STUDIES ON THE EFFECT OF SOME NITROGENOUS MATERIALS ON THE FOOD INTAKE OF SHEEP

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PREFACE

The problem of how mammals regulate their food intake is in itself, of sufficient scientific interest to justify its intensive study. To those concerned with human biology, additional impetus is provided by the prevalence in affluent societies of disorders of health associated with the excessive intake of food. To those concerned with animal production, there is the realisation that optimum food intake is the major determinant of efficient production. In practice, the nutritive value of many of the foodstuffs the ruminant is called on to convert to useable products is limited by the amount eaten.

It is generally recognised that the central nervous system, particularly the hypothalamus, is concerned with processing and integration of sensory information relating to the control of food intake. Despite much effort, the actual mechanisms concerned have not yet been defined. However, in the case of the simple stomached animals (non-ruminants) evidence in support of a number of stimuli, both physical and chemical, has been obtained. The relevance of these findings to food intake regulation in the ruminant is uncertain. The specialised anatomy and the physiological adaptations of these animals would suggest that in their case, stimuli involved in food intake regulation are either different from those operative in animals with a simpler stomach, or are of different relative importance. The latter appears the more likely. Thus, it is probable that gastric distension provides stimuli evoking satiety in both ruminants and non-ruminants. On the other hand, whereas the quality and quantity of protein in the diet appears to be important in determining how much of that diet is consumed by simple stomached animals, there is little evidence to indicate that the food intake of the ruminant may be limited by the quality or quantity of protein reaching the intestines. Recently, however, it has been shown (Egan & Moir, 1965; Egan, 1965a,b,c) that the intraduodenal administration of protein solutions may result in an increased consumption of a low quality roughage by sheep. It was suggested that the food intake of ruminants may depend in part, on the nitrogen status of the animal.

The work described in this thesis had three objectives:

1. to define better the effects of intraduodenal infusions of protein on the food intake of sheep;
2. to identify factors influencing those effects; and
3. to establish the site of action and the mechanisms involved in the effects.

In the initial stages of the work described, it was found that, in contrast to published reports, the intraduodenal infusion of protein was accompanied by effects on food intake that ranged from a marked increase to an equally marked decrease. While this variability proved to be one of the major difficulties in pursuing these investigations, its study proved most valuable in pointing to the large number of factors influencing the response. It was largely from a consideration of these factors that the importance of physical factors, particularly those likely to influence osmoreceptors, was realised. It is considered that the suggestion arising from later experiments that changes in electrolyte and water metabolism contribute to the regulation of food intake by the ruminant is the most important outcome of this thesis.

It was not possible to establish either the mechanisms whereby the protein infusions influenced intake or the site at which they were acting. The simple experiments undertaken in which different amino acid-containing materials were delivered to different sites in the body, while failing in this regard, have indicated that there are a number of mechanisms involved with a variety of receptors situated at various sites in the body. Furthermore, they indicate that a change of food intake is but one of a number of effects of protein supplementation; they emphasise the possibility that the observed changes of food intake may be secondary to one or more of the other effects rather than a direct one of the amino acid-containing material administered.

The explanation of the effects on food intake of post-ruminal protein supplementation will be complex. So too will be the explanation of its effects on wool and body growth. The success in practical terms of such a procedure is doubtful unless this complexity i realised and effort is made to first establish a firm foundation of basic knowledge.

A preliminary account of this work was presented to the 1967 meeting of the Nutrition Society of New Zealand.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

The regulation of food intake has of recent years been the subject of intensive study in monogastric animals. In this review, therefore, a brief outline of the present state of knowledge for monogastric animals will be given before the more specialised but less advanced field of ruminant food intake regulation is considered.

The consideration given to the various topics may not be taken as an indication of their relative importance: rather it is a reflection of their applicability to this study, the extent of coverage in recent reviews and the direction taken by recent research. While an endeavour has been made to include references to recent major contributions, a complete bibliography has not been attempted.

The terminology used here is that of Tepperman (1962, p.170):

> "Hunger is the awareness of the need to ingest food, and it may be accompanied by a complex set of phenomena including hunger pangs, anticipatory salivation, increased food-searching behaviour and others. In sum, hunger is a malaise, a disagreeable combination of sensations which, as it progresses, acquires a frantic character. Appetite is the desire to ingest food. Unlike hunger, which occurs when the body's store of nutrients becomes depleted below a certain preset maintenance level, appetite may persist even when hunger has been appeased. Appetite is strongly influenced by emotion, by the presence or absence of conditioning or distracting stimuli, and by discriminatory choices of various kinds. Satiety is the lack of desire to eat which occurs after the ingestion of food. Anorexia describes a situation in which the physiological state which would ordinarily produce a sensation of hunger is present, but all the available signals call in vain for the resumption of eating behaviour."

A. THE REGULATION OF FOOD INTAKE IN NON-RUMINANTS

1. Role of the central nervous system

The regulation of food intake is thought to involve the nervous system in an integrative manner similar to that established for other somatic activities such as respiration, posture and locomotion. Recent reviews on the subject have been provided by Brobeck (1960a), Anand (1961), Tepperman (1962, p.168), Kennedy (1964, 1966) and Stevenson (1964).

Three levels of control have been postulated (Brobeck, 1960a, Anand, 1961).

(i) The basic patterns are reflex in nature, food being the stimulus evoking the feeding responses. Brobeck (1955) has suggested a tentative classification of the reflexes but "it is not clear how much feeding behaviour can be designated as reflex and how much may be either conditioned responses or learned behaviour or some other type of more highly organised behaviour" (Brobeck, 1960a).

(ii) At a higher level than the reflex feeding responses is the hypothalamus which, on the basis of stimulation and ablation experiments, is postulated to contain a facilitatory "feeding" centre in the lateral areas and an inhibitory "satiety" centre located in the ventromedial nuclei. It has been suggested that the "feeding" centre serves as an integrative centre for the reflexes associated with feeding behaviour. Tepperman (1962, p.172) believes that satiety information reaches the "satiety" centre and this structure acts to inhibit the "feeding" centre.

The concept of two discrete centres is a gross simplification. In this regard, Stevenson (1964) has stated, "the neurophysiological systems for the regulation of food and water intake are very complex being evident as well in other parts of the hypothalamus and indeed in other parts of the nervous system." The concept of a lateral feeding centre has been questioned on the grounds that the effects on food intake from experimental interference in this area may be a result of damage to, or stimulation of, tracts passing through this area on the way to or from higher centres (Anderson, Gale & Sundsten, 1963). There is uncertainty as to whether the primary effect of lesions in the lateral areas is adipsia or aphagia (Kennedy, 1966), and whether it is due to a motivational failure or a development of a motor deficit. The latter possibility is suggested by the observations of Baillie & Morrison (1963) who found that in rats conditioned to obtain all their food by pressing a lever, lesions in the lateral hypothalamus led to the rats continuing to press the lever for food which they did not eat. If pressure on the lever caused food to be delivered intragastrically, rats with certain lateral hypothalamic lesions continued to press and so to feed themselves: the same animals would not ingest either food or water by mouth. It was concluded that the lesions caused a motor rather than a motivational defect. Rodgers, Epstein & Teitelbaum (1965) rejected this conclusion; they performed similar experiments but offered highly palatable food by mouth, and found that their rats began to eat from one to fifteen days after operation, always before bar pressing returned. Perhaps these two groups of workers studied different effects for there may be distinct "hunger" and "feeding" motivating systems in the lateral hypothalamus (Morgane, 1961).

Considerations of this nature led Andersson et al. (1963) to suggest that the role of the hypothalamus in food intake regulation may be to modify the hunger "drive" by determining the degree of satiety. Anand (1961) describes hunger as a "basic urge".

