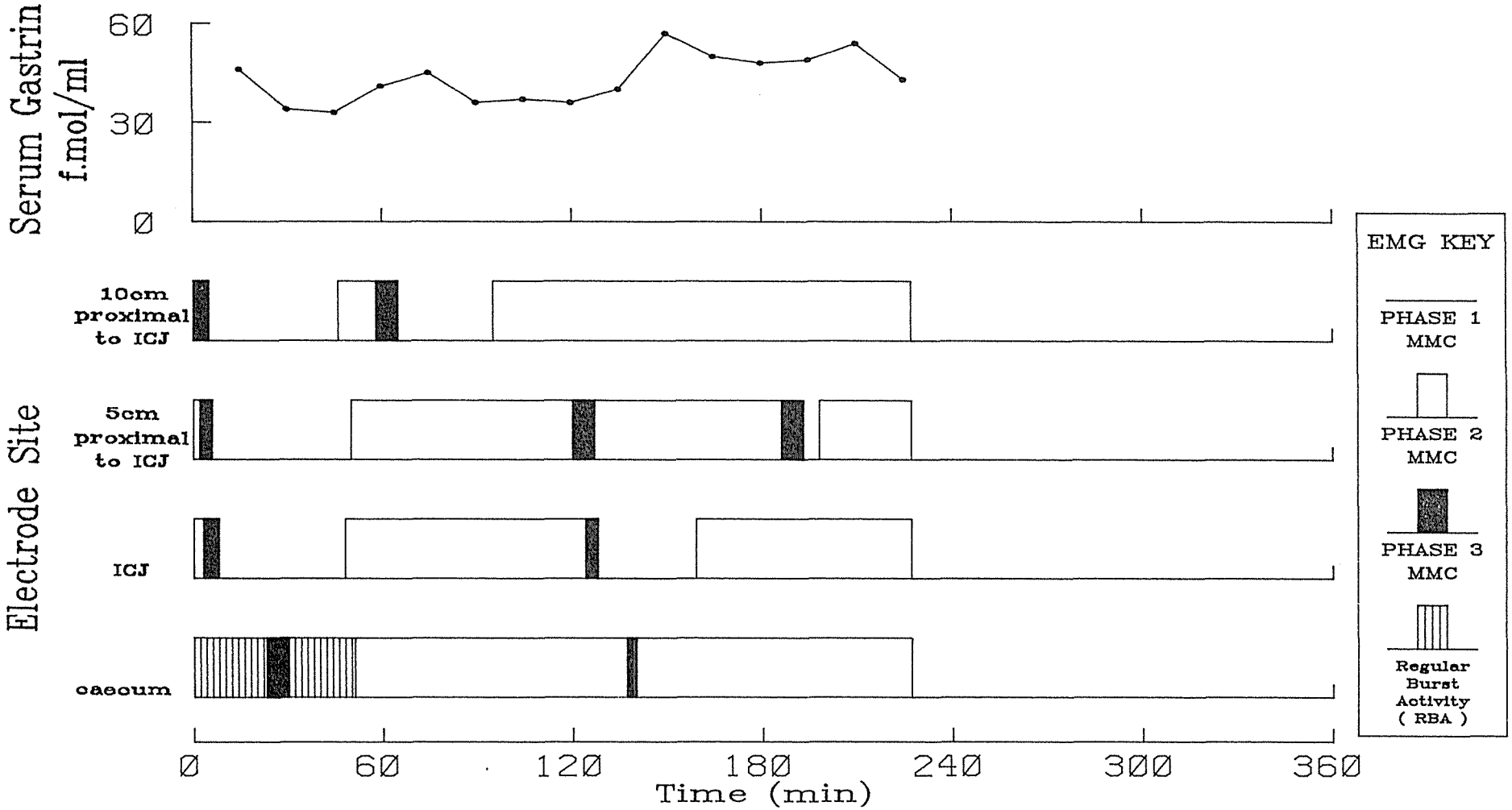


APPENDIX B FIGURE 8 Sheep 1, fourth feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 68 minutes. An increase in serum gastrin was observed following feeding. EMG recording ended at T = 252 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
15	46	0	3	0	2	0	2	0	4
30	34	5	1	2	3	3	3	23	3
45	33	46	2	6	1	8	1	30	4
60	41	58	3	50	2	48	2	51	2
75	45	65	1	120	3	124	3	137	3
90	36	95	2	127	2	128	1	140	2
105	37			186	3	159	2		
120	36			193	1				
135	40			198	2				
150	57								
165	50								
180	48								
195	49								
210	54								
225	43								

Feeding commenced at T = 46 minutes
EMG recording ended at T = 227 minutes

TABLE XXI
Data for Sheep 1 Feeding Experiment 5

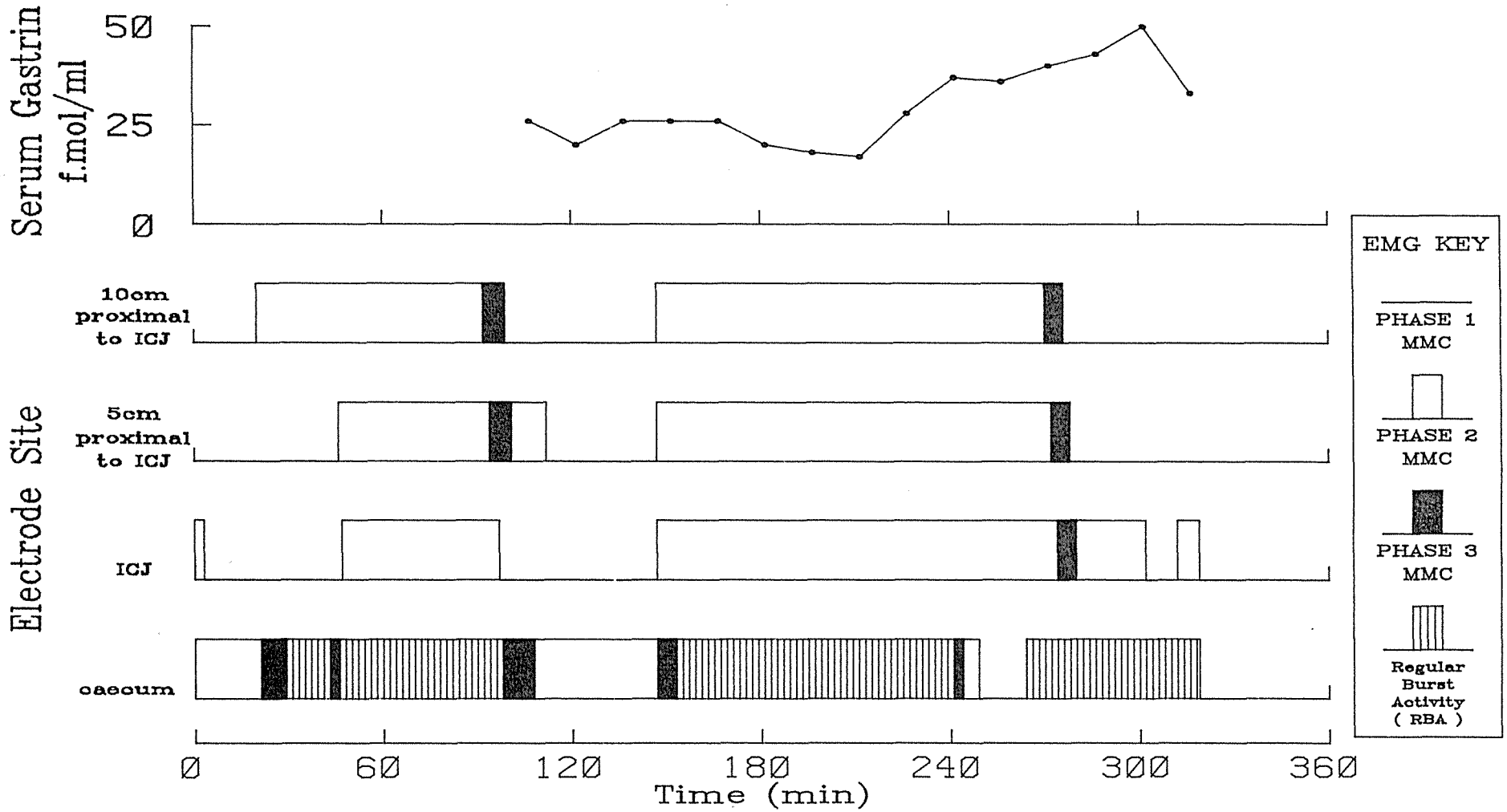


APPENDIX B FIGURE 9 Sheep 1, fifth feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 46 minutes. EMG recording ended at T = 227 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
107	26	0	1	0	1	0	2	0	2
122	20	20	2	46	2	3	1	21	3
137	26	92	3	94	3	47	2	29	4
152	26	99	1	101	2	97	1	43	3
167	26	147	2	112	1	147	2	46	4
182	20	270	3	147	2	274	3	98	3
197	18	276	1	272	3	280	2	108	2
212	17			278	1	302	1	147	3
227	28					312	2	153	4
242	37							241	3
257	36							244	2
272	40							249	1
287	43							264	4
302	50								
317	33								

Pentagastrin administered at T = 138 minutes
EMG recording ended at T = 319 minutes

TABLE XXII
Data for Sheep 1 Pentagastrin Experiment 1

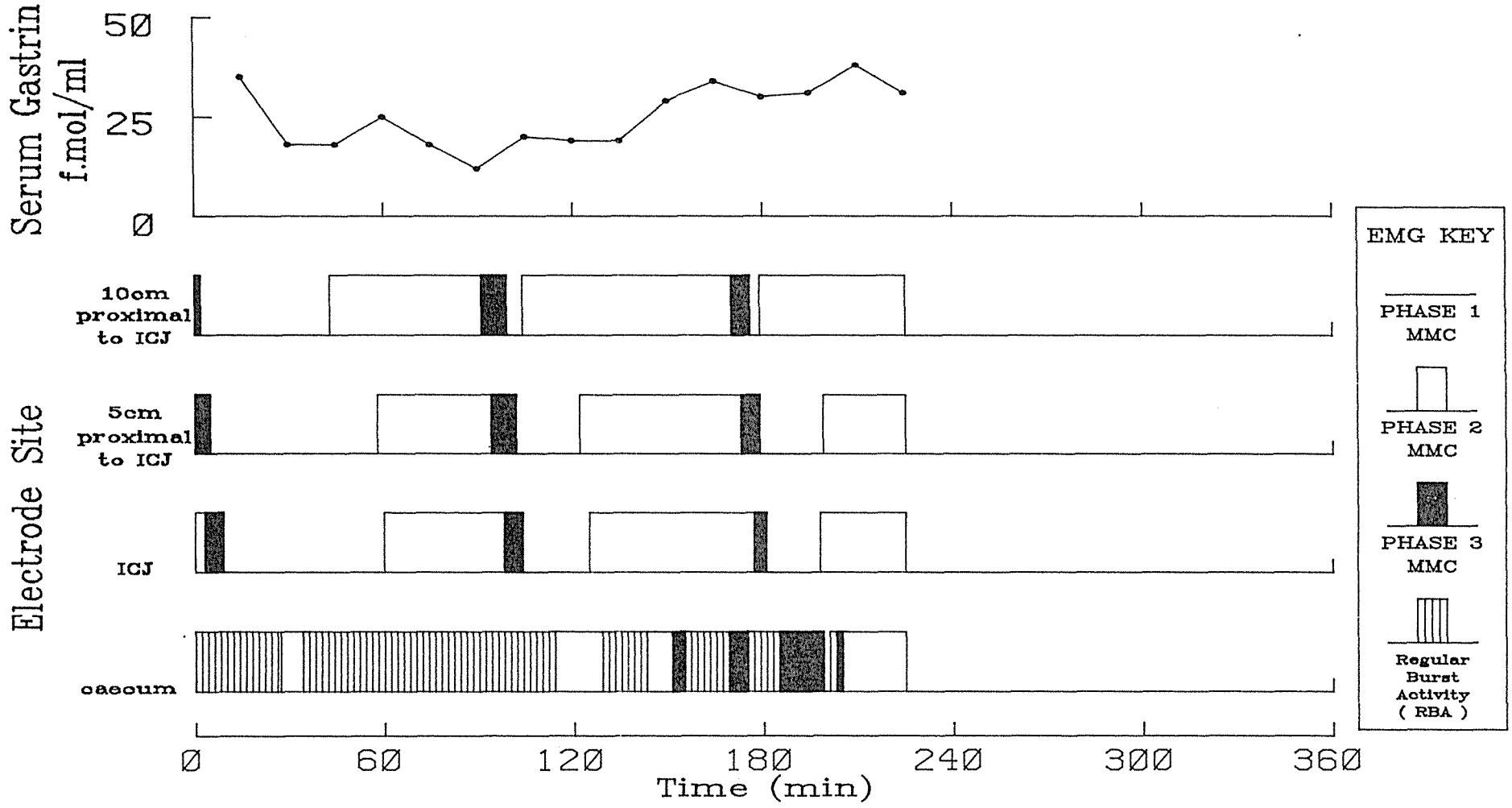


APPENDIX B FIGURE 10 Sheep 1, first pentagastrin experiment: graph of EMG activity and serum gastrin levels. 7 µg.kg pentagastrin was administered subcutaneously at T = 138 minutes. EMG recording ended at T = 319 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
15	35	0	3	0	3	0	2	0	4
30	18	2	1	5	1	3	3	27	2
45	18	43	2	58	2	9	1	34	4
60	25	91	3	94	3	60	2	114	2
75	18	99	1	102	1	98	3	129	4
90	12	104	2	122	2	104	1	143	2
105	20	170	3	173	3	125	2	151	3
120	19	176	1	179	1	177	3	155	4
135	19	179	2	199	2	181	1	169	3
150	29					198	2	175	4
165	34							185	3
180	30							199	1
195	31							201	2
210	38							203	3
225	31							205	2

Pentagastrin administered at T = 46 minutes
EMG recording ended at T = 225 minutes