(iii) The importance of cerebral structures has been studied in several species (see Anand, 1961; Stevenson, 1964): their influence is regarded mainly of a discriminative nature (Anand, 1961).

While the "integrative action" of the central nervous system in the regulation of food intake is generally recognised, there is little agreement as to the nature of the changes taking place within the body which act as signals for the integrating system. Several theories which have been proposed are briefly considered below. There are two components involved in the regulation of food intake, a short term one which tends to equate daily food intake and energy expenditure and a long term regulation which acts to maintain a constant body weight through adjustments to stored energy, work and heat production as well as food intake. Although there is no sharp division, this review will deal mainly with short term regulation.

2. Nature of stimuli and location of receptors involved in the regulation of food intake by non-ruminants.

In addition to those cited above, recent reviews are those of Grossman (1960), Andersson & Larsson (1961), Balch & Campling (1962) and Mayer (1963).

(a) Role of gastrointestinal tract.

(i) Hunger contractions.

Much of the early work has been discussed by Alvarez (1948, p.679) and Quigley (1955): some of these findings, based chiefly on recordings of activity made with the balloon, water manometer technique must be considered with caution in view of the observation that with such a technique of recording, the procedure may lead to the stimulation of the gastric contractions recorded (Penick, Smith, Wienske & Hinkle, 1963). It is now generally agreed that although the contractions seem to be associated with the sensation of hunger in man, their role in regulating food intake is of minor importance (Grossman, 1955; Mayer, 1955; Anand, 1961). Davenport (1966, p.47) concludes there is no type of gastric contraction uniquely associated with hunger.

(ii) Oropharyngeal metering.

The passage of food through the oropharyngeal regions, according as it does stimulus to receptors associated with taste, chewing and swallowing may result in satiety. The effect is transient but is reinforced when associated with gastric distension (Janowitz & Grossman, 1949; Share, Martyniuk & Grossman, 1952; Grossman, 1955, 1960). As measured by the rate of performing a conditioned response (Kohn, 1951), consumption of food (Berkun, Kessen & Miller, 1952) or by the reward value in learning new responses (Miller & Kessen, 1952), the oral ingestion of food by rats has been found more effective in reducing hunger than feeding through a gastric fistula. These results suggest that stimulation of the oropharyngeal regions is an important element of the factors normally contributing to satiety.

(iii) Gastric distension.

Experiments undertaken on dogs by Janowitz & Grossman (1949) and Share et al. (1952) have often been quoted as evidence for the involvement of gastric distension in signalling for the cessation of eating (Grossman, 1960; Anand, 1961; Balch & Campling, 1962). However, the general applicability of these observations is uncertain: an animal which becomes satiated after 2.5 min of feeding (Janowitz & Grossman, 1949) may respond to satiety signals different from those operative in animals which eat in a more leisurely manner and take a longer period of the day to ingest their food.

According to Janowitz (1958), metering by gastric distension is probably mediated by vagal afferent fibres arising from gastric stretch receptors (Paintal, 1954). Evidence that gastric distension increases the activity of the "satiety" centre but not the "feeding" centre has been discussed by Anand (1961).

The "setting" of the gastric distension mechanism is subject to alteration as shown by the ability of rats to compensate within limits for dilution of their food with non-nutritious bulk or water (Adolph, 1947; Stominger, Brobeck & Cort, 1953).

Since animals with "denervated" gastrointestinal tracts show almost normal regulation of food intake (see Andersson & Larsson, 1961; Anand, 1961) it has been concluded that the gastric distension mechanisms are dispensible (Grossman, 1955).

(iv) Gastric emptying.

Some reviewers have given scant consideration to the contribution made to food intake regulation by that portion of the gastrointestinal tract caudal to the pylorus; for example "Food after it leaves the stomach does not produce further inhibition." (Anand, 1961). Balch & Campling (1962) make only one reference to it.

The rate of stomach emptying may be considered of importance in the regulation of food intake for not only is gastric distension relieved by the passage of food into the intestines but regulated emptying prevents the excessive accumulation of nutrients in the intestines, the flooding of the extracellular spaces with glucose and other metabolites, and the excessive transfer of extracellular fluid constituents into the intestines (Hunt, 1959; Rogers & Harper, 1964). It has been postulated that the control of gastric emptying is dependent on varying degrees of inhibition of gastric motility by the stimulation of cephalic, pre- and post-cardial receptors (Thomas, 1957; Hunt, 1959). Particularly important in this regard are the post-cardial receptors which are said to be stimulated by the presence of chyme in the duodenum. Rapid emptying of the stomach occurs when the chyme is diverted through an open fistula located in the first portion of the duodenum (see Alvarez, 1948, p.399). Alvarez (loc.cit., Chapter 17) has discussed in detail the older literature concerned with the properties of chyme involved in delaying gastric emptying. It was concluded that particle size, viscosity, osmotic pressure, acidity, volume and chemical composition could all contribute to the control of gastric emptying. More recent reviews on factors controlling gastric emptying are those of Thomas (1957), Hunt (1959, 1963) and Davenport (1966, Chapter 3).

Gastric motility of conscious dogs has been shown to be inhibited by the presence of protein, peptones, casein hydrolysate or amino acids in the proximal duodenum (Thomas & Crider, 1939). Thomas (1942) found that of the amino acids, only monoamino, monocarboxy acids regularly caused inhibition when infused into the duodenum in neutral solutions. Dicarboxy and diamino acids were ineffective in neutral solution but were inhibitory when administered as free acids. Thomas was unable to determine whether amino acids owed their activity to their ionic properties or to their amino acid structure. With rats fed a single meal of a low fat diet, the addition of casein to the diet delayed the rate of, and at times caused the complete cessation of stomach emptying (Peraino, Rogers, Yoshida, Chen & Harper, 1959). The effect is modified by the type of protein (Rogers, Chen, Peraino & Harper, 1960) and the nature of the carbohydrate portion of the diet (Rosenthal & Nasset, 1958). Slower emptying of casein from the stomach resulted when sucrose instead of dextrin was included in the diet (Peraino *et al.*, 1959). Rosenthal & Nasset (1958) found stomach emptying equally rapid whether a high or low protein diet was fed. This result has been explained on the basis of the physical state of the diet, the small quantity fed and the long fast to which the rats were subjected prior to their being offered food (Rogers & Harper, 1964). Mayer (1955) has suggested that delayed gastric emptying may partially explain the low intake of diets containing a high proportion of protein.

Davenport (1966, p.56) has stated that three pathways of inhibition are involved: (a) Blood borne inhibitory hormones liberated from the intestinal mucosa, (b) Reflexes not involving higher centres but operating through intrinsic plexuses or the coeliac plexus and (c) Reflex mechanisms whose afferent and efferent pathways are in the vagus. The pathway utilized by any one factor is still uncertain except possibly for that of acid in the duodenum. This has bean assigned to an enterogastric reflex whose efferent limb consists of post ganglionic sympathetic fibres (Schapiro & Woodward, 1959). Since the inhibitory effects of peptones appeared after a delay of about 15 seconds and were abolished by vagotomy (Thomas & Crider, 1939) it has been concluded that a reflex mechanism is involved (Thomas, 1957). However, other mechanisms may be involved for the intraduodenal instillation of peptones in dogs inhibits the motility of fundic pouches deprived of sympathetic and vagal innervation (Johnson & Magee, 1965) and of transplanted (i.e. denervated) gastric pouches (Brown, Johnson & Magee, 1967).

The humoral inhibition of gastric motility, induced by fat in the duodenum has been well established. It is postulated that enterogastrone, the chalone involved, is liberated from the duodenal mucosa (Grossman, 1950; Thomas, 1957; Gregory, 1962). Attempts to isolate the pure compound have failed. The demonstration by Johnson and coworkers that a preparation of cholecystokinin and pancreozymin (hormones obtained from duodenal mucosa) inhibited gastric motility in dogs (Johnson

& Magee, 1965) and man (Johnson, Brown & Magee, 1966) has led these workers to suggest that this preparation may possess the physiological activity of enterogastrone. McLeay (1967, p.94) has discussed in detail the "attractive proposal" that enterogastrone, pancreozymin and cholecystokinin are one and the same hormone.

The role of enterogastrone in food intake regulation has not been established. MacLagan (1937) reduced the food intake of rabbits by subcutaneous or intravenous injections of an enterogastrone preparation. This may have been a non-specific effect as the preparation was crude and the effects were transient. Pre-feeding small amounts of carbohydrates, fat or protein to dogs was without significant effect on subsequent food intake (Janowitz & Grossman, 1951). The inhibitory effects of larger doses of these materials were attributed to bulk and not chemical composition. Rather than providing evidence that enterogastrone does not contribute to satiety as is often assumed (Grossman, 1960; Anand, 1961), the experiments can only be described as unsatisfactory. Not only were the results confounded by the effects of gastric distension and caloric substitution, but as suggested elsewhere in this review, the feeding habits of dogs suggests their unsuitability for this kind of study.

Recently, Schally, Redding, Lucien & Meyer (1967) have reported that in mice fasted for 17 hr, the intravenous or subcutaneous administration of an enterogastrone preparation purified from hog duodenum reduced food intake. This effect was greatest during the first 30 min from when food was available but continued for at least 4 hr. Injection of hog duodenum preparation which did not contain enterogastrone was ineffective. It was concluded that enterogastrone may have a role in food intake regulation.

It has been postulated that an osmoreceptor mechanism, located in or beyond the duodenum contributes to the control of gastric emptying (Hunt & Pathak, 1960; Hunt, 1963). In general, there is a direct relationship between the osmolality of liquid test meals and the extent they retard gastric emptying. Some solutions however, (e.g., NaCl, urea) empty fastest if their osmotic pressure is 200-250 milliosmolar. Menguy (1960) has suggested that one of the factors involved in the inhibition of gastric function when fat has been introduced into the duodenum may be an increase in osmolality of the duodenal contents. The inhibitory effects of hypertonic saline, sugars, peptones and fat on the gastric response to histamine may involve two separate mechanisms, one sensitive to changes in pH and the other, to changes in osmolality (Sircus, 1958). As inhibition of gastric secretion was found with autotransplanted pouches, Sircus concluded that the osmoreceptor may cause the release of a humoral agent. Such a concept removes many of the difficulties associated with an understanding and interpretation of how fat, amino acids and other products of hydrolytic digestion lead to an inhibitory effect. It makes it clear how there may be a common response evoked by a variety of substances without there being "specific" receptors.

The importance of the osmoreceptor mechanism in food intake regulation is discussed further in a later section.

Hill, Ison, Jones & Archdeacon (1952) have reported that distension of the small intestine of the dog by non-nutritive fibre introduced through a fistula at the duodeno-jejunal junction reduced food intake. This may be an experimental demonstration that conditions in one part of the lower digestive tract can influence the activity of another part by reflex mechanisms (Davenport, 1966, p.67).

Although motility and secretion are important aspects of gut function, there is little definite evidence relating their inhibition with reduced food intake. A hormone such as glucagon which inhibits gut motility in man (Dotevall & Kock, 1963) and reduces the sensation of hunger (Andersson & Larsson, 1961; Tepperman, 1962, p. 173), has other effects which may explain these relationships. Similarly, the reduction in food intake reported by Schmidt, Moak & Van Meter (1958) when rats were given a subcutaneous injection of atropine may not have been due entirely to an effect on gut motility; a non-specific effect on the central nervous system may have been involved. It is pertinent to recall here that there is evidence that the hypothalamic feeding and satiety centres may exert a direct control on gastric motility and secretion (Ridley & Brooks, 1965).

(b) <u>Metabolic and chemostatic hypotheses</u>.

As satiety usually precedes absorption of nutrients, it may be reasoned that mechanisms exist for sensing the quantity of nutrients ingested before they are metabolised. In an attempt to place these considerations in context with other factors, Grossman (1960) has suggested that the oropharyngeal and gastric metering mechanisms are "set" at sensitivities determined or influenced by other factors.

The concentration of metabolites in the blood or metabolic changes consequent upon feeding have been proposed as the primary stimuli affecting the satiety centre, i.e., in contrast to the oropharyngeal or gastric metering mechanisms being the primary determinants of food intake. Some of the mechanisms which have been proposed are briefly discussed below:-

(i) <u>Glucostatic</u>.

Mayer (1953, 1955) has suggested a short term mechanism for regulating food intake may operate through glucose sensitive receptors, possibly located in the satiety centre. The rate of utilization is postulated to affect the receptor, satiety being experienced when utilization is high.

Using arteriovenous differences in blood glucose concentration as a measure of glucose utilization, it has been possible to relate the sensation of hunger with diminished glucose utilization (Van Itallie, Beaudoin & Mayer, 1953; Stunkard, Wolff & Plescia, 1956). In other cases, neither increased utilization (Fryer, Moore, Williams & Young, 1955, Quaade, 1962; Quaade & Juhl, 1962) nor hyperglycemia (Janowitz & Ivy, 1949; Smith & Duffy, 1957; Schally et al. 1967) decreased hunger sensations or food intake. The absence of effect has been explained (Anand, 1961; Mayer, 1963, 1964) on the basis that neither the absolute levels nor arteriovenous differences in blood glucose concentration necessarily indicate glucose utilization at the site of the glucoreceptors.

The effect of the pancreatic hormone, glucagon, on gastric contractions and hunger feelings has been interpreted as support for the glucostatic theory (Anand, 1961; Mayer, 1963). Because of its effect on liver glycogen, glucagon increases blood glucose concentration and presumably, utilization (Stunkard, Van Itallie & Reiss, 1955). However, the reduction in gastric motility and hunger feelings may be explicable in terms of the effect of glucagon on fat and amino acid metabolism (Haugaard & Haugaard, 1954; Ganong, 1965) and heat production (Brobeck, 1960b), rather than on glucose utilization.

Gold thioglucose has been used to induce lesions, and hence obesity, in the ventromedial satiety centre of mice (see Mayer, 1963). The accumulation of the toxic gold moiety in this region is said to indicate the affinity of the neurones therein for the glucose component of the molecule. As the lesions are not necessarily confined to the satiety centre (Liebelt & Perry, 1957), the effect on food intake may be associated with the destruction of neurones in other areas of the brain (Brobeck, 1960b).

The hyperphagia of diabetes mellitus has necessitated the postulate that the glucose receptors, unlike the rest of the brain, require insulin (Mayer, 1963). This concept has been criticised in detail by Grossman (1955) who believes that hypoglycomia is an emergency mechanism in the regulation of hunger, not operative in the physiological range of blood glucose variations.

Kennedy (1964) has cited evidence to show that the glucostatic theory does not explain why in rats and mice with hypothalamic lesions, food intake is increased in the immediate post-operative period but eventually returns to normal ("static obesity").

More recently Anand, Chhina, Sharma, Dua & Singh (1964) have presented evidence that the activity of the neurones in the satiety centre increases at the time of increased glucose utilisation in the body (induced by the intravenous infusion of glucose, insulin or both). Activity of the neurones in the feeding centre showed an inverse relationship.

Thus, although Mayer (1963) concluded that "The glucostatic component in the regulation of food intake appears to be the main mechanism whereby the metabolic state of the organism exercises a regulatory role in the overall regulation", there are many who would dispute such a claim.

(ii) Thermostatic.