TABLE XXIII
Data for Sheep 1 Pentagastrin Experiment 2

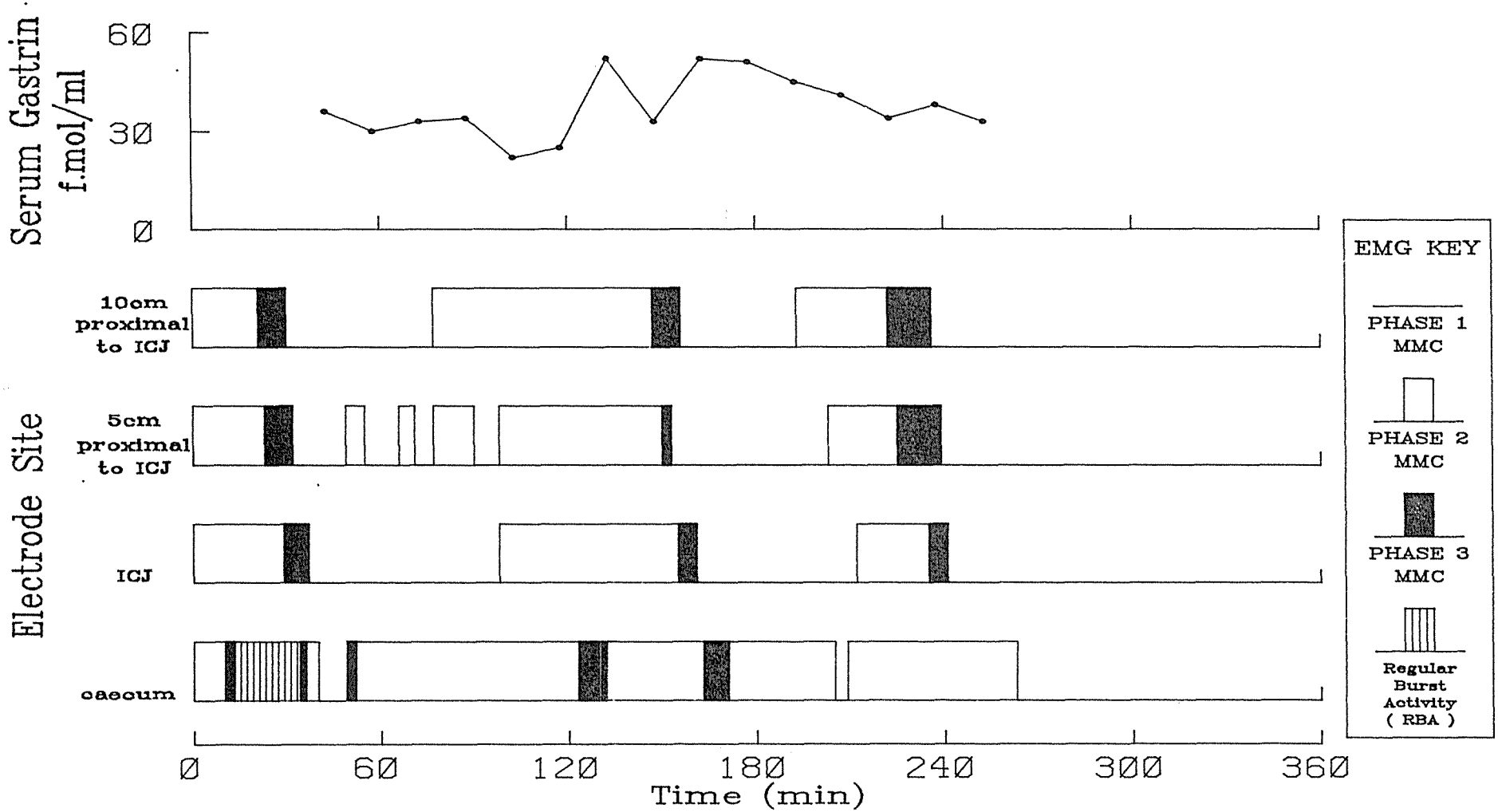


APPENDIX B FIGURE 11 Sheep 1, second pentagastrin experiment: graph of EMG activity and serum gastrin leve
7 µg.kg pentagastrin was administered subcutaneously at T = 46 minutes.
EMG recording ended at T = 225 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
43	36								
58	30	0	2	0	2	0	2	0	2
73	33	21	3	23	3	29	3	10	3
88	34	30	1	32	1	37	1	13	4
103	22	77	2	49	2	98	2	34	3
118	25	147	3	55	1	155	3	36	2
133	52	156	1	66	2	161	1	40	1
148	33	193	2	71	1	212	2	49	3
163	52	222	3	77	2	235	3	52	2
178	51	236	1	90	1	241	1	123	3
193	45			98	2	263	1	132	2
208	41			150	3			163	3
223	34			153	1			171	2
238	38			203	2			205	1
253	33			225	3			209	2
				239	1			263	1

Pentagastrin administered at T = 74 minutes
EMG recording ended at T = 263 minutes

TABLE XXIV
Data for Sheep 1 Pentagastrin Experiment 3

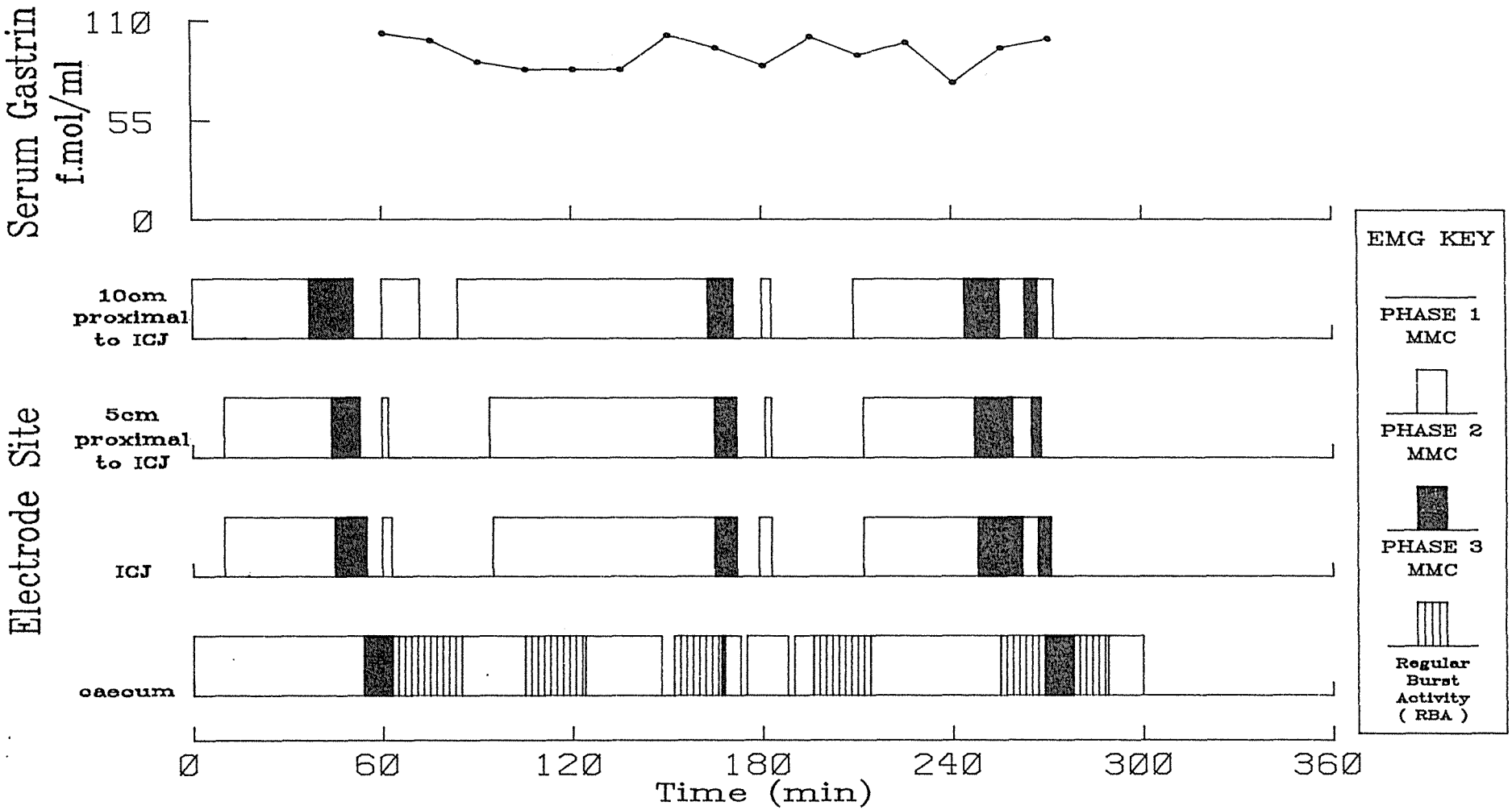


APPENDIX B FIGURE 12 Sheep 1, third pentagastrin experiment: graph of EMG activity and serum gastrin levels 7 µg.kg pentagastrin was administered subcutaneously at T = 74 minutes. EMG recording ended at T = 263 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
61	103	0	2	0	1	0	1	0	2
76	99	37	3	10	2	10	2	54	3
91	87	51	1	44	3	45	3	63	4
106	83	60	2	53	1	55	1	85	2
121	83	72	1	60	2	60	2	105	4
136	83	84	2	62	1	63	1	124	2
151	102	163	3	94	2	95	2	148	1
166	95	171	1	165	3	165	3	152	4
181	85	180	2	172	1	172	1	167	3
196	101	183	1	181	2	179	2	168	2
211	91	209	2	183	1	183	1	173	1
226	98	244	3	212	2	212	2	175	2
241	76	255	2	247	3	248	3	188	1
256	95	263	3	259	2	262	2	190	2
271	100	267	2	265	3	267	3	196	4
		272	1	268	1	271	1	214	2
								255	4
								269	3
								278	4
								289	2

High gastrin levels due to nematode infestation
EMG recording ended at T = 300 minutes

TABLE XXV
Data for Sheep 2 Control Experiment 1

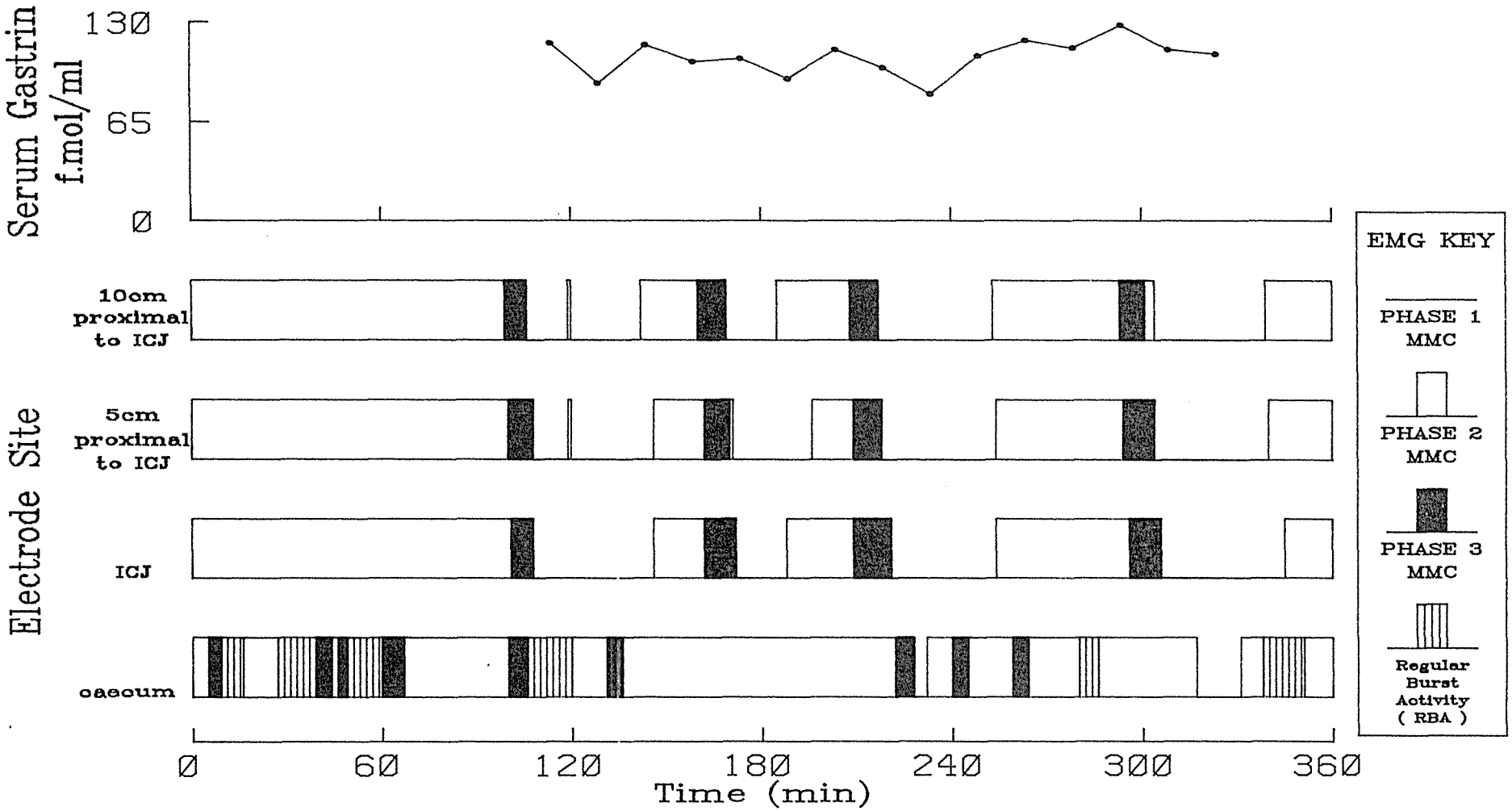


APPENDIX B FIGURE 13 Sheep 2, first control experiment: graph of EMG activity and serum gastrin levels. The high serum gastrin levels indicate a nematode infestation. EMG recording ended at T = 300 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
114	116	0	2	0	2	0	2	0	2
129	90	99	3	100	3	101	3	5	3
144	115	106	1	108	1	108	1	9	4
159	104	119	2	119	2	146	2	16	2
174	106	120	1	120	1	162	3	27	4
189	93	142	2	146	2	172	1	39	3
204	112	160	3	162	3	188	2	44	2
219	100	169	1	170	2	209	3	46	3
234	83	185	2	171	1	221	1	49	4
249	108	208	3	196	2	254	2	60	3
264	118	217	1	209	3	296	3	67	2
279	113	253	2	218	1	306	1	100	3
294	128	293	3	254	2	345	2	106	4
309	112	301	2	294	3			120	2
324	109	304	1	304	1			131	3
		339	2	340	2			136	2
								222	3
								228	1
								232	2
								240	3
								245	2
								259	3
								264	2
								280	4
								286	2
								317	3
								331	2
								338	4
								351	2

High gastrin levels due to nematode infestation
EMG recording ended at T = 360 minutes

TABLE XXVI
Data for Sheep 2 Control Experiment 2

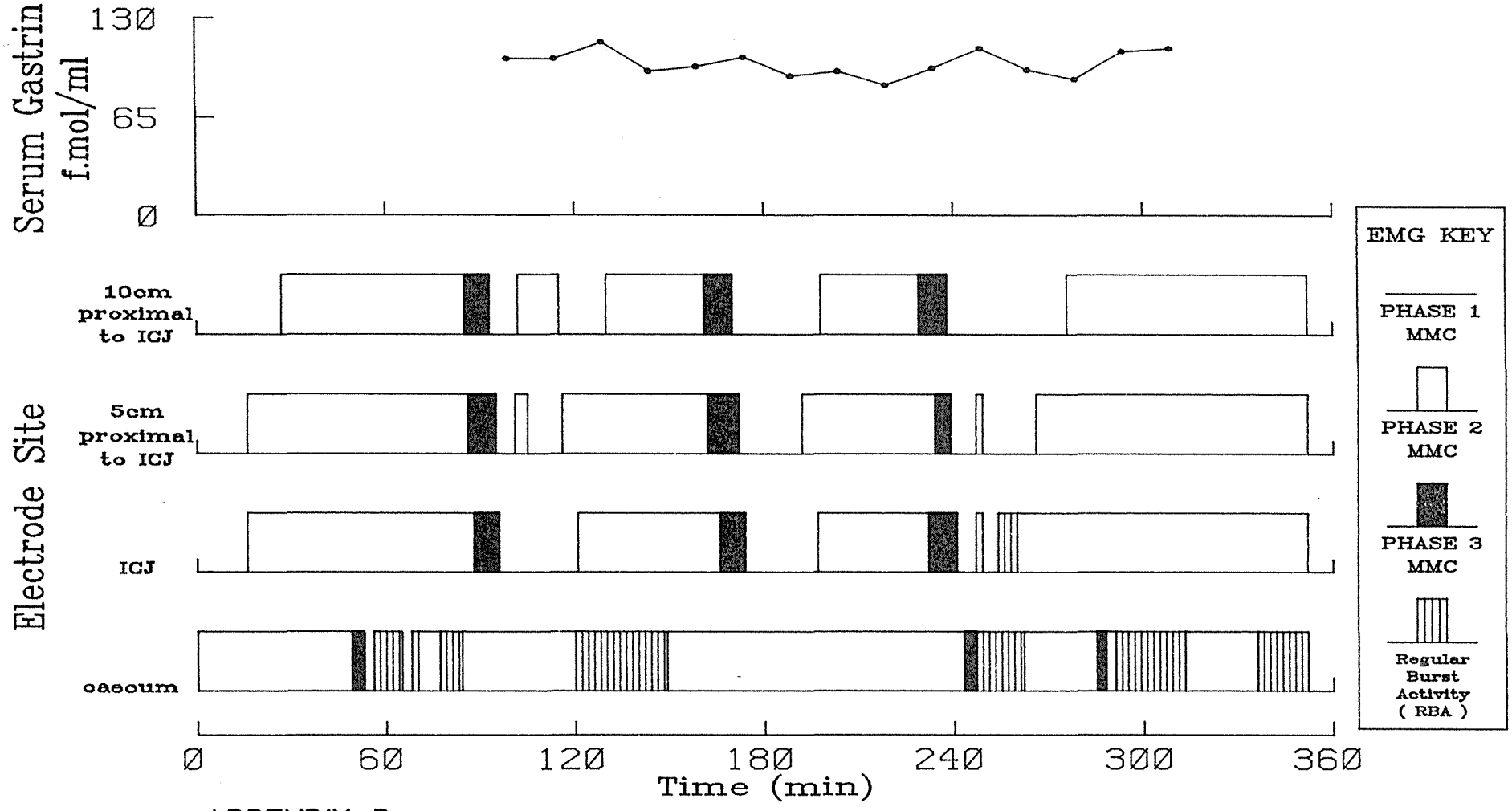


APPENDIX B FIGURE 14 Sheep 2, second control experiment: graph of EMG activity and serum gastrin levels. The high serum gastrin levels indicate a nematode infestation. EMG recording ended at T = 360 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
99	103	0	1	0	1	0	1	0	2
114	103	27	2	16	2	16	2	49	3
129	114	85	3	86	3	88	3	53	1
144	95	93	1	95	1	96	1	56	4
159	98	102	2	101	2	121	2	65	1
174	104	115	1	105	1	166	3	68	4
189	92	130	2	116	2	174	1	70	2
204	95	161	3	162	3	197	2	77	4
219	86	170	1	172	1	232	3	84	2
234	97	198	2	192	2	241	1	120	4
249	110	229	3	234	3	247	2	149	2
264	96	238	1	239	1	249	1	243	3
279	90	276	2	247	2	254	4	247	4
294	108			249	1	260	2	262	2
309	110			266	2			285	3
								288	2
								291	4
								313	2
								336	4

High gastrin levels due to nematode infestation
EMG recording ended at T = 352 minutes

TABLE XXVII
Data for Sheep 2 Control Experiment 3

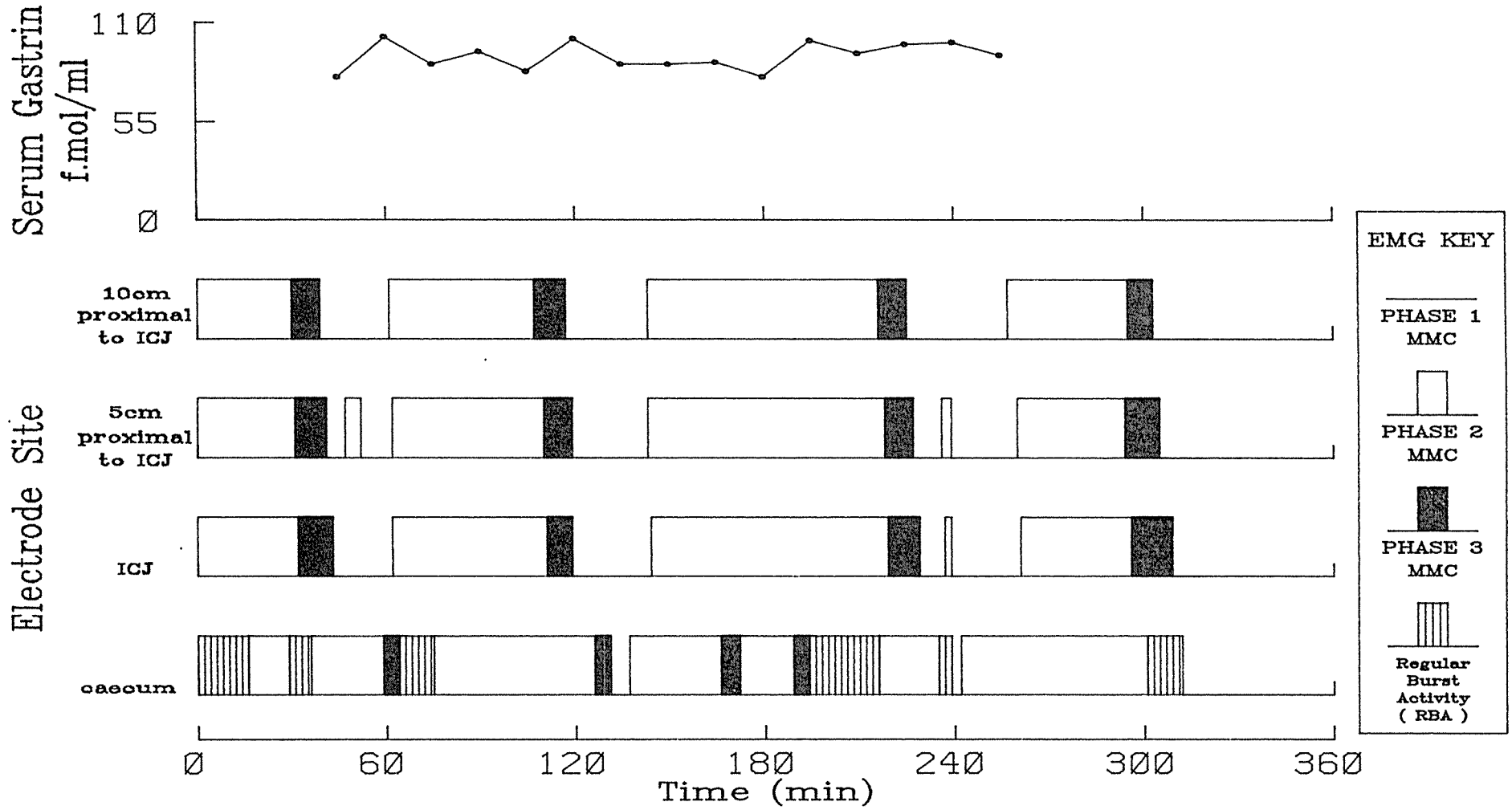


APPENDIX B FIGURE 15 Sheep 2, third control experiment: graph of EMG activity and serum gastrin levels. The high serum gastrin levels indicate a nematode infestation. EMG recording ended at T = 352 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
45	80	0	2	0	2	0	2	0	4
60	102	30	3	31	3	32	3	16	2
75	87	39	1	41	1	43	1	29	4
90	94	61	2	47	2	62	2	36	2
105	83	107	3	52	1	111	3	59	3
120	101	117	1	62	2	119	1	64	4
135	87	143	2	110	3	144	2	75	2
150	87	216	3	119	1	219	3	126	3
165	88	225	1	143	2	229	1	131	1
180	80	257	2	218	3	237	2	137	2
195	100	295	3	227	1	239	1	166	3
210	93	303	1	236	2	261	2	172	2
225	98			239	1	296	3	189	3
240	99			260	2	309	1	194	4
255	92			294	3			216	2
				305	1			235	4
								239	1
								242	2
								301	4

High gastrin levels due to nematode infestation
EMG recording ended at T = 312 minutes

TABLE XXVIII
Data for Sheep 2 Control Experiment 4

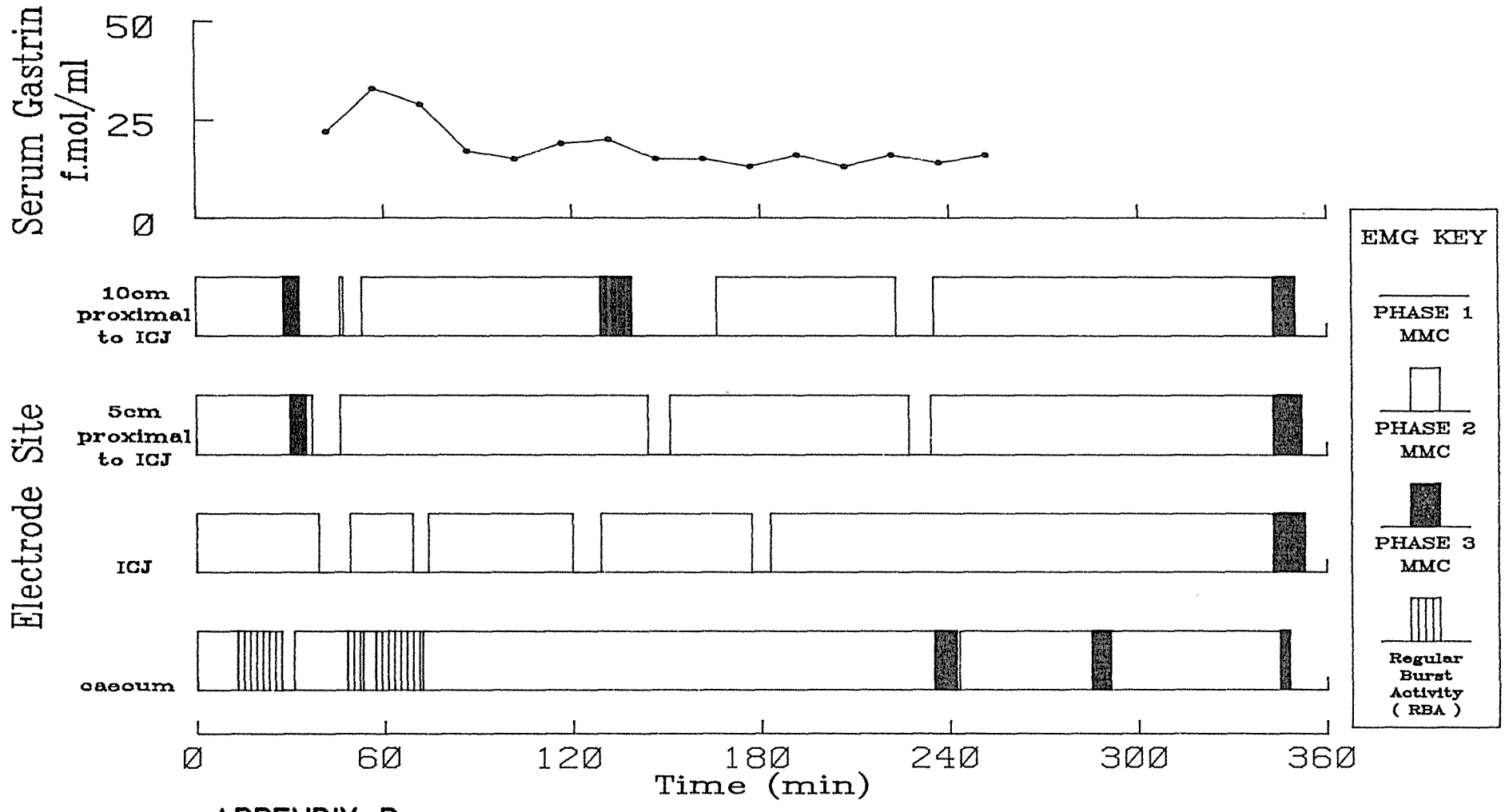


APPENDIX B FIGURE 16 Sheep 2, fourth control experiment: graph of EMG activity and serum gastrin levels. The high serum gastrin levels indicate a nematode infestation. EMG recording ended at T = 312 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
42	22	0	2	0	2	0	2	0	2
57	33	28	3	30	3	39	1	13	4
72	29	33	1	35	2	49	2	27	1
87	17	46	2	37	1	69	1	31	2
102	15	47	1	46	2	74	2	48	4
117	19	53	2	144	1	120	1	53	2
132	20	129	3	151	2	129	2	57	4
147	15	139	1	227	1	177	1	72	2
162	15	166	2	234	2	183	2	235	3
177	13	223	1	343	3	343	3	242	1
192	16	235	2	352	1	353	1	243	2
207	13	343	3					285	3
222	16	350	1					291	2
237	14							345	3
252	16							348	1

EMG recording ended at T = 360 minutes

TABLE XXIX
Data for Sheep 2 Control Experiment 5

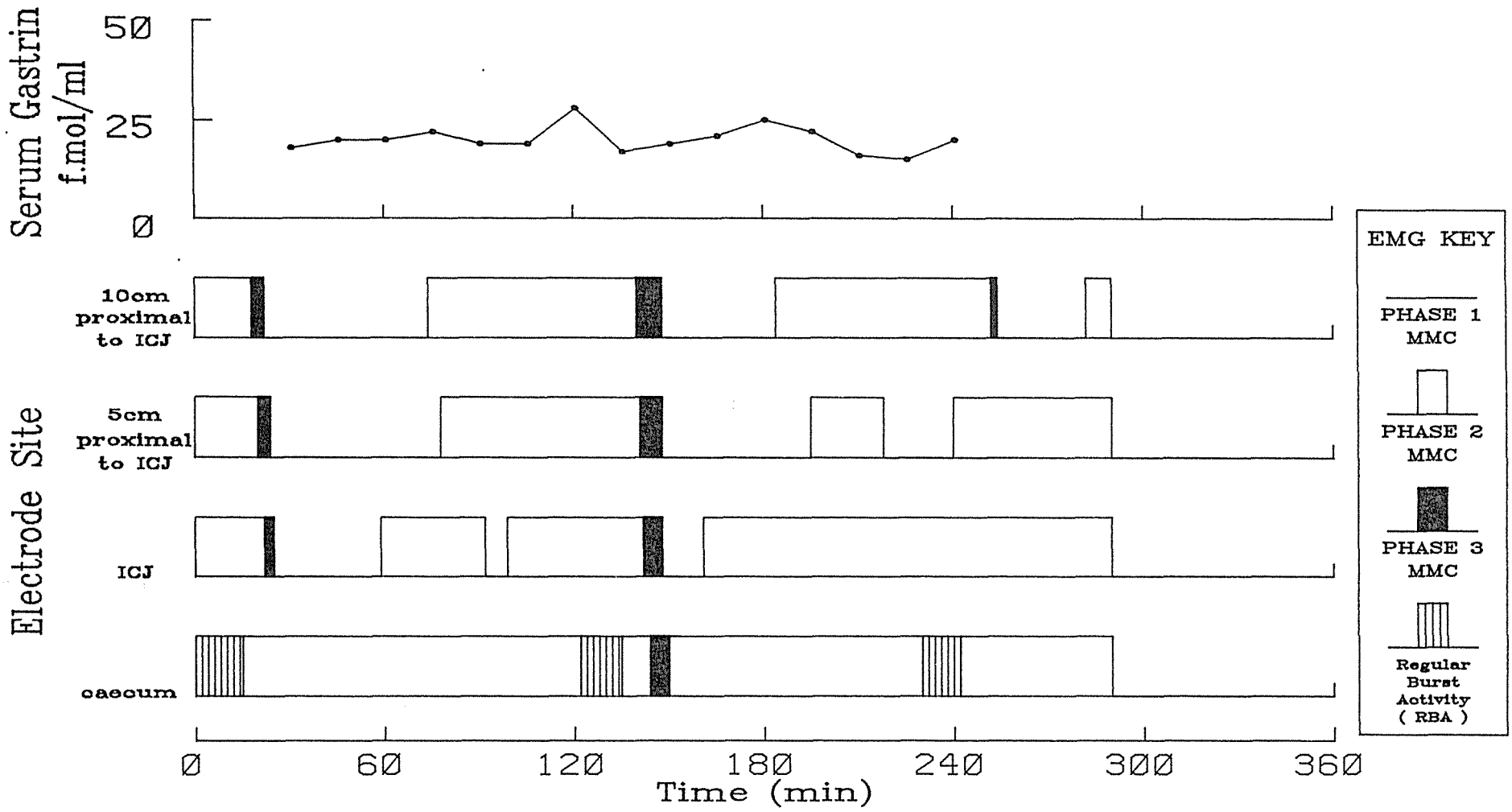


APPENDIX B FIGURE 17 Sheep 2, fifth control experiment: graph of EMG activity and serum gastrin levels. Serum gastrin levels are in the normal range after anthelmintic treatment for a nematode infestation. EMG recording ended at T = 360 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
31	18	0	2	0	2	0	2	0	4
46	20	18	3	20	3	22	3	15	2
61	20	22	1	24	1	25	1	122	4
76	22	74	2	78	2	59	2	135	2
91	19	140	3	141	3	92	1	144	3
106	19	148	1	148	1	99	2	150	2
121	28	184	2	195	2	142	3	230	4
136	17	252	3	218	1	148	1	242	2
151	19	254	1	240	2	161	2		
166	21	282	2						
181	25								
196	22								
211	16								
226	15								
241	20								