Brobeck (1948, 1960a,b) has suggested that heat, particularly that released during the assimilation of food, may be an important factor. Hunger is said to be reduced by a rise in temperature in the satiety centre or by stimulation of heat sensitive neurones in the anterior hypothalamus. The observations that animals tend to eat more at low than at high environmental temperatures (see Brobeck, 1960b) and that warming the heat sensitive neurones reduces food intake whereas cooling tends to have the reverse effect (Andersson et al. 1963), may be interpreted in favour of this theory. Andersson et al.(1963) have cautioned that these stimuli may be non-specific, or alternatively, a reflection of changes in the hormonal status of the animals. Anand (1961) has pointed out that "According to this hypothesis, diets rich in calories but low in S.D.A. (Specific Dynamic Action) should cause obesity, but excessive consumption of such diets by young rats is only transient. Also, this theory fails to explain how the hypothalamic receptors could distinguish between the heat released from the S.D.A. of a meal and the far greater amount of heat released during muscular exercise. Instead of being a signal to eat more, the metabolism of exercise should satisfy hunger".

Even so, the thermostatic hypothesis has been accepted to be of some importance in food intake regulation (Anand, 1961; Andersson et al. 1963; Hamilton & Brobeck, 1964; Stevenson, 1964).

(iii) Lipostatic.

Kennedy (1950, 1953) suggested that the primary regulation carried out by the satiety centre is stabilization of the fat stores, signalled by the concentration of some complex of circulating metabolites. Stevenson (1964) has suggested the importance of innervation of fat tissue ("liponeurostatic" stimuli).

The lipostatic theory has been invoked to explain why the food intake of rats and mice with hypothalamic lesions in the satiety centre returns to normal after an initial period of hyperphagia. Obesity is said to develop until the size of the fat stores is sufficient to elicit stimuli of sufficient strength to either trigger the damaged satiety centre or, by some other mechanism, prevent hyperphagia (Kennedy, 1961, 1963). Hervey (1959) studied the effects of hypothalamic lesions in rats which were members of pairs joined in parabiosis - in this case the union was between peritoneal cavities and between scapulae and the exchange of plasma between members of pairs was demonstrated. The animals in which the lesions were made showed hyperphagia and obesity while their normal but parabiotic partners became thin. It was suggested that they became thin because they ate little; they ate little because of stimulation of their hypothalamic satiety centres by signals crossing the parabiotic union. As the effects were most marked when the lesioned animals became very obese, it was suggested that the results pointed to a form of lipostatic control.

Van Itallie & Hashim (1960) observed a reciprocal relationship between plasma nonesterified fatty acid (NEFA) and glucose utilization and suggested that they (NEFA) may serve as an indicator of "satiety-hunger state". As Hales & Kennedy (1964) found that plasma NEFA levels increased both in hunger and in obesity, Kennedy (1966) regarded them as "no more useful than glucose utilisation as the likely satiety signal to the hypothalamus".

It has recently been suggested that the satiety signal may be hormonal (Kennedy, 1966). The work of Hales & Kennedy (1964) was cited as evidence that plasma insulin levels may signal satiety. These workers found plasma insulin levels were high after feeding. Hunter, Friend & Strong (1966) and Hunter & Rigal (1966) found that growth hormone levels in the plasma of children and adults were so low after a meal as to be undetectable but rose 3-4 hr later when, it was suggested, the hormone helped to make fat available as a source of energy. Thus, although Kennedy (1966) has stated that "It may be that the hypothalamus received a post-prandial signal of satiety associated with lipogenesis (or insulin) followed later by a hunger signal of lipolysis (or growth hormone)", there is as yet, little direct evidence to support such a claim.

(iv) The effect of protein on food intake.

Diets containing 27.5% of the total calories as protein were found to have a higher subjective satiety value than those in which the protein contribution was 7.8% (Fryer et al. 1955), an effect possibly attributable to the S.D.A. of the protein (Brobeck, 1960b). Ashida & Harper (1961) found that within one day after the protein content of the diet was raised from 25% to 45 or 70%, food intake of rats fed the high protein diets was depressed and their growth rate retarded.

The food intake of normal rats and those rendered hyperphagic by bilateral electrolytic lesions in the ventromedial nucleus was successively reduced as the casein content of a semi-synthetic diet was increased from 50 to 90% (Krauss & Mayer, 1963, 1965). In the 10-40% range, the protein level did not influence food intake. When the diet contained 60-90% protein, food intake was depressed so that the amount of protein ingested by each rat remained relatively constant at 10-11g/day. It was suggested that because of "a limitation imposed by the animal's ability to metabolise large quantities of dietary nitrogen within a short period of time", food intake was limited so that the amount of protein ingested did not exceed a certain threshold. As diets of high protein content reduced food intake of normal and lesioned rats, it was further concluded that the effects were independent of the satiety centres. These workers apparently did not consider the possibility that the rate of gastric emptying, and thus food intake, may have been reduced by the high protein diets (Mayer, 1955; Peraino et al. 1959).

Depression of food intake and retarded growth are characteristic signs in rats fed a diet in which there is an imbalance of amino acids

(Kumta & Harper 1960, 1961). In the protein depleted animal, a depression in food intake may be evident within 6 hr after the addition to the diet of the amino acid mixture causing the imbalance (Kumta & Harper, 1962). Sanahuja, Rio & Lede (1965) used the concept of "appetite quotient" developed by Carpenter (1953) to determine whether decreased intake of an imbalanced diet was a primary effect or whether it was only a consequence of low nutritive value of the diet. Appetite quotients were lower for imbalanced than for the basal or supplemented diets. It was suggested that the effect was a primary one and that food intake was depressed by an imbalanced diet because of "some specific physiological effects that affect directly the food intake" and not as a consequence of the low nutritive value of those diets.

Of the possible explanations of the reduced food intake (Harper, 1964), the one considered most likely by Harper is that an amino acid imbalance affects some basic mechanism regulating food intake. Mellinkoff (1957) suggested that an abnormal plasma amino acid pattern may cause a fluctuation in the desire for food. The plasma amino acid pattern of rats fed an imbalanced diet exhibits a disproportionate fall in the concentration of the amino acid most limiting for growth (Kumta & Harper, 1962) and these changes precede or occur concurrently with changes in food intake (Sanahuja & Harper, 1963). It is not known how the appetite depressing mechanism is triggered. On diets containing 3-7% leucine, there is an alteration in the pattern of plasma amino acids and a depression of food intake in normal rats (Rogers, Spolter & Harper, 1962). As similar results were obtained by Krauss & Mayer (1965) with rats in the "dynamic phase" of hypothalamic hyperphagia, it was concluded that the depressive effects on food intake of imbalanced diets are independent of the satiety centre.

Nasset, Ganapathy & Goldsmith (1963) expressed doubts that blood amino acid patterns are critical in determining food intake. It is claimed that the amino acid pattern of plasma and gut contents do not reflect that of the diet because of digestion of endogenous protein (Twombly & Meyer, 1961; Nasset & Ju, 1961), metabolic activity of the intestinal wall and differences in rate of absorption of the individual amino acids (see Gitler, 1964). Nasset et al. (1963) concluded that there may be receptors in the intestinal wall sensitive to gut contents which signal the hypothalamus early in the digestion of ingested foods. Direct support for some such chemoreceptor has been claimed (Sharma & Nasset, 1962). However as Nasset and co-workers have shown that the amino acid composition of the gut contents does not resemble that of the diet, it is obscure how such a receptor detects the deficiencies of the diet. In addition, these concepts are not readily reconciled with successful development of a method for the evaluation of protein quality based on the pattern of plasma amino acids after feeding the protein under test (Longenecker, 1963).

Using rats fed diets ranging from 6 - 34% protein, Meyer (1958) found that compared with high protein diets, those of low protein content resulted in lower food intake and gains of fat free tissue and a higher proportion of gain as fat. It was suggested that the intake of the low protein diets was limited by the ability to dispose of excess energy as fat and by heat production. When the "energy portion" of the diet, sucrose, was replaced by cellulose, food intake increased. This was explained on the basis that less energy had to be disposed of and therefore food intake could increase. The subsequent demonstration (Meyer & Hargus, 1959) that exercise or cold environment increased the intake of a high sucrose, low protein diet was interpreted in favour of this belief. It was considered that these conditions facilitated the rats' ability to dispose of excess energy thereby allowing food intake to increase.

These conclusions have been criticised in detail (Anon., 1959). In addition, they must be regarded as speculative in view of the evidence discussed below that osmotic factors may limit the intake of low protein diets to which sucrose has been added. The increased food intake when sucrose was partly replaced with casein or cellulose may be explained on this basis rather than by increased protein and reduced energy intake respectively as suggested by Meyer. The increased food intake induced by cold environment or exercise may be explained by an increased energy loss as suggested by Meyer & Hargus (1959) but concurrent changes in water metabolism and temperature regulation may have been involved.

Diets low in both protein and calories or low in protein with adequate or even excessive calories may result in protein deficiency. The food intake of rats, dogs (Platt, Heard & Stewart, 1964) and children (Viteri, Béhar, Arroyave & Schrimshaw, 1964) is reduced when they are fed these diets. Anorexia is, in fact, one of the early signs of protein deficiency. The causes of the reduced food intake of low protein diets is unlikely to be simple for the body's response to changes in protein intake are complex and related to many factors (see Munro, 1964).

3. Relationship between food and water intake.

The regulation of water intake has been recently reviewed by Brobeck (1960a), Andersson & Larsson (1961), Gregerson & Cizek (1961), Stevenson (1964) and Fitzsimons (1966). Chew (1965) has reviewed many aspects of mammalian water metabolism and includes a bibliography in excess of 600 references.

As with food intake, the control of water intake is vested in the central nervous system. Fitzsimons (1966) concludes that the hypothalamus is important and appears to be organised into a lateral drinking centre and a medial satiety centre. However, the latter has not been demonstrated in so far that excess drinking has not been observed as a consequence of hypothalamic or other central lesions (Brobeck, 1960a; Andersson & Larsson 1961).

A difficulty in interpreting the results of experiments where various regions of the brain are lesioned or stimulated is that feeding and drinking are usually affected concurrently. There is often uncertainty as to the primary effect. However, it has been concluded (Anand, 1961; Fitzsimons, 1966) that the hypothalamic mechanisms controlling food and water intake act separately and independently.

Various stimuli have been shown to be of importance in the initiation and satiation of thirst. Oropharyngeal metering (Towbin, 1949; Gregerson & Cizek, 1961), gastric distension (Towbin, 1949, 1955), salivary secretion (Gregerson & Cizek, 1961), temperature change (Andersson et al., 1963), increase in effective osmotic pressure of extracellular fluid and decrease in extracellular fluid volume (Fitzsimons 1961a,b; Stricker, 1966) have been implicated.

A close, direct relationship between food and water intake has been shown in short term observations on the "normal" rat (Adolph, 1947; Strominger, 1947), the hyperphagic rat (Strominger, 1947) and in long term observations on the dog (Cizek, 1959), rabbit (Cizek, 1961) chicken (Kellerup, Parker & Arscott, 1965) and the rat (Cizek & Nocenti, 1965). The diurnal patterns of food and water intake are also closely related in the rat (Strominger, 1947; Siegel & Stuckey, 1947; Young & Richey, 1952) and the dog (Robinson & Adolph, 1943). The thirst accompanying the ingestion of food presumably arises from a decrease in volume and an increase in tonicity of extracellular fluid owing to the entry of secretions and water into the digestive tract (Andersson & Larsson, 1961).

This aspect of digestion appears to have been little studied. Lepkovsky, Lyman, Fleming, Nagumo & Dimick (1957), found that rats fed for 2 hr in the absence of water, regulated their food intake so that a constant water:food ratio could be maintained in the gastric contents by the mobilisation of water from tissues. The skin appeared to supply the majority of the water. The tissues of rats fed with water available showed a less pronounced fall in water content. Lepkovsky _et al_. suggested that temporary withdrawal of water from selected tissues and consequent dehydration may be one of the factors causing the cessation of eating.

Using intragastric injection of glucose, NaCl and sodium saccharine solutions of osmotic concentrations from 0.98-3.10 osmol/1., Schwartzbaum & Ward (1958) found that the food intake of rats over a 90 min period immediately following the preloading was related to the tonicity of the preload, irrespective of the substance used. Hypertonic solutions reduced food intake by as much as 50%. Hypo- and isotonic solutions tended to increase intake above control levels during the first 45 min when water was not available, but the difference was not apparent after a further 45 min during which water was available. The inhibitory effects of intragastric hypertonic solutions on rate of bar pressing as an index of hunger (Smith & Duffy, 1955) and on food intake (Smith & Duffy, 1957) have led these workers to postulate that blood tonicity is one of the important factors in food intake regulation. Subsequent work (Smith, Pool & Weinberg, 1959) has shown that intragastric preloading with water caused a small but consistent increase in food intake during the subsequent 2 hr feeding period. Evidence to show that blood tonicity changes were involved was that intraperitoneal injections of hypertonic solutions or the withdrawal of a hypertonic intragastric injection 5 min after its introduction caused a reduction of food intake. When food was available for

24 hr, an intragastric preload of 10 ml. of a one molar solution of either glucose or sucrose resulted in a depression of food intake for the first 3 hr followed by a partial or complete recovery. Intravenous injections of water and hypertonic sugar solutions (Smith, 1966) caused small increases and decreases respectively of food intake. These experiments provide some support for the suggestion (Brobeck, 1955) that osmoreceptors are involved in the regulation of food intake. The physiological normality of rapid intragastric injections of hypertonic solutions must be questioned and is perhaps similar to the "dumping syndrome", unpleasant symptoms of which are induced in gastrectomised patients by the passage of hyperosmolar solutions into the duodenum (Weidner, Scott, Bond & Shull, 1959). Even in normal man, the ingestion of a sufficient volume of a solution of high osmotic activity such as casein hydrolysate has rapidly appearing and debilitating effects (Free & Leonards, 1944).

A number of other reports offer indirect support for the suggestion that osmotic factors may be involved in the regulation of food intake. When rats were fed for 2 hr each day on a diet containing 10% casein and 80% of dextrin, sucrose, maltose or glucose, Harper & Spivey (1958) found that food intake and rate of body weight gain were inversely related to the capacity of the carbohydrates to exert osmotic pressure. The volume and moisture content of the stomach contents were directly related to osmotic capacity of the dietary carbohydrate. MacDonald (1963) also observed an inverse relationship between osmotic capacity of dietary carbohydrate and food intake of rabbits.

The reduction of food intake induced by preloading with hypertonic solutions or the inclusion of large amounts of osmotically active carbohydrates such as sucrose in the diet may involve:-

(a) delayed gastric emptying by an osmoreceptor mechanism as proposed by Hunt (1963)

(b) stomach distension by the entry and retention of water

(c) redistribution of water within the body tissues causing cessation of eating by an unknown mechanism but possibly involving osmoreceptors outside the digestive tract

(d) metabolic changes as suggested by changes in body composition (see Romberg & Benton, 1965).

No progress appears to have been made in determining the relative importance of these and other factors.