EMG recording ended at T = 290 minutes

TABLE XXX
Data for Sheep 2 Control Experiment 6

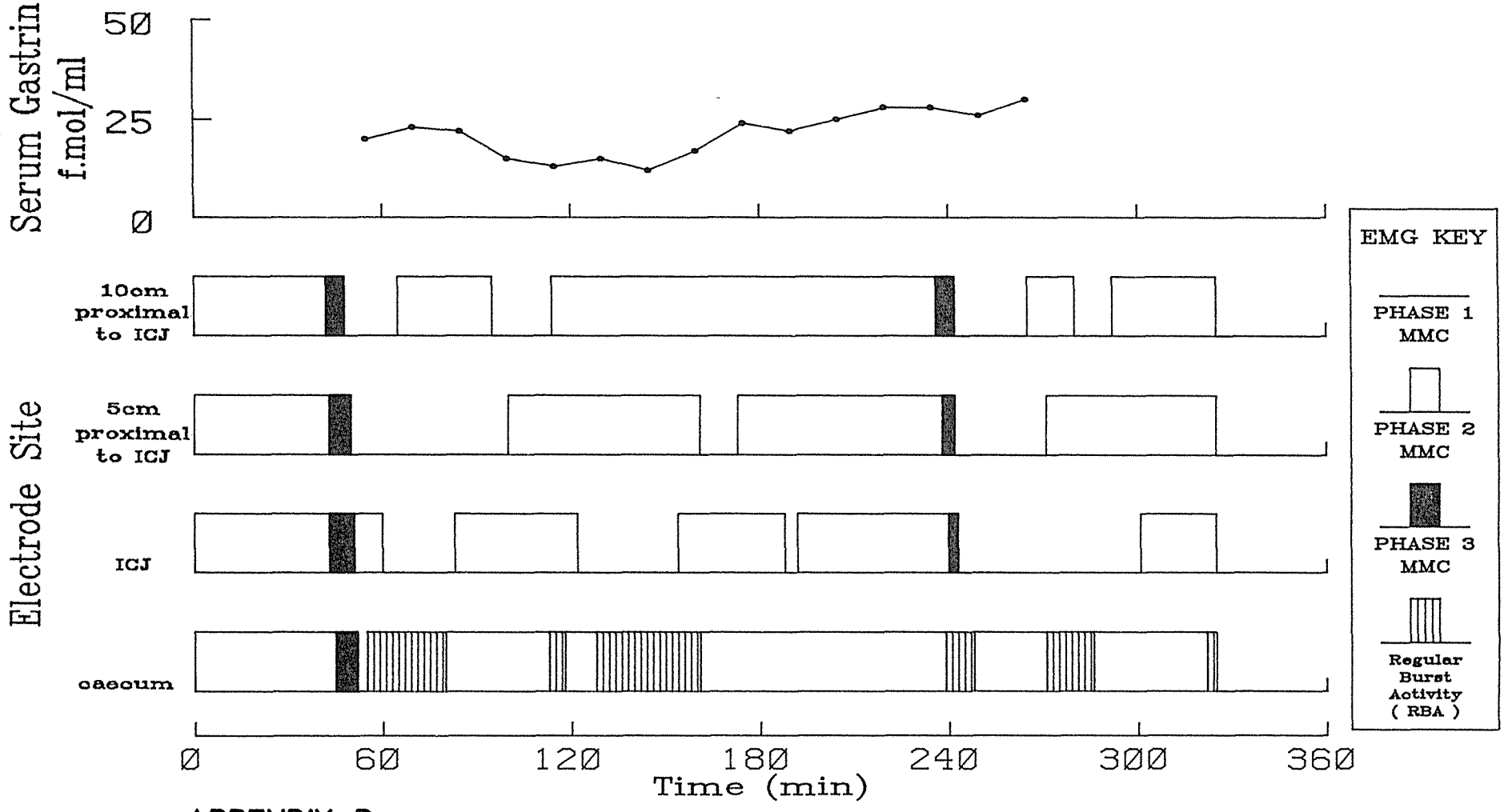


APPENDIX B FIGURE 18 Sheep 2, sixth control experiment: graph of EMG activity and serum gastrin levels. Serum gastrin levels are in the normal range after anthelmintic treatment for a nematode infestation. EMG recording ended at T = 290 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
55	20	0	2	0	2	0	2	0	2
70	23	42	3	43	3	43	3	45	3
85	22	48	1	50	1	51	2	52	1
100	15	65	2	100	2	60	1	55	4
115	13	95	1	161	1	83	2	80	2
130	15	114	2	173	2	122	1	113	4
145	12	236	3	238	3	154	2	118	2
160	17	242	1	242	1	188	1	128	4
175	24	265	2	271	2	192	2	161	2
190	22	280	1			240	3	239	4
205	25	292	2			243	1	248	2
220	28					301	2	271	4
235	28							286	2
250	26							322	4
265	30								

EMG recording ended at T = 325 minutes

TABLE XXXI
Data for Sheep 2 Control Experiment 7

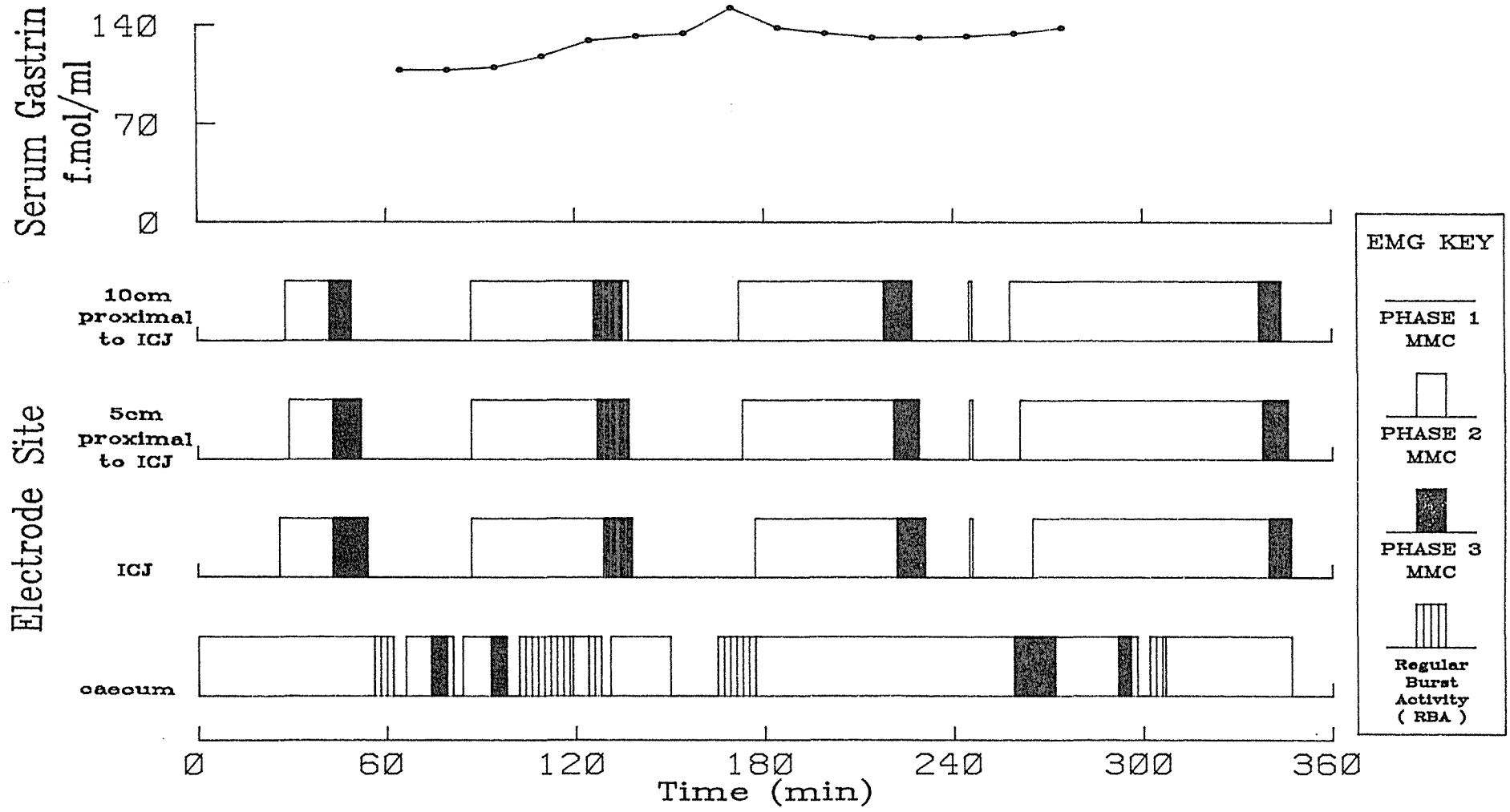


APPENDIX B FIGURE 19 Sheep 2, seventh control experiment: graph of EMG activity and serum gastrin levels. Serum gastrin levels are in the normal range after anthelmintic treatment for a nematode infestation. EMG recording ended at T = 325 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
65	108	0	1	0	1	0	1	0	1
80	108	28	2	29	2	26	2	56	4
95	110	42	3	43	3	43	3	62	1
110	118	49	1	52	1	54	1	66	2
125	129	87	2	87	2	87	2	74	3
140	132	126	3	127	3	129	3	79	4
155	134	135	2	137	1	138	1	81	1
170	152	137	1	173	2	177	2	84	2
185	138	172	2	221	3	222	3	93	3
200	134	218	3	229	1	231	1	98	1
215	131	227	1	245	2	245	2	102	4
230	131	245	2	246	1	246	1	119	2
245	132	246	1	361	2	265	2	124	4
260	134	258	2	338	3	340	3	128	1
275	138	337	3	346	1			131	2
		344	1					150	1
								165	4
								177	2
								259	3
								272	2
								292	3
								296	2
								298	1
								302	4
								307	2

High gastrin levels due to nematode infestation
Feeding commenced at T = 96 minutes .
EMG recording ended at T = 347 minutes

TABLE XXXII
Data for Sheep 2 Feeding Experiment 1

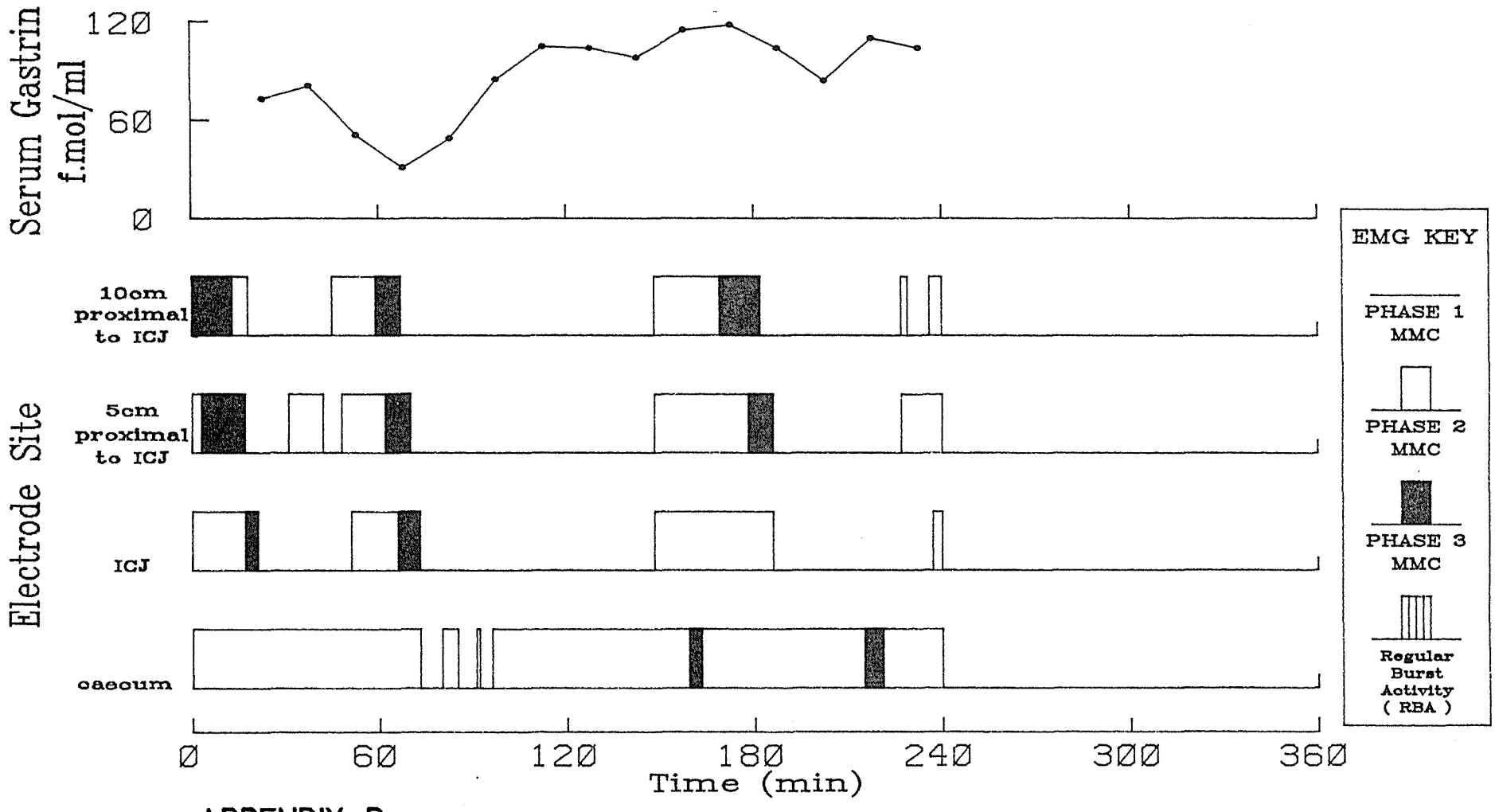


APPENDIX B FIGURE 20 Sheep 2, first feeding experiment: graph of EMG activity and serum gastrin levels. The high serum gastrin levels indicate a nematode infestation. Feeding commenced at T = 96 minutes. EMG recording ended at T = 347 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
23	73	0	3	0	2	0	2	0	2
38	81	13	2	3	3	17	3	73	1
53	51	18	1	17	1	21	1	80	2
68	31	45	2	31	2	51	2	85	1
83	49	59	3	42	1	66	3	91	2
98	85	67	1	48	2	73	1	92	1
113	105	148	2	62	3	148	2	96	2
128	104	169	3	70	1	186	1	159	3
143	98	182	1	148	2	237	2	163	2
158	115	227	2	178	3			215	3
173	118	229	1	186	1			221	2
188	104	236	2	227	2				
203	84								
218	110								
233	104								

High gastrin levels due to nematode infestation
Feeding commenced at T = 54 minutes
EMG recording ended at T = 240 minutes