If osmotic factors are important in determining food intake it is pertinent to enquire as to the effects of adding water to a diet. Adolph (1947) has shown that within certain limits, the dilution of a milk diet with water has no effect on the intake of milk by rats. In contrast, Archdeacon & Allen (1948) found that when dogs were fed for 30 min each day on a "Purina chow" diet with water freely available, the addition of an equal weight of water to the chow increased its consumption during the 15 day experimental period. Strominger et al. (1953) observed that when a calf meal diet fed ad lib. to rats was diluted with two parts by weight of water, caloric intake was increased by 30%. The effect was transient, occurring only during the 3 days following dilution.

Using rats, Keane, Smutko, Krieger & Denton (1962) found that the addition of 20% of water to purified diets containing 6-12% protein resulted in increased growth rate and a higher body weight gain per gram of protein consumed (protein efficiency ratio, P.E.R.) than that obtained when no water was added to the diet. Subsequent paired feeding studies with rats (Keane et al., 1963) indicated that the effects were not due to an increased protein intake. These workers also found that although P.E.R.

was increased by the 5 - 35% addition of water to a diet containing sucrose, when the sucrose was replaced by either cornstarch or dextrin, a similar effect was achieved with 50 but not 20% addition of water. Confirmation of these findings has been reported by Reussner, Mazura & Thiessen (1964).

Evidence for the participation of osmotic factors in food intake regulation is strong. It is sufficient to warrant its detailed investigation. As Schwartzbaum & Ward (1958) suggest, herein may lie one of the important mechanisms for short term regulation.

It is clear that hunger, satiety and long term energy balance are the consequence of the activity of a number of mechanisms. It appears that there are a number of stimuli delivered to receptors in different regions of the body, the afferent signals to which they give rise being integrated in the central nervous system to result in coordinated behaviour. While there may be circumstances in which an individual stimulus or mechanism may dominate, more usually a multiplicity of factors is involved, the relative importance of which may vary according to the conditions prevailing at a given time.

B. REGULATION OF FOOD INTAKE IN RUMINANTS

When considering the regulation of food intake in ruminants, there are several peculiarities of the anatomy and the nutrition of these animals which must be taken into account. Some important points in this regard are listed below.

(a) The dependence of the host animal on the microorganisms inhabiting the gut, particularly the reticulorumen: the nutrients of the host are mainly the metabolic waste products of these organisms and the organisms themselves.

(b) The range of diets is wide, the concentration of energy contained therein is often low, as little as 40% being digestible.

(c) Large amounts of digesta are contained in the gut and the complex stomach (and its associated motility) has a marked effect on the passage of the digesta along the gut.

(d) The digestion of fibrous food is dependent in part, on rumination.

(e) The ingestion of food occupies a large portion of the day.

(f) The amount of roughage eaten increases with increasing digestible energy content (i.e., quality) of the roughage.

(g) Short chain fatty acids are the main source of energy.

(h) There is no marked post-prandial hyperglycemia when the diet is one of roughage.

(i) The ease with which adult ruminants fatten suggests an unusual adjustment of caloric intake and energy expenditure.

A consideration of the factors listed above suggests that the stimuli involved in the regulation of ruminant food intake are either different from those thought to be important in animals with a simple stomach, or as is more likely, of different relative significance.

Recent reviews are those of Balch & Campling (1962), Blaxter (1962, ch. 15), Ulyatt (1964), Egan (1964), Campling (1966) and Conrad (1966).

1. Role of the central nervous system.

The presence of feeding and satiety centres in the ruminant hypothalamus has not been established with certainty. Larsson (1954) and Wyrwicka & Dobrzecka (1960) have produced feeding and satiety responses of a few minutes duration by stimulating certain areas of the hypothalamus. However, neither aphagia leading to death of the lesioned animal or hyperphagia leading to obesity - effects repeatedly shown in rats and mice (see Kennedy, 1966) - have been produced in the ruminant. Holmes &

Frazer (1965) were unsuccessful in attempts to establish hyperphagia in sheep by electrolytic ablation of the ventromedial region: species difference in location of the satiety centre was considered by them to be an unlikely explanation. In some circumstances the limitation of food intake may be independent of the satiety centre (Krauss & Meyer, 1965). The factor(s) limiting the intake of the wheaten-lucerne chaff fed by Holmes and Frazer, possibly capacity of the reticulorumen, may likewise not have involved these centres.

In contrast to monogastric animals, vagal innervation of the stomach is essential for the well-being of the ruminant (Duncan, 1953; Phillipson & Cuthbertson, 1956). The movements of the rumen, reticulum, omasum and oesophagus are essentially reflex responses and the vagus nerves contain both afferent and efferent fibres of the relevant reflex arcs (see Habel, 1956; Comline & Titchen, 1961; Titchen & Reid, 1965). The coordinating centre(s) for these activities is presumably located in the medulla oblongata (Bell & Lawn, 1955; see Comline & Titchen, 1961).

2. Role of gastrointestinal tract.

(a) Oropharyngeal regulation.

Balch & Campling (1962) have reviewed the limited evidence available suggesting that food intake is not limited by metering of the time spent eating or number of chews, or by exhaustion of salivary secretion or jaw muscles.

The rate of food consumption is directly related to the voluntary intake of the roughage (Hesselbarth, 1954; Krüger, Müller, Ginkel & Schulze, 1955; Freer, Campling & Balch, 1962), the time elapsed since the start of feeding (Reid & Cornwall, 1959) and time of access to the roughage (Freer *et al.*, 1962). Freer *et al.* found that when the voluntary intake of straw was increased by the intraruminal infusion of urea, the rate of straw consumption was also increased suggesting there was no relationship between the latter and palatability. These relationships may reflect the degree of hunger rather than an association between the rate of breakdown of the food in the reticulorumen and the rate of food consumption as was suggested by Freer and co-workers. Differences in the availability of body fluid and solutes for the production of digestive secretions, particularly saliva, may contribute to the difference observed in the rates of consumption of various roughages. The decline in rate of food intake as feeding progresses may be a result of the partial inhibition of saliva production by inhibitory stimuli elicited by the accumulation of digesta in, and the consequent distension of, the reticulorumen (Wilson, 1963; Kay, 1966).

(b) Gastric distension.

Balch & Campling (1962) have reviewed a considerable volume of direct and indirect evidence suggesting that a relationship exists between the bulk of a food and the amount of it voluntarily consumed by ruminants. It was concluded that physical distension of the reticulorumen is an important factor regulating food intake. This concept of the importance of bulk was expressed in the generalisation made by Blaxter (1950) that the amount of food dry matter consumed increased with increasing concentration of the ration, expressed as net energy/kg DM. Subsequently, a close positive relationship between the apparent digestibility and the voluntary intake of roughages has been shown (Crampton, Donefer & Lloyd, 1960; Blaxter, Wainman & Wilson, 1961; Blaxter & Wilson, 1962; Blaxter, Wainman & Davidson, 1966; Campling, 1966). Cattle digest the same roughage better than do sheep and also consume more of it (Blaxter et al., 1966). This relationship may not apply to a whole range of roughages for Conrad, Pratt & Hibbs (1964) found that for diets of high roughage

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content and between 52 and 66% digestibility, food intake was related to body weight (reflecting gut capacity) and to dry matter digestibility. Food intake increased with increasing digestibility up to about 67% digestibility beyond which food intake decreased with increasing digestibility.