TABLE XXXIII
Data for Sheep 2 Feeding Experiment 2

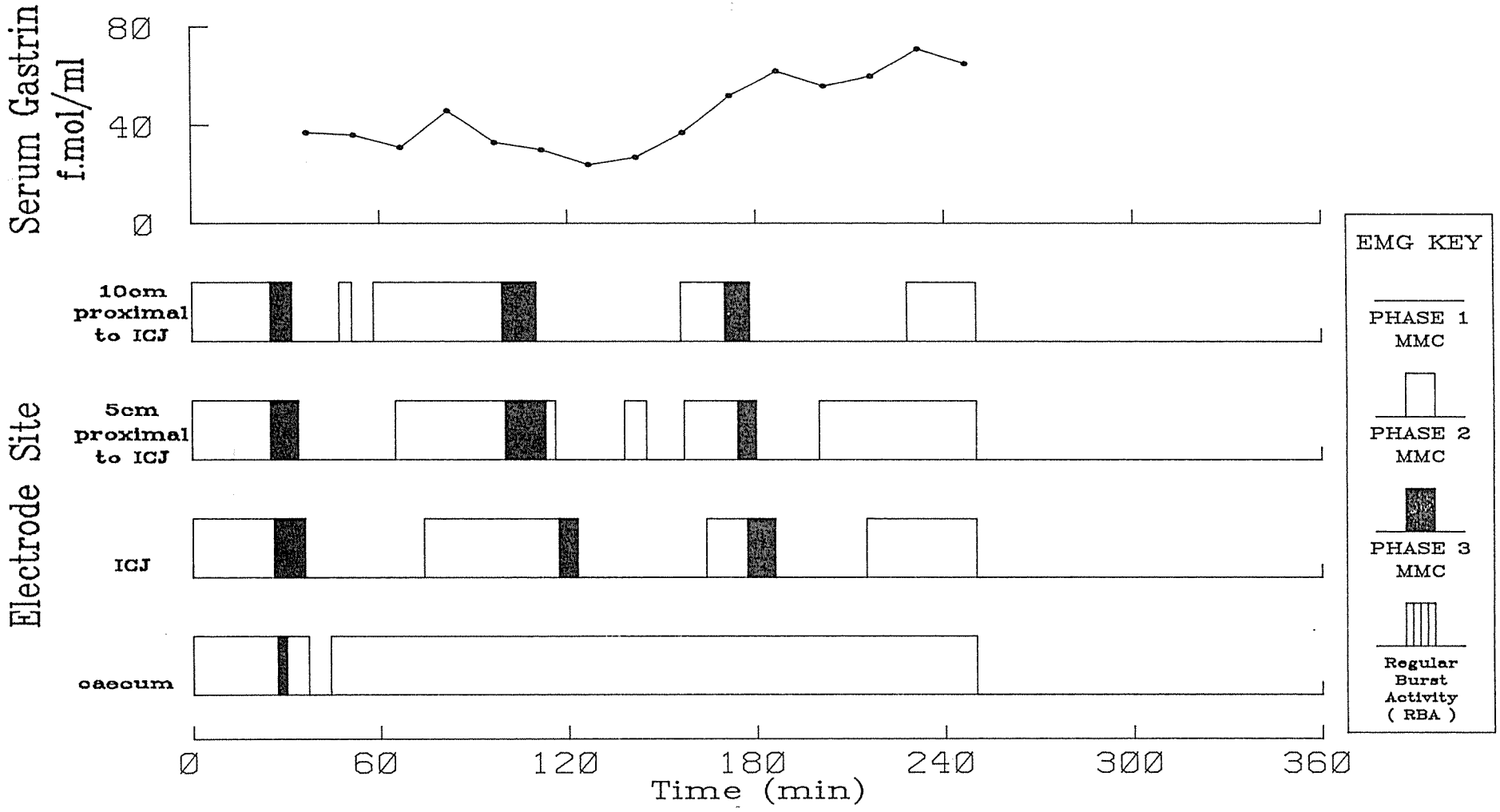


APPENDIX B FIGURE 21 Sheep 2, second feeding experiment: graph of EMG activity and serum gastrin levels. The high serum gastrin levels indicate a nematode infestation. Feeding commenced at T = 54 minutes. EMG recording ended at T = 240 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
37	37	0	2	0	2	0	2	0	2
52	36	25	3	25	3	26	3	27	3
67	31	32	1	34	1	36	1	30	2
82	46	47	2	65	2	74	2	37	1
97	33	51	1	100	3	117	3	44	2
112	30	58	2	113	2	123	1		
127	24	99	3	116	1	164	2		
142	27	110	1	138	2	177	3		
157	37	156	2	145	1	186	1		
172	52	170	3	157	2	215	2		
187	62	178	1	174	3				
202	56	228	2	180	1				
217	60			200	2				
232	71								
247	65								

Feeding commenced at T = 68 minutes
EMG recording ended at T = 250 minutes

TABLE XXXIV
Data for Sheep 2 Feeding Experiment 3

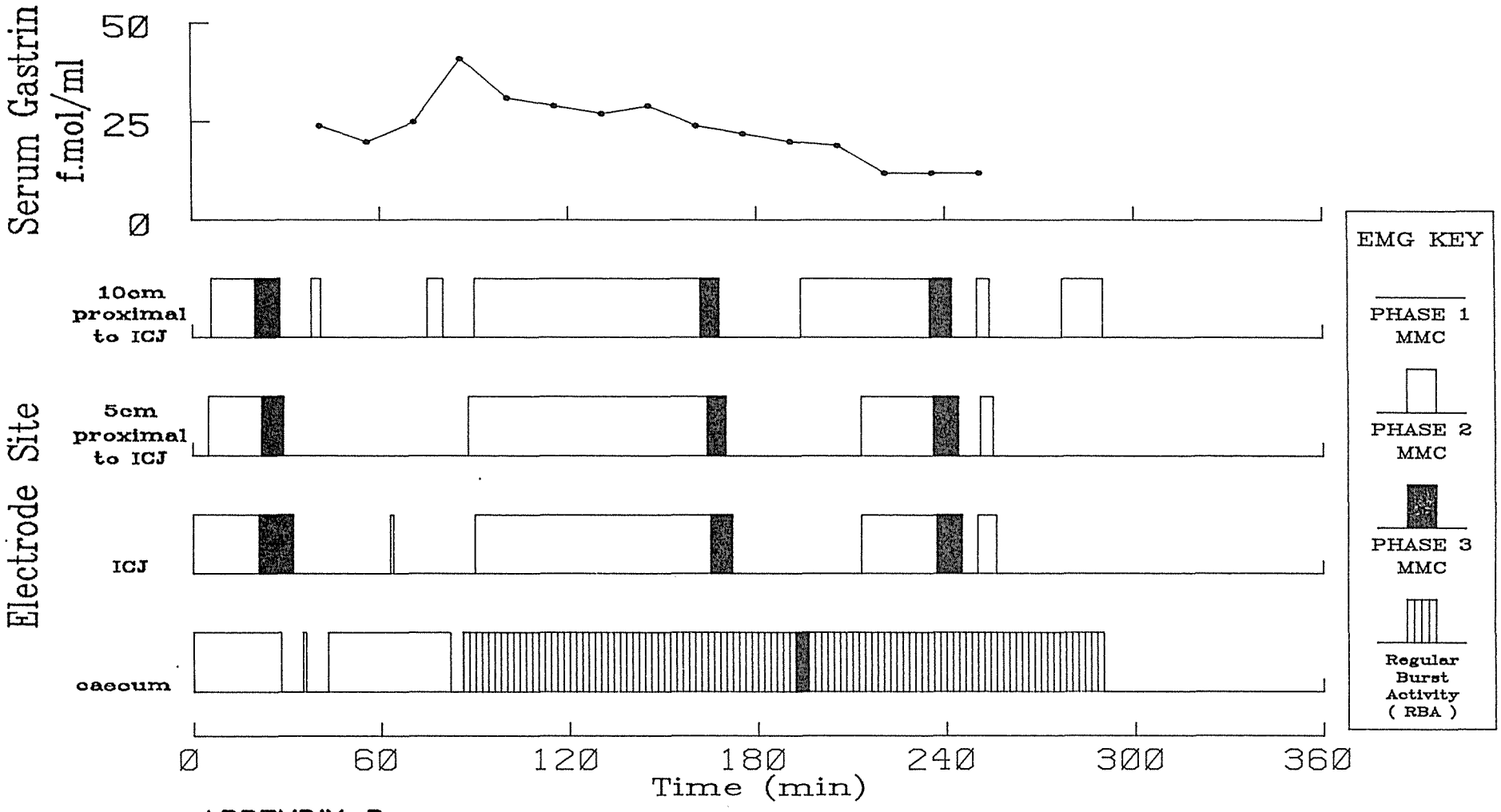


APPENDIX B FIGURE 22 Sheep 2, third feeding experiment: graph of EMG activity and serum gastrin levels. Serum gastrin levels are in the normal range after anthelmintic treatment for a nematode infestation. Feeding commenced at T = 68 minutes. EMG recording ended at T = 250 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
41	24	0	1	0	1	0	2	0	2
56	20	6	2	5	2	21	3	28	1
71	25	20	3	22	3	32	1	35	2
86	41	28	1	29	1	63	2	36	1
101	31	38	2	88	2	64	1	43	2
116	29	41	1	164	3	90	2	82	1
131	27	75	2	170	1	165	3	86	4
146	29	80	1	213	2	172	1	192	3
161	24	90	2	236	3	213	2	196	4
176	22	162	3	244	1	237	3		
191	20	168	1	251	2	245	1		
206	19	194	2			250	2		
221	12	235	3			256	1		
236	12	242	1						
251	12	250	2						
		254	1						
		277	2						

Feeding commenced at T = 72 minutes
EMG recording ended at T = 290 minutes

TABLE XXXV
Data for Sheep 2 Feeding Experiment 4

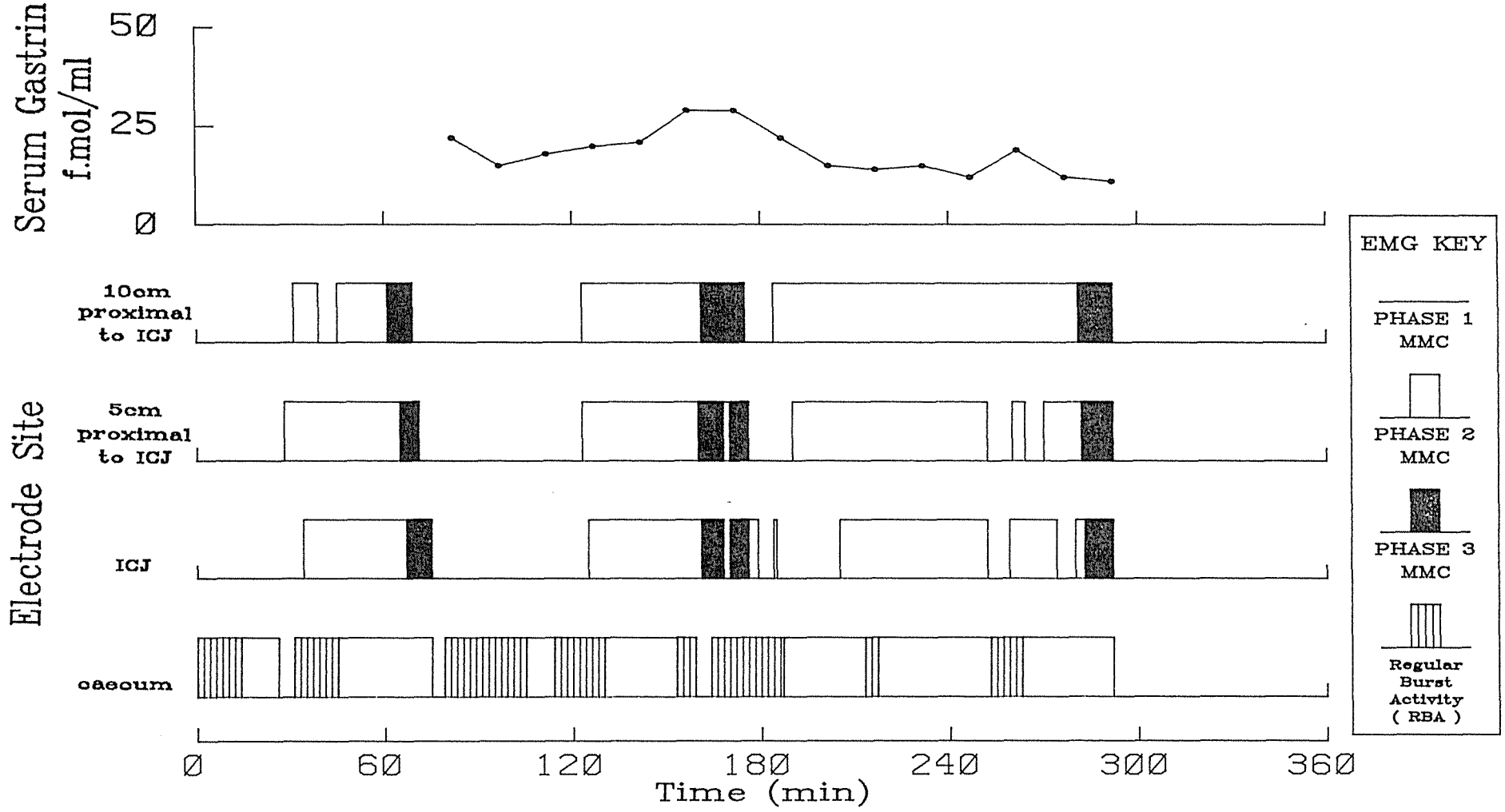


APPENDIX B FIGURE 23 Sheep 2, fourth feeding experiment: graph of EMG activity and serum gastrin levels. Serum gastrin levels are in the normal range after anthelmintic treatment for a nematode infestation. Feeding commenced at T = 72 minutes. EMG recording ended at T = 290 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
82	22	0	1	0	1	0	1	0	4
97	15	31	2	28	2	34	2	14	2
112	18	39	1	65	3	67	3	26	1
127	20	45	2	71	1	75	1	31	4
142	21	61	3	123	2	125	2	45	2
157	29	69	1	160	3	161	3	75	1
172	29	123	2	168	2	168	1	79	4
187	22	161	3	170	3	170	3	105	2
202	15	175	1	176	1	176	2	114	4
217	14	184	2	190	2	179	1	130	2
232	15	281	3	252	1	184	2	153	4
247	12			260	2	185	1	159	1
262	19			264	1	205	2	164	4
277	12			270	2	252	1	187	2
292	11			282	3	259	2	213	4
						274	1	217	2
						280	2	253	4
						283	3	263	2

Feeding commenced at T = 113 minutes
EMG recording ended at T = 292 minutes

TABLE XXXVI
Data for Sheep 2 Feeding Experiment 5

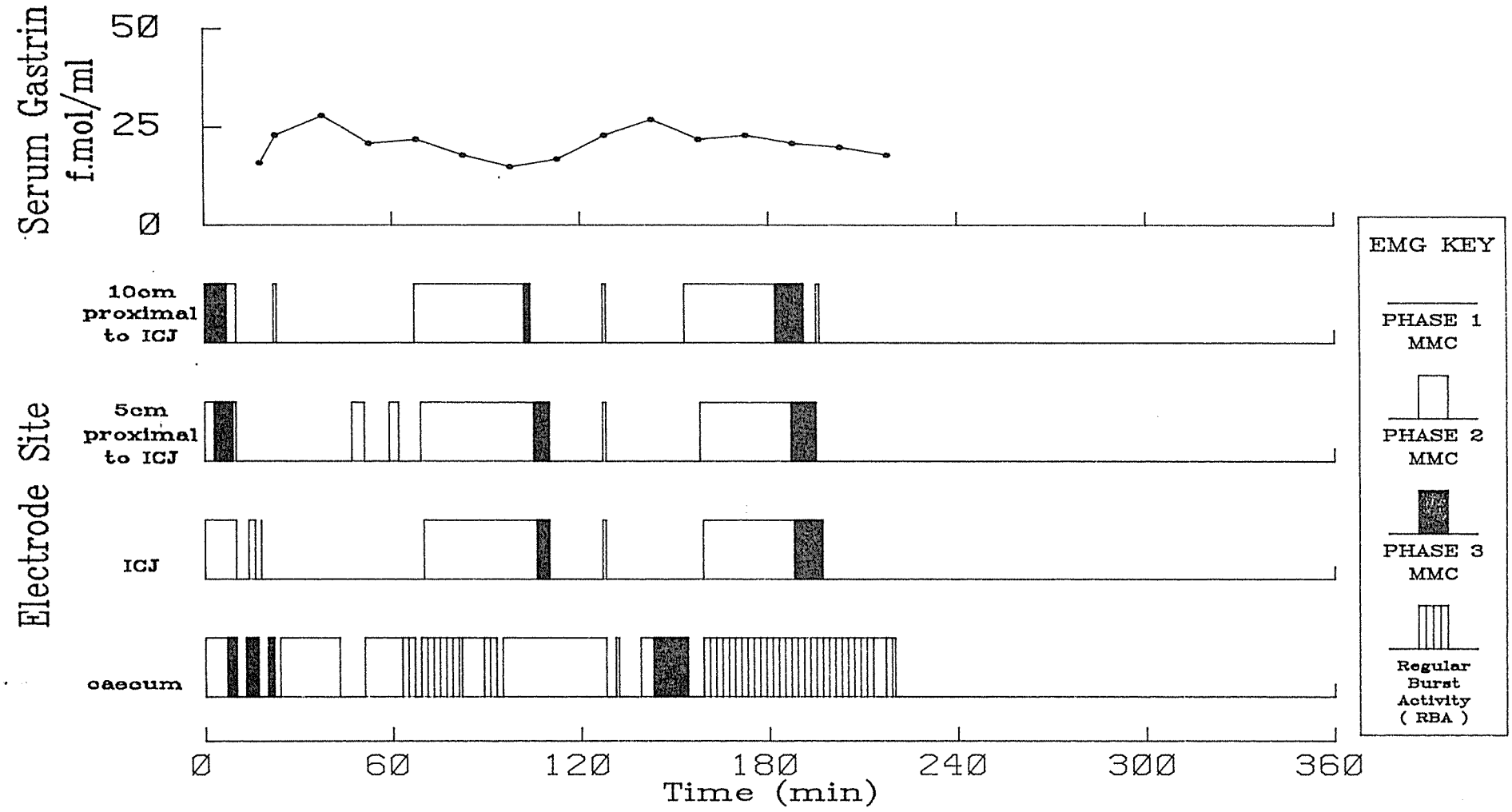


APPENDIX B FIGURE 24 Sheep 2, fifth feeding experiment: graph of EMG activity and serum gastrin levels. Serum gastrin levels are in the normal range after anthelmintic treatment for a nematode infestation. Feeding commenced at T = 113 minutes. EMG recording ended at T = 292 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
18	16	0	3	0	2	0	2	0	2
23	23	7	2	3	3	10	1	7	3
38	28	10	1	9	2	14	2	10	1
53	21	22	2	10	1	16	1	13	3
68	22	23	1	47	2	18	2	17	1
83	18	67	2	51	1	18	1	20	3
98	15	102	3	59	2	70	2	22	1
113	17	104	1	62	1	106	3	24	2
128	23	127	2	69	2	110	1	43	1
143	27	128	1	105	3	127	2	51	2
158	22	153	2	110	1	128	1	63	4
173	23	182	3	127	2	159	2	67	1
188	21	191	1	128	1	188	3	69	4
203	20	195	2	158	2	197	1	82	2
218	18	196	1	187	3			89	4
				195	1			93	1
								95	2
								128	1
								131	4
								132	1
								139	2
								143	3
								154	1
								159	4
								213	2
								217	4

EMG recording ended at T = 220 minutes

TABLE XXXVII
Data for Sheep 3 Control Experiment 1

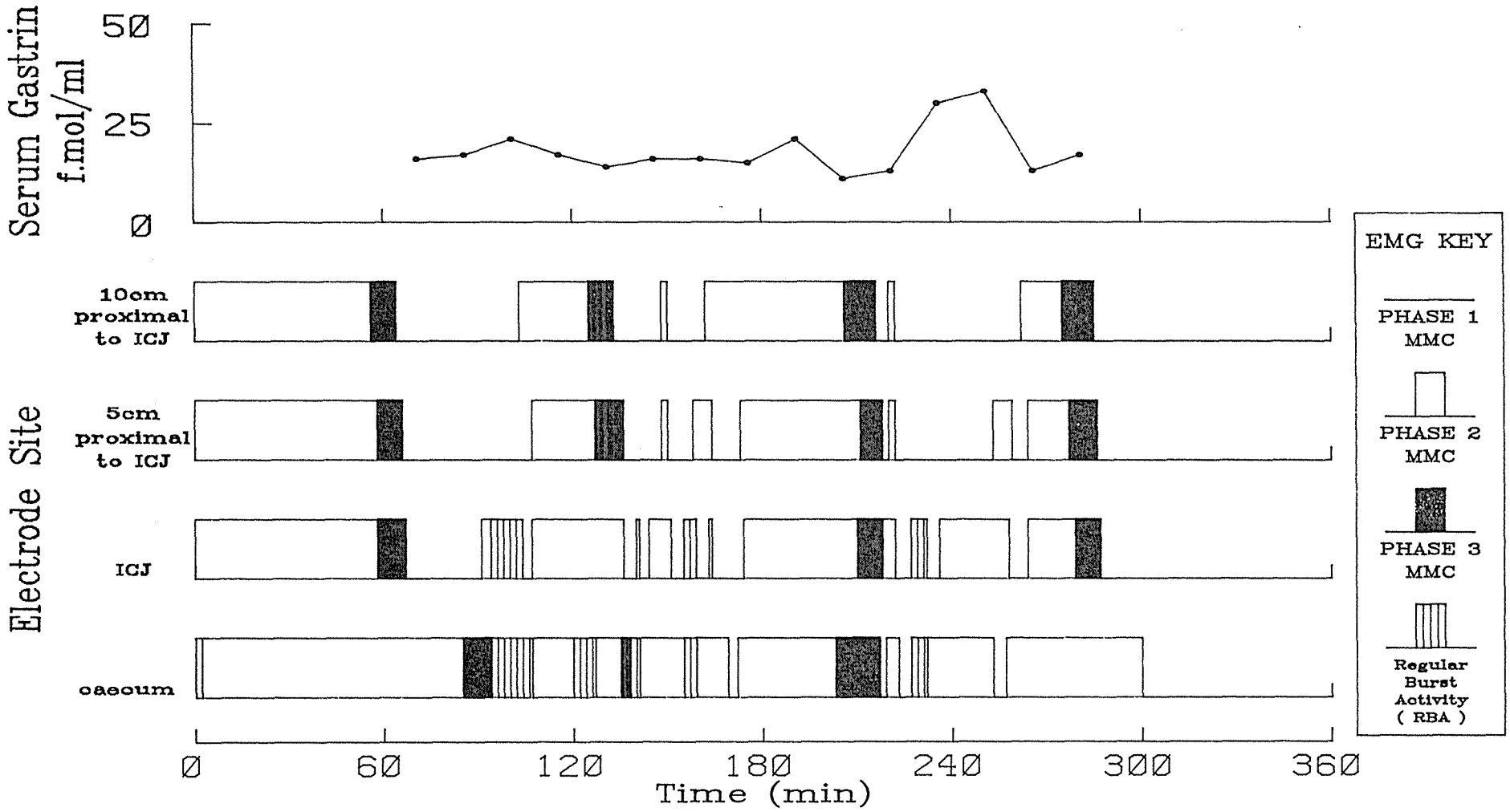


APPENDIX B FIGURE 25 Sheep 3, first control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 220 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
71	16	0	2	0	2	0	2	0	4
86	17	56	3	58	3	58	3	2	2
101	21	64	1	66	1	67	1	85	3
116	17	103	2	107	2	91	2	94	4
131	14	125	3	127	3	94	4	107	2
146	16	133	1	136	1	104	1	120	4
161	16	148	2	148	2	107	2	127	2
176	15	150	1	150	1	136	1	135	3
191	21	162	2	158	2	140	4	138	4
206	11	206	3	164	1	141	1	141	2
221	13	216	1	173	2	144	2	155	4
236	30	220	2	211	3	151	1	159	2
251	33	222	1	218	1	155	4	169	1
266	13	262	2	220	2	159	1	172	2
281	17	275	3	222	1	163	2	203	3
		285	1	253	2	164	1	217	1
				259	1	174	2	219	2
				264	2	210	3	223	1
				277	3	218	2	227	4
				286	1	222	1	232	2
						227	4	253	1
						232	1	257	2
						236	2		
						258	1		
						264	2		
						279	3		
						287	1		

EMG recording ended at T = 300 minutes

TABLE XXXVIII
Data for Sheep 3 Control Experiment 2

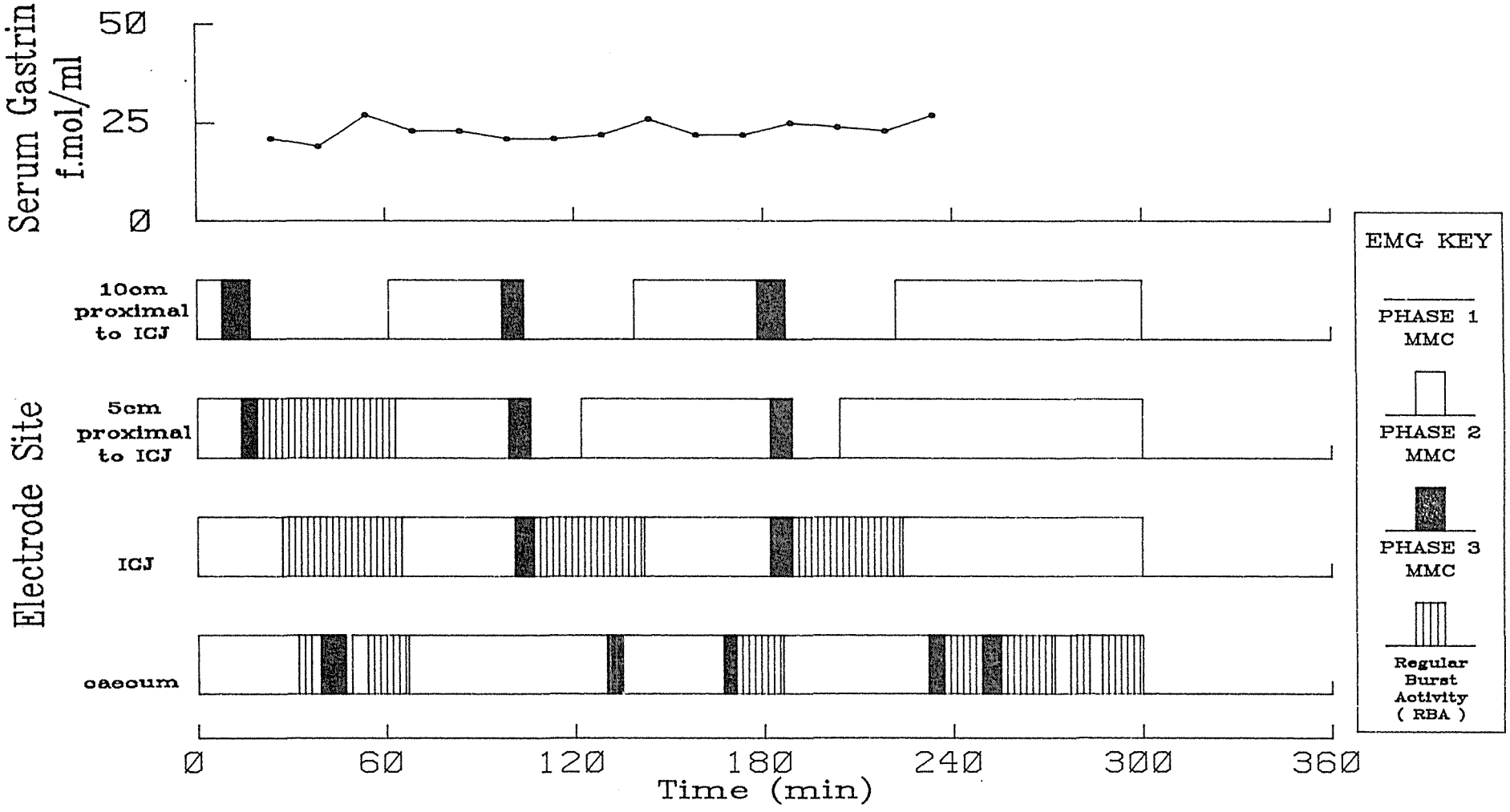


APPENDIX B FIGURE 26 Sheep 3, second control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 300 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
24	21	0	2	0	2	0	2	0	2
39	19	8	3	14	3	27	4	32	4
54	27	17	1	19	4	65	2	36	2
69	23	61	2	63	2	101	3	39	3
84	23	97	3	99	3	107	4	47	1
99	21	104	1	106	1	142	2	49	2
114	21	139	2	122	2	182	3	54	4
129	22	178	3	182	3	189	4	67	2
144	26	187	1	189	1	224	2	130	3
159	22	222	2	204	2			135	2
174	22							167	3
189	25							171	4
204	24							186	2
219	23							232	3
234	27							237	4
								249	3
								255	4
								272	2
								277	4
								283	2
								287	4

EMG recording ended at T = 300 minutes

TABLE XXXIX
Data for Sheep 3 Control Experiment 3

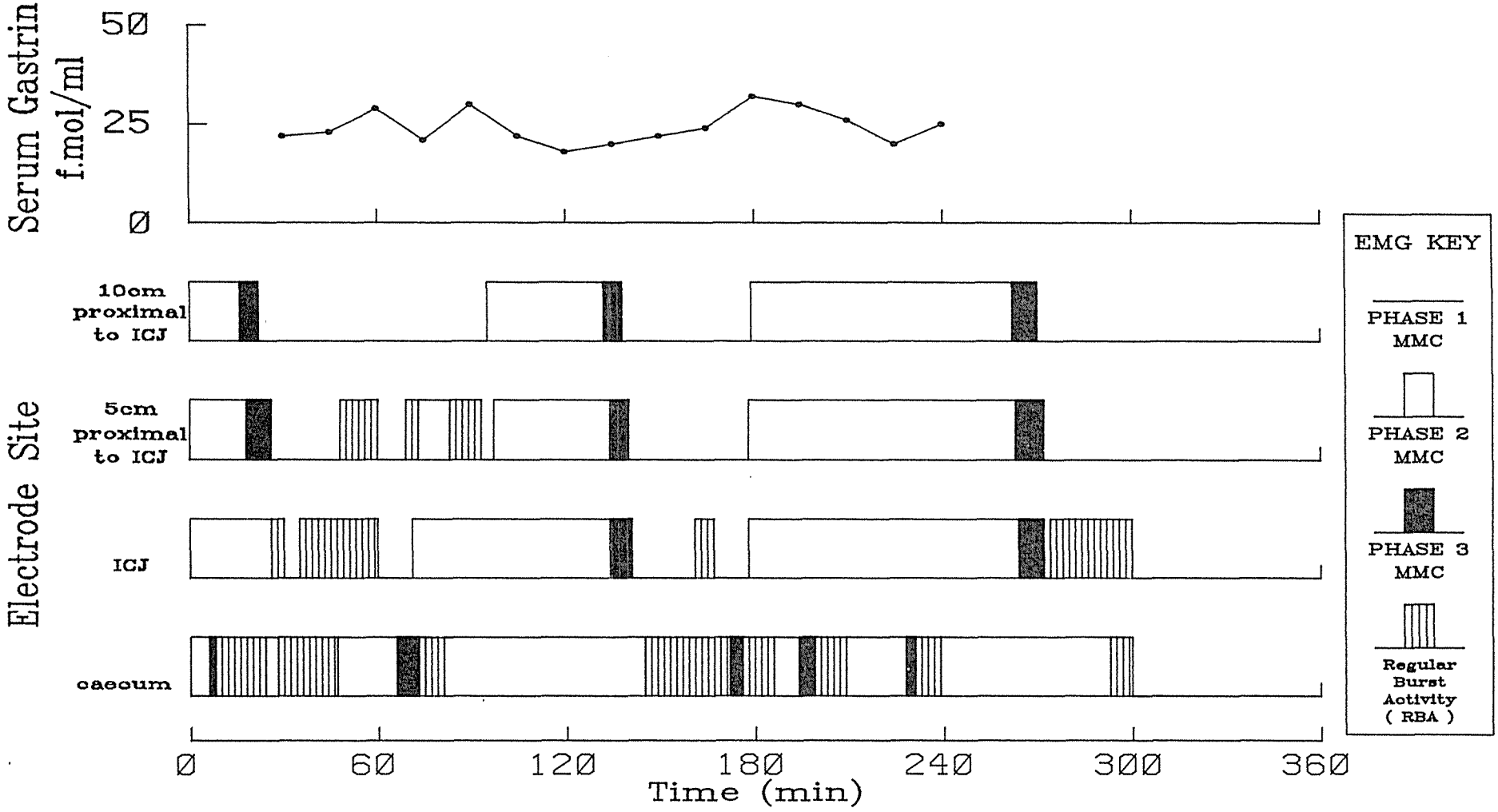


APPENDIX B FIGURE 27 Sheep 3, third control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 300 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
30	22	0	2	0	2	0	2	0	2
45	23	16	3	18	3	26	4	6	3
60	29	22	1	26	1	30	1	8	4
75	21	95	2	48	4	35	4	24	2
90	30	132	3	60	1	60	1	28	4
105	22	138	1	69	4	71	2	47	2
120	18	179	2	73	2	134	3	66	3
135	20	262	3	83	4	141	1	73	2
150	22	270	1	93	1	161	4	75	4
165	24			97	2	167	1	81	2
180	32			134	3	178	2	145	4
195	30			140	1	264	3	172	3
210	26			178	2	272	1	176	4
225	20			263	3	274	4	186	2
240	25			272	1			194	3
								199	4
								209	2
								228	3
								231	4
								239	2
								293	4