Direct evidence that the amount of digesta contained in the reticulorumen of cows ("fill") is important in determining the amount of food eaten has been obtained by Campling & Balch (1961). Collection at the cardia and removal from the rumen of the food swallowed during the first 3 hr of a meal increased the amount of hay consumed by 70-82%. Changes in the amount of reticulorumen contents by removal or addition of digesta were partly compensated for by an increase and decrease respectively of the amount of food eaten. The presence of water filled balloons in the rumen also resulted in a decrease in intake, an observation supported by Davies (1962). The addition of food or sawdust to the rumens of sheep immediately before feeding was found by Weston (1966a) to result in an almost fully compensatory decrease in food intake except on the first day of the intraruminal feeding. It was suggested that the sensitivity of the mechanisms was insufficient to maintain the *status quo* during the 24 hr following the first additions. The addition of either 500 or 1,000 g of finely ground polyvinyl chloride had relatively little effect on intake; this was attributed to its rapid passage out of the rumen.

Blaxter *et al*. (1961) estimated that the dry matter content of the digestive tract of sheep at the end of a meal was similar with three different roughages offered *ad lib*. and suggested that the animals eat to a constant distension of their digestive tracts. The reticulorumen was suggested as the compartment making the major contribution to this effect.

(c) Rate of disappearance of digesta from the reticulorumen

Campling, Freer & Balch (1961) observed that when four cows were given hay or straw ad lib. in one meal daily, they ate more than twice as much hay as straw. Immediately after the meal the dry weight of the digesta in the reticulorumen was 35% greater with hay than with straw but just before the next meal, the difference was less than 6%. If urea was infused into the rumens of cows fed straw (Campling, Freer & Balch, 1962) the amount of digesta in the reticulorumen after a meal was increased but the amount present before the next meal was the same as when straw alone was fed. These results do not confirm that the critical fill determining satiety is at the end of the meal. Subsequent observations (Freer & Campling, 1963) suggested that with different roughages having a mean daily rate of disappearance from the reticulorumen of about 18 lb DM, eating ceased when the reticulorumen contained equal amounts of digesta, a limit possibly set by the capacity of the organ. With roughages having a slower rate of disappearance, it appeared that eating ceased when the reticulorumen contained less digesta, the amount present at the end of feeding being that which would be reduced to about 19 lb DM immediately before the next meal.

The rate of disappearance of digesta from the reticulorumen is regarded an a function of the rate at which the food material is broken down while it is retained there and the rate of flow of material out of the rumen. Variation in either of these components may be expected to affect intake.

(d) Rate of breakdown of digesta in the reticulorumen.

Rate of breakdown is used in this review to describe the rate at which the digesta in the reticulorumen is altered to that state in which it can pass out of the organ either by absorption or by passage along the tract. Rate of passage, expressed as retention time, and rate of flow have been defined by Balch & Campling (1962, 1965).

Size is probably the main factor in determining whether or not undigested food residues leave the reticulorumen (Balch, 1950; Castle, 1956; Balch & Campling, 1965) but specific gravity of the particles is also important (Balch & Campling, 1965). Reduction of particle size by finely grinding the roughage may on occasions result in an increase in food intake, possibly by increasing the rate of passage of the particles out of the rumen (see Campling & Freer, 1966).

Factors affecting the rate of breakdown include rumination, activity of the microorganisms, quality of the diet and reticulorumen motility.

One of the important factors responsible for reduction of the food particles to a size sufficiently small to pass through the reticulo-omasal orifice is chewing during rumination and eating. The significance of rumination has been investigated by Pearce & Moir (1964). They used a chaffed ration on which rumination was either allowed to occur normally or was restricted by muzzling the animal and the same diet finely ground with rumination normal for that ration or stimulated by the addition of polythene flakes. Retention time was increased by muzzling. By contrast, it was decreased when the ground roughage was fed compared with the chaffed ration, and a further decrease resulted from the addition of polythene flakes. Freer et al. (1962) found a direct relationship between the total time spent eating and ruminating per pound of roughage and the rate at which the organic matter passed from the rumen. Factors affecting the extent and pattern of rumination have recently been studied by Pearce (1965a,b).

The relationship between apparent digestibility of the feed and voluntary food intake is partly explicable in terms of the ability of the food to satisfy the nutrient requirements of the rumen microorganisms and hence the rate at which it is broken down in the rumen. It is now well established that digestion of poor quality roughages in the rumen may be hampered by a shortage of nitrogen. The provision of small amounts of nitrogen, often as an intraruminal infusion of urea, increases the rate of breakdown, rate of passage and voluntary intake of the food (Campling et al. 1962; Hemsley & Moir, 1963; Coombe & Tribe, 1963; Egan, 1965a): in some instances an additional energy source is also required (see Faichney, 1965; Hemsley, 1966). Other substances such as certain fatty acids (Hemsley & Moir, 1963) and yeast (Tosic, 1949) increase food intake probably by enhancing cellulose digestion. Substances which depress microbial activity such as antibiotics, cause a reduction in food intake (Bell, Whitehair & Gallup, 1951; Oyaert, Quin & Clark, 1951).

Attributes of the food affecting the availability of nutrients such as amount of lignification, cell wall content (Van Soest, 1965) and particle size (Meyer, Gaskill, Stoewsand & Weir, 1959; Meyer, Kromann & Garrett, 1965), may also affect the rate and extent of digestion.

Reid (1963) has discussed the role of reticulorumen motility in aiding digestion in that organ.

An outcome of the relationship between rate of breakdown and rate of disappearance is that there is an inverse relationship between rate of passage of undigested food residues, expressed as retention time, and voluntary food intake (Blaxter et al. 1961; Coombe & Tribe, 1963; Campling, 1966). It is not known with certainty however, whether the higher food intake is caused by, or is the cause of, a faster rate of passage. The effects of the amount of digesta in the reticulo-rumen on rate of passage has been discussed by Balch & Campling (1965).

(e) Flow of digesta from the reticulorumen.

Variations in the flow of material out of the reticulorumen may be expected to alter the rate of disappearance of digesta from that organ.

The importance of the reticulorumen movements in aiding the passage of digesta is indicated by the effects of vagotomy. The resultant loss of reticulorumen movements leads to distension of the reticulorumen and ultimately, death (see Phillipson & Cuthbertson, 1956).

The relationship between reticulorumen motility and digesta transfer has not been widely studied over long periods, due mainly to the difficulty in measuring flow of digesta into and out of the omasum (see Phillipson & Ash, 1965).

The mean weight of organic matter passing through the reticulo-omasal orifice with each biphasic contraction of the reticulum has been calculated for a number of roughages (Freer et al. 1962). It was concluded that the amount of organic matter transferred per contraction depended on the weight of organic matter consumed and not on the nature of the roughage and that it was unlikely that the intake of roughages offered ad lib. was limited by the amount of organic matter transferred per reticulum contraction. These conclusions may be regarded as speculative.

Stevens, Sellers & Spurrell (1960) recorded reticulo-omasal pressure changes and attempted to relate them to ingesta flow. They found that reticulum contents may enter the omasum mainly during the height of the second of the biphasic reticulum contractions; their exit to the abomasum was less regular. Whether the material in the omasal body passed to the abomasum depended on the contractions of the omasum and whether the omaso-abomasal orifice was open during those contractions. Omasal body contractions did not regularly occur with each biphasic reticulum contraction and on some occasions when it did, the omaso-abomasal orifice was closed and omasal contents were returned to the reticulum. Kay & Hobson (1963) have argued that, due to the absorption of water by the omasum, the outflow of digesta from that organ is probably less than inflow from the reticulum, but as the omasum has little holding capacity, the pattern of outflow may be similar to that of inflow. The exit of material from the omasum shows no regular relationship to the motility of the reticulum and rumen (Phillipson & Ash, 1965).

That the physical nature of the diet affects reticulorumen motility and therefore digesta transfer from that organ is suggested by the work of Colvin & Daniels (1965). These workers measured the effects of grinding a roughage plus concentrate diet on the rumen motility of fasted calves. As the degree of fineness of grinding increased, the amplitude and frequency of rumen contractions decreased and the resting intraruminal pressure increased. Pharr, Colvin & Noland (1967) reported similar results for sheep with the exception that the frequency of contraction was not affected.