EMG recording ended at T = 300 minutes

TABLE XXXX
Data for Sheep 3 Control Experiment 4

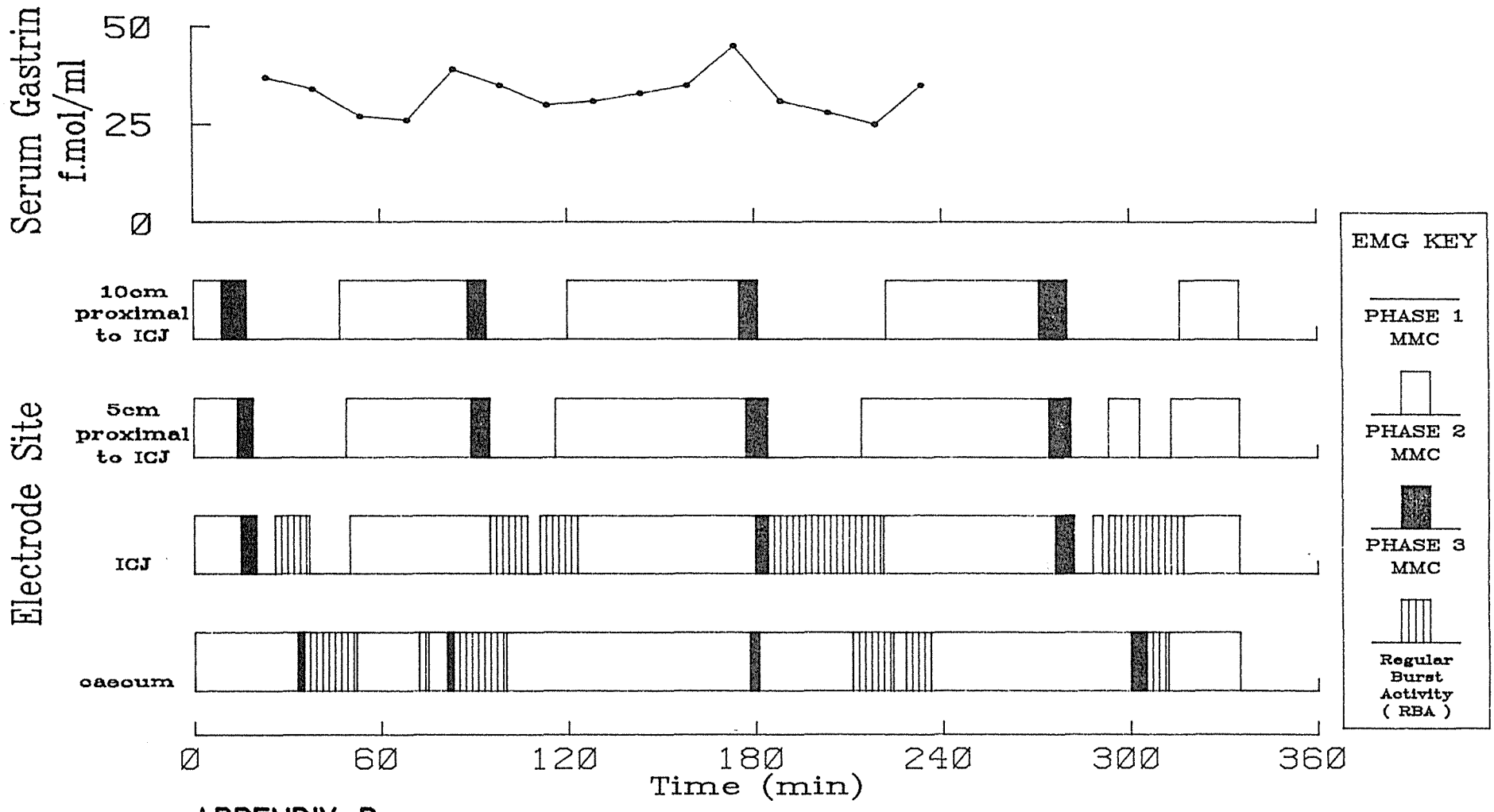


APPENDIX B FIGURE 28 Sheep 3, fourth control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 300 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
24	37	0	2	0	2	0	2	0	2
39	34	9	3	14	3	15	3	33	3
54	27	17	1	19	1	20	1	35	4
69	26	47	2	49	2	26	4	52	2
84	39	88	3	89	3	37	1	72	4
99	35	94	1	95	1	50	2	75	2
114	30	120	2	116	2	95	4	81	3
129	31	175	3	177	3	107	1	83	4
144	33	181	1	184	1	111	4	100	2
159	35	222	2	214	2	123	2	178	3
174	45	271	3	274	3	180	3	181	2
189	31	280	1	281	1	184	4	211	4
204	28	316	2	293	2	221	2	224	2
219	25			303	1	276	3	228	4
234	35			313	2	282	1	236	2
						288	2	300	3
						291	1	305	4
						293	4	312	2
						317	2		

EMG recording ended at T = 335 minutes

TABLE XXXXI
Data for Sheep 3 Control Experiment 5

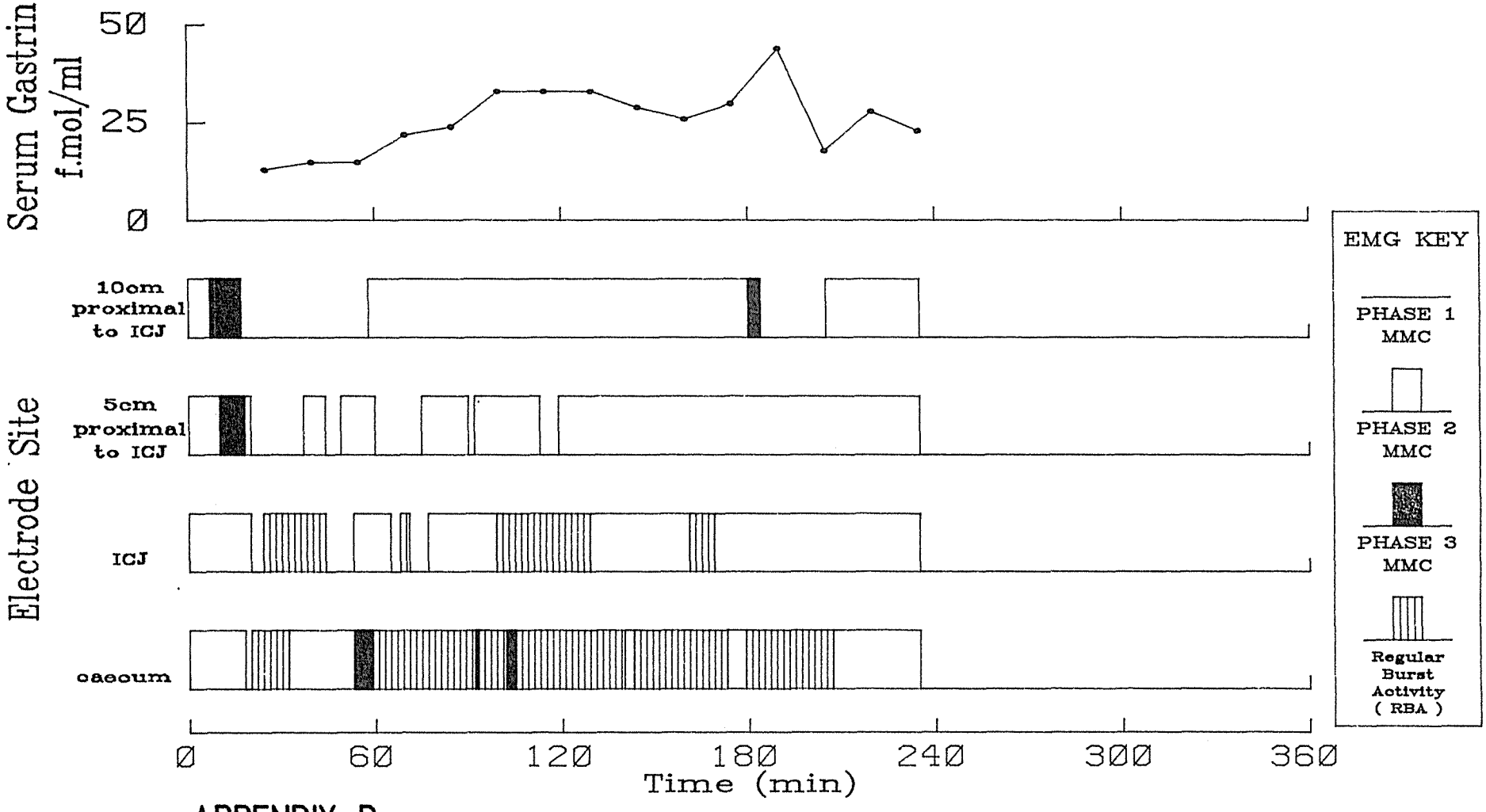


APPENDIX B FIGURE 29 Sheep 3, fifth control experiment: graph of EMG activity and serum gastrin levels. EMG recording ended at T = 335 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
25	13	0	2	0	2	0	2	0	2
40	15	7	3	10	3	20	1	18	1
55	15	17	1	18	2	24	4	20	4
70	22	58	2	20	1	44	1	32	2
85	24	180	3	37	2	53	2	53	3
100	33	184	1	44	1	65	1	59	4
115	33	205	2	29	2	68	4	92	3
130	33			60	1	71	1	93	4
145	29			75	2	77	2	102	3
160	26			90	1	99	4	105	4
175	30			92	2	129	2	140	2
190	44			113	1	161	4	143	4
205	18			119	2	169	2	173	2
220	28							179	4
235	23							207	2

Feeding commenced at T = 56 minutes
EMG recording ended at T = 235 minutes

TABLE XXXXII
Data for Sheep 3 Feeding Experiment 1

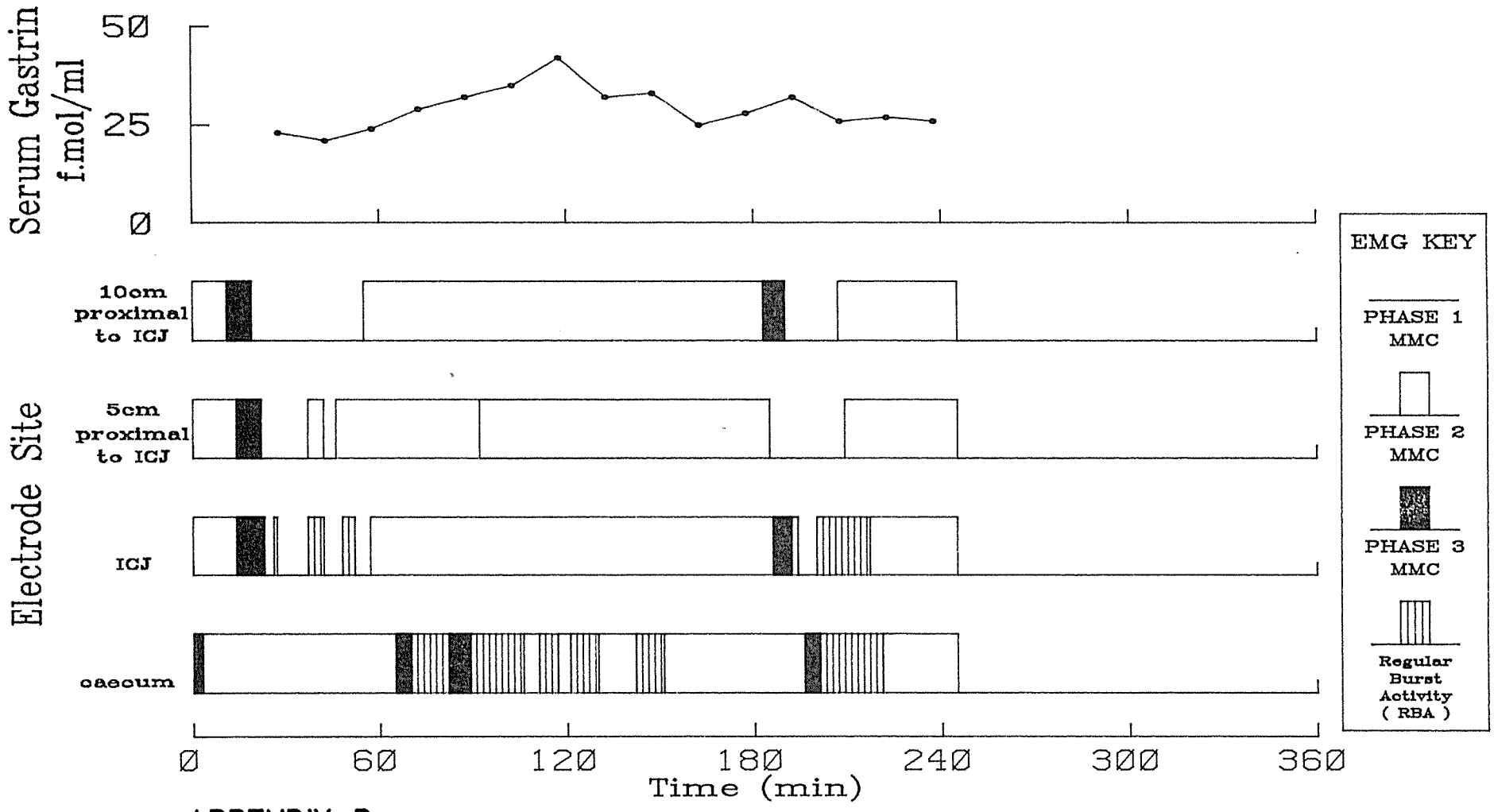


APPENDIX B FIGURE 30 Sheep 3, first feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 56 minutes. EMG recording ended at T = 235 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
28	23	0	2	0	2	0	2	0	3
43	21	11	3	14	3	14	3	3	2
58	24	19	1	22	1	23	1	65	3
73	29	55	2	37	2	26	4	70	4
88	32	183	3	42	1	27	1	82	3
103	35	190	1	46	2	37	4	89	4
118	42	207	2	185	3	42	1	106	2
133	32			192	1	48	4	111	4
148	33			209	2	52	1	117	2
163	25					57	2	121	4
178	28					186	3	130	2
193	32					192	4	142	4
208	26					194	1	151	2
223	27					200	4	196	3
238	26					217	2	201	4
								221	2