Evidence to show that on occasions there may be a limit to the rate at which digesta particles are expelled from the reticulorumen through the reticulo-omasal orifice is found in the work of Pearce (1967). By measuring size and distribution of particles of the reticulorumen contents of sheep at various intervals after a single feed, he found evidence to suggest that at times, the rate of formation of small particles was greater than their rate of removal from the rumen.

(f) Influence of the gut beyond the reticulorumen.

Distension of the abomasum inhibits contractions of the rumen and reticulum (Titchen, 1958, 1960), omasal outflow (Ash, 1962a) and reduces the strength of contraction of the omasum (Stevens et al. 1960). Distension of the duodenum inhibits abomasal outflow (Phillipson, 1952) and the frequency and force of the reticulum contractions (Phillipson & Ash, 1965). Total obstruction of the flow of digesta into the duodenum

(Ash, 1962b) or through the terminal ileum (Goodall & Kay, 1965) rapidly leads to complete loss of appetite. Chemical stimuli are also important, affecting reticulorumen motility (Titchen, 1958) and abomasal secretion (Ash, 1961; Hill, 1965). The existence of inhibitory stimuli originating in the duodenum and affecting flow of digesta from the abomasum is indicated by the increased flow rate observed when digesta are collected from a pyloric fistula and not returned to the duodenum (Hogan & Phillipson, 1960). As in simple stomached animals, the intraduodenal instillation of acid, fat or protein digestion products is followed by varying degrees of inhibition of abomasal motility and secretion (Singleton, 1951; McLeay, 1967). The stimuli so induced by fat and fatty acids can on occasions depress food intake (Titchen, Reid & Vlieg, 1966).

The importance of reflex and other mechanisms discussed in this section in the regulation of food intake are unknown. Some evidence suggests that where flow of material out of the reticulorumen is enhanced by finely grinding roughage, the amount of digesta in the lower gut may limit food intake (Campling & Freer, 1966). Any increase in the amount of digesta passing to the intestines results in distension rather than an increase in dry matter concentration (Goodall & Kay, 1965). More rapid propulsion also occurs and this may vary depending on the diet (Coombe & Kay, 1965).

There is thus a considerable body of evidence to suggest that the amount of digesta in the reticulorumen, its rate of disappearance from that organ, and its presence in the more distal regions of the gut are important factors in controlling the voluntary intake of roughages. It is pointed out, however, that a characteristic of the majority of the studies on which these conclusions are based has been the disregard of the contribution that liquid makes to rumen fill. Water accounts for about 90% of the weight of digesta in the reticulorumen (Reid, Bailey & Glenday, 1967) and hence must make the major contribution to effects involving distension. According to Kay (1966), the absence of appreciable effects on food intake of additions of large volumes of water to the rumen (Campling & Balch, 1961; Davies, 1962; Holmes & Lang, 1963), may merely be a reflection of the rapid exit of this excess water. Kay (1966) stated that "it seems that 'osmotically-free' water - in practice the water drunk in excess of the quantity required to render isotonic the salts released from the food - is absorbed quite rapidly." This concept appears incompatible with recent work for although the rumen contents tend towards hypotonicity (Engelhardt, 1963a; Warner & Stacey, 1965), the solute concentration in rumen liquor rises very rapidly on feeding to a peak of about 400mosm/kg water in sheep (Warner & Stacey, 1965) and cows (Reid, C.S.W. & Little, D., unpublished) and returns to hypotonicity only after several hours. An equally gradual return to hypotonicity is evident when the solute concentration is lowered by the rapid addition of large volumes of water into the rumen of the fasted animal (Warner & Stacy, loc.cit., Reid & Little, loc.cit.). There appears to be no evidence as to the immediate fate of liquid added to the rumen. Until a detailed study of the factors involved is made, it must remain uncertain, for example, why an intraruminal infusion of 10 l. daily of either artificial saliva or 1% NaCl, but not a similar volume of water, results in the inhibition of parotid saliva secretion, an effect which was presumed due to a distension mechanism (Wilson & Tribe, 1963).

Changes in the distribution and amount of water within the reticulorumen may have considerable effects on rumen function as discussed below. Factors affecting the distribution of dry matter within the rumen have been little studied (Reid et al., 1967) and even less is known about the importance of this phenomenon. One consequence may be that all receptors in the reticulorumen are not uniformly stimulated.

The possibility of receptors sensitive to changes in the volume of the rumen, the tension in its walls or both have been discussed by Kay & Hobson (1963). Distension of the rumen by inflation or by balloons inhibits both rumination and saliva secretion (Kay & Phillipson, 1959; Ash & Kay, 1959) and at intrarumen gas pressure of 14 cm H_2O or more, inhibition of reticulum and rumen contractions is evident in decerebrate sheep (Reid & Titchen, 1965). The inhibitory effects of distension may occur during feeding causing a reduction in saliva secretion as feeding progresses and at the beginning of the rest period which follows feeding (see Wilson, 1963). Inhibition of rumination after feeding by a similar mechanism has been suggested (Pearce, 1965a). Whether these effects are involved in satiety is unknown but their similarity is striking.

The assumption has been made (Kay & Hobson, 1963), that distension of the rumen stimulates the hypothalamic "satiety" centre just as stretch does in other species (Sharma, Anand, Dua & Singh, 1961). The possibility cannot be excluded however that the hypothalamic "satiety centre" is not involved as discussed earlier, and that satiation due to distension is a reflex phenomenon similar to the inhibition by distension of saliva secretion and rumination.

The manner in which the animal can adjust its food intake to the rate of disappearance of digesta from the reticulorumen is even less certain. Two possibilities are suggested. The "setting" of the level of gut distension at which satiety occurs may depend on the diet. Egan (1965a,b) has proposed a relationship between level of fill of the reticulorumen and the nitrogen status of the animal to account for the increased food intake induced by intraduodenal casein supplements. Secondly, it is probable that it is not the rate of disappearance of digesta from the reticulorumen that is monitored but rather some aspects of the processes on which it depends - the rate of digestion of food material, absorption of soluble products and passage of gut contents along the digestive tract. The nature, location and function of the reticulo-omasal orifice suggests that further study of the factors controlling its activity may be fruitful.

3. Food intake and water metabolism.

(a) Relationship between food and water intake.

Factors affecting the consumption of water by cattle include type and dry matter content of the ration, environmental temperature and requirements for growth, lactation and pregnancy (Leitch & Thomson, 1944; Winchester & Morris, 1956). Presumably similar factors are important in the sheep. Restriction of water decreases the food intake of cattle and sheep (Balch, Balch, Johnson & Turner, 1953; English, 1966). The decrease in food intake may not become apparent during the first 1-3 days of water restriction, even in the complete absence of drinking water (Bianca, Findlay & McLean, 1965; Gordon, 1965).

It is not apparent why for many roughages including grass but excluding silage a direct relationship should exist between the dry matter content of the food and its voluntary consumption (Arnold, 1962; Davies, 1962; Calder, Nicholson & Cunningham, 1964; Halley & Dougall, 1962; Lloyd, Donefer, Bowman & Crampton, 1962). On the other hand, the positive relationship between the rate of cellulolysis and water content of the surrounding rumen contents (Balch & Johnson, 1950) suggests that an increase in the water content of the food may result in conditions conducive to an increase in rate of breakdown of the digesta in the drier dorsal regions of the rumen where the majority of the dry matter is located (Reid et al. 1967). Balch (1950) found a positive between-cow relationship between percentage dry matter content of the total food and water intake and rate of passage of undigested residues through the hind gut.

(b) The consistency of rumen contents and food intake.

After a week of limited water intake by a cow