Feeding commenced at T = 59 minutes
EMG recording ended at T = 245 minutes

TABLE XXXXIII
Data for Sheep 3 Feeding Experiment 2

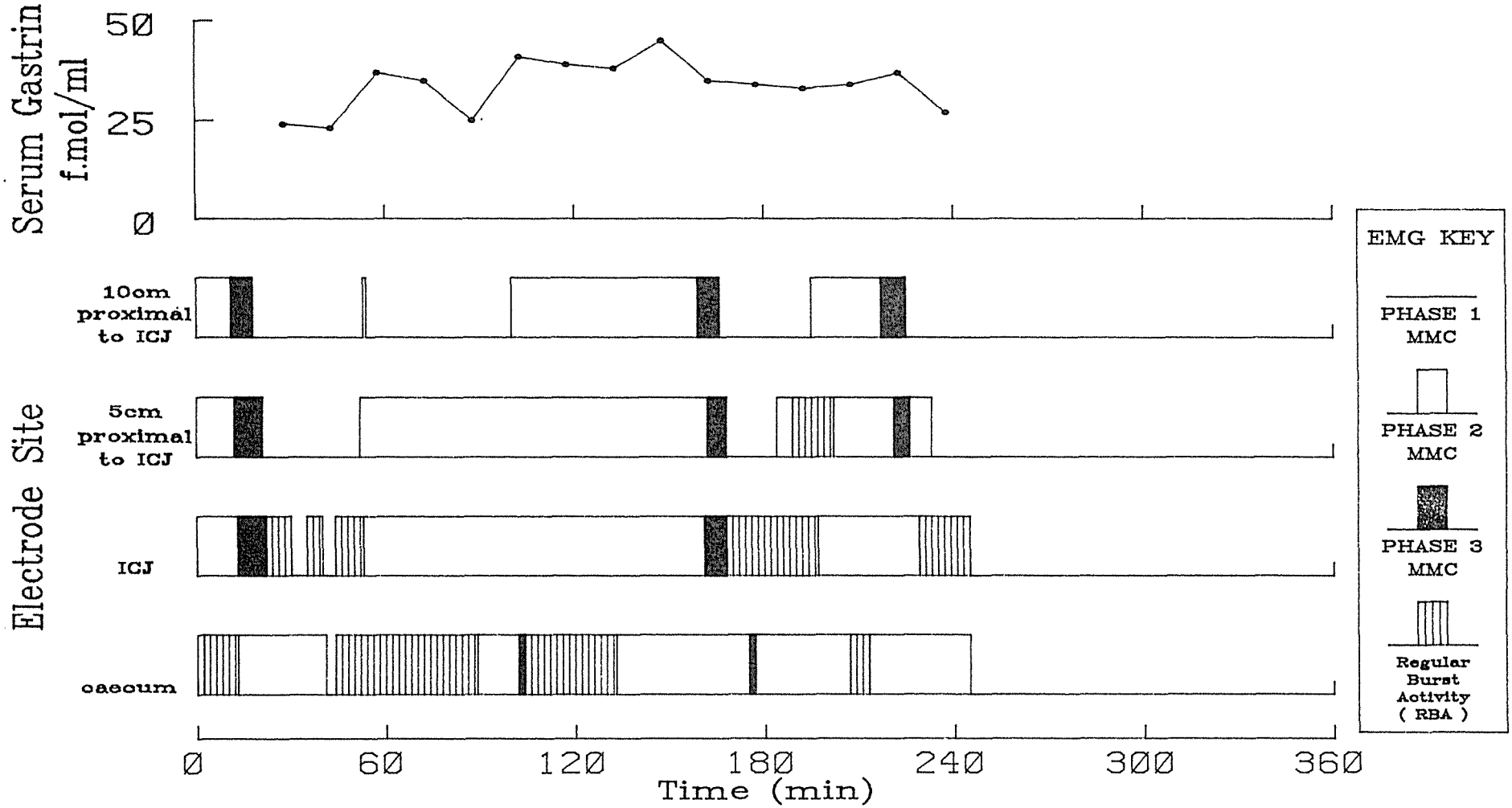


APPENDIX B FIGURE 31 Sheep 3, second feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 59 minutes. EMG recording ended at T = 245 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
28	24	0	2	0	2	0	2	0	2
43	23	11	3	12	3	13	3	2	4
58	37	18	1	21	1	22	4	13	2
73	35	53	2	52	2	30	1	41	1
88	25	54	1	162	3	35	4	44	4
103	41	100	2	168	1	40	1	89	2
118	39	159	3	184	2	44	4	102	3
133	38	166	1	189	4	53	2	104	4
148	45	195	2	202	2	161	3	133	2
163	35	217	3	221	3	168	4	175	3
178	34	225	1	226	2	197	2	177	2
193	33			233	1	229	4	207	4
208	34							213	2
223	37								
238	27								

Feeding commenced at T = 59 minutes
EMG recording ended at T = 245 minutes

TABLE XXXXIV
Data for Sheep 3 Feeding Experiment 3

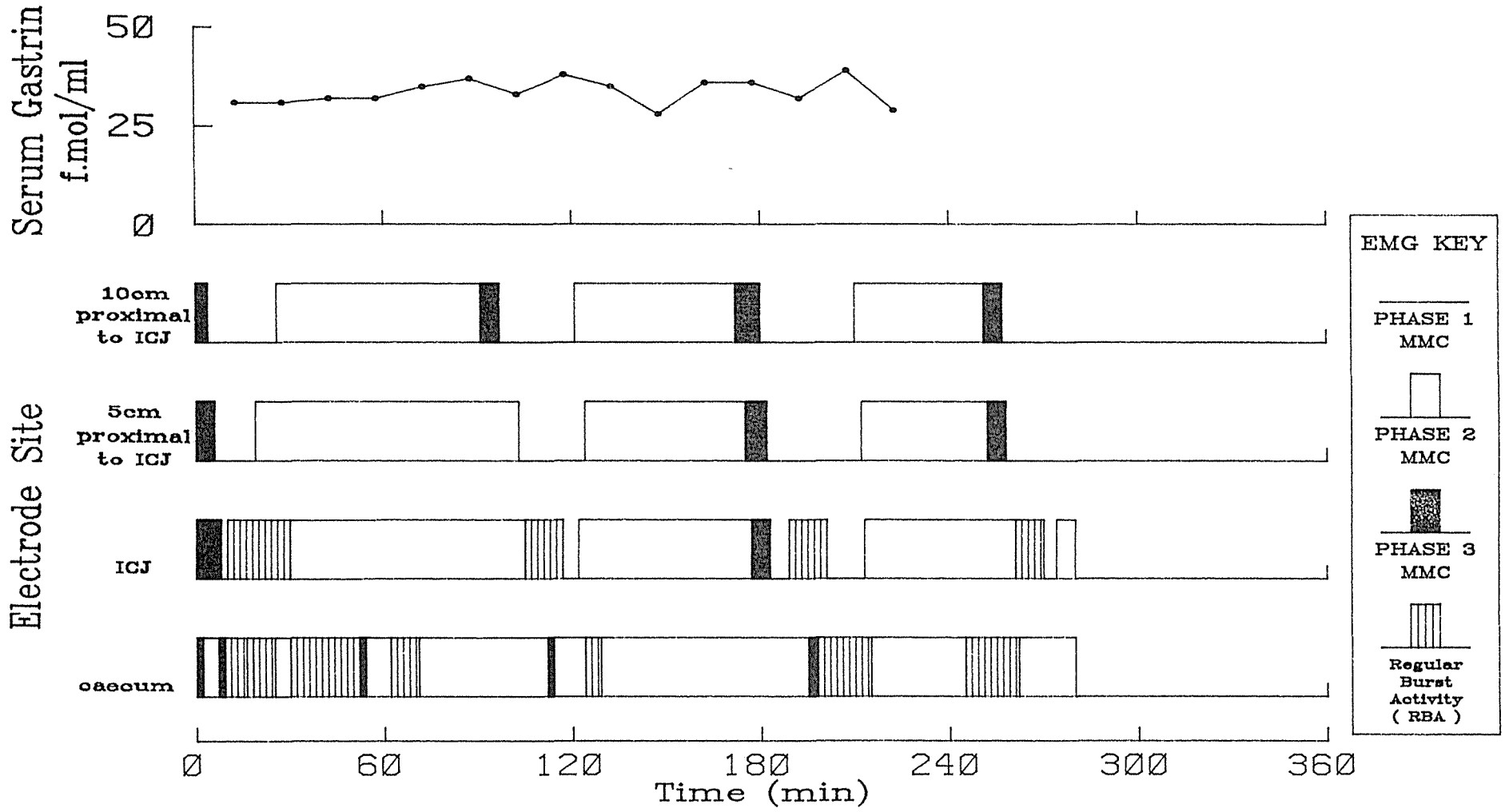


APPENDIX B FIGURE 32 Sheep 3, third feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 59 minutes. EMG recording ended at T = 245 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
13	31	0	3	0	3	0	3	0	3
28	31	4	1	6	1	8	1	2	2
43	32	26	2	19	2	10	4	7	3
58	32	91	3	103	1	30	2	9	4
73	35	97	1	124	2	105	4	16	2
88	37	121	2	175	3	117	1	18	4
103	33	172	3	182	1	122	2	25	2
118	38	180	1	212	2	177	3	30	4
133	35	210	2	252	3	183	1	52	3
148	28	251	3	258	1	189	4	54	2
163	36	257	1			201	1	62	4
178	36					213	2	71	2
193	32					261	4	112	3
208	39					270	1	114	2
223	29					274	2	124	4
								129	2
								195	3
								198	4
								215	2
								245	4
								262	2

Feeding commenced at T = 44 minutes
EMG recording ended at T = 280 minutes

TABLE XXXXV
Data for Sheep 3 Feeding Experiment 4

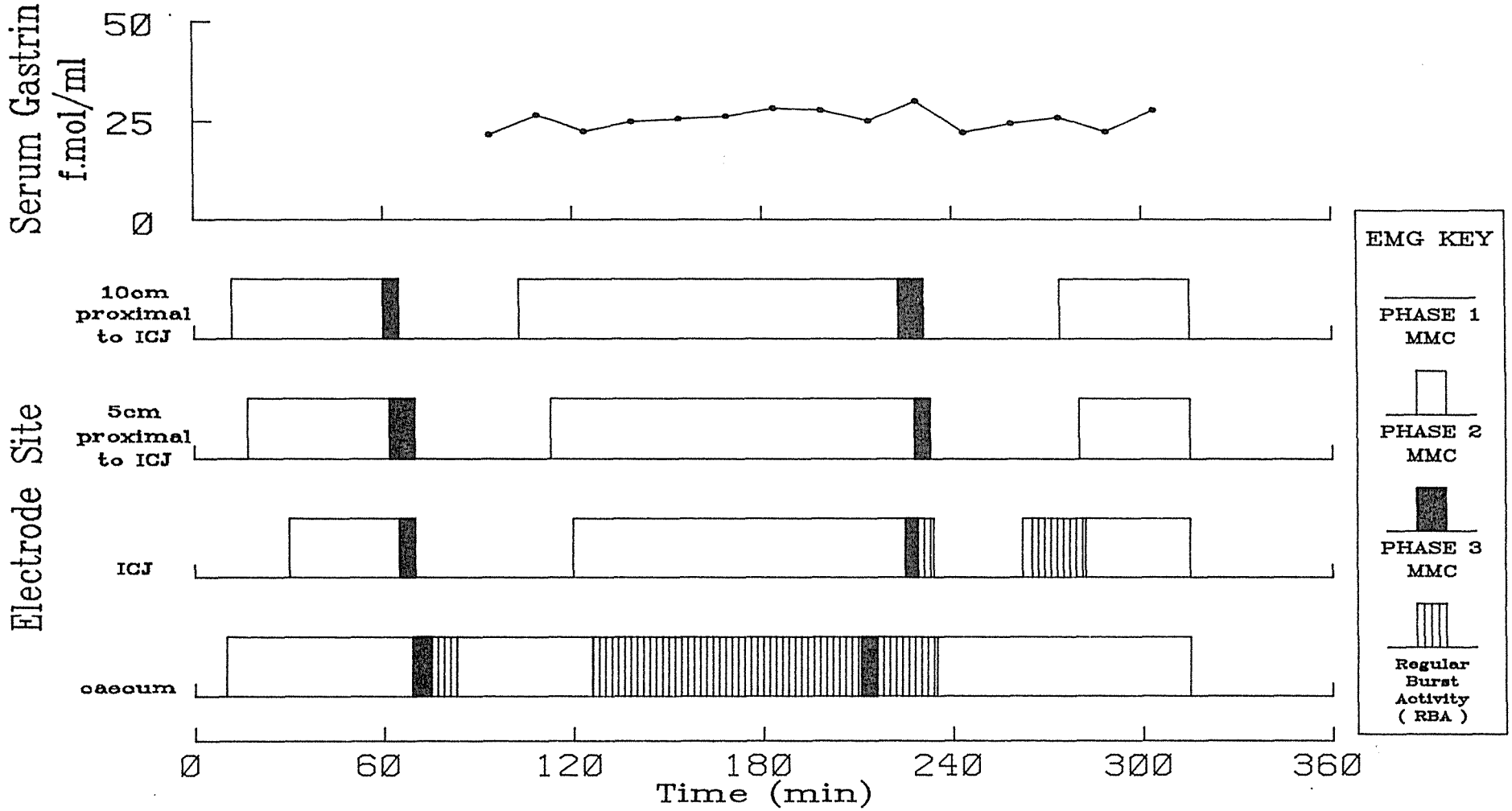


APPENDIX B FIGURE 33 Sheep 3, fourth feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 44 minutes. EMG recording ended at T = 280 minutes.

Time (Mins)	Serum Gastrin (fmol ml^{-1})	EMG							
		10cm Ileum		5cm Ileum		ICJ		Caecal	
		Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
94	22	0	1	0	1	0	1	0	1
109	27	12	2	17	2	30	2	10	2
124	22	60	3	62	3	65	3	69	3
139	25	65	1	70	1	70	1	75	4
154	26	103	2	113	2	120	2	83	2
169	26	223	3	228	3	225	3	126	4
184	28	231	1	233	1	229	4	211	3
199	28	274	2	280	2	234	1	216	4
214	25					262	2	235	2
229	30					265	4		
244	22					282	2		
259	24								
274	26								
289	22								
304	28								

Feeding commenced at T = 125 minutes
EMG recording ended at T = 315 minutes

TABLE XXXXVI
Data for Sheep 3 Feeding Experiment 5



APPENDIX B FIGURE 34 Sheep 3, fifth feeding experiment: graph of EMG activity and serum gastrin levels. Feeding commenced at T = 125 minutes. EMG recording ended at T = 315 minutes.

EMG							
10cm Ileum		5cm Ileum		ICJ		Caecal	
Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
0	2	0	2	0	4	0	4
4	3	6	3	48	1	35	2
10	1	14	1	75	2	54	3
68	2	36	4	123	3	57	4
120	3	47	1	129	1	81	2
126	1	70	2	150	4	131	4
168	2	122	3	158	1	160	2
		128	1				
		174	2				

EMG recording ended at T = 175 minutes

TABLE XXXXVII
Data for Sheep 3 Pentagastrin Experiment 1

EMG							
10cm Ileum		5cm Ileum		ICJ		Caecal	
Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
0	2	0	2	0	2	0	2
7	3	13	3	26	4	30	4
16	1	18	1	60	2	35	2
73	2	76	2	131	3	38	3
128	3	81	1	138	4	43	1
135	1	88	2	160	2	48	2
189	2	130	3			53	4
		137	1			70	2
		178	2			134	4
						141	2
						186	3
						191	4

EMG recording ended at T = 196 minutes

TABLE XXXXVIII
Data for Sheep 3 Pentagastrin Experiment 2

EMG							
10cm Ileum		5cm Ileum		ICJ		Caecal	
Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase	Time (Mins)	MMC phase
0	1	0	1	0	1	0	2
40	2	41	2	12	2	30	3
81	3	85	3	18	4	33	4
86	1	89	1	27	2	52	2
120	2	123	2	34	4	65	4
188	3	191	3	41	2	78	2
194	1	197	1	87	3	83	4
235	2	232	2	91	1	90	2
				100	4	160	3
				118	2	163	2
				128	1	211	4
				132	2	226	2
				192	3	230	4
				197	4	242	2
				209	2		
				232	4		

EMG recording ended at T = 250 minutes

TABLE IL
Data for Sheep 3 Pentagastrin Experiment 3

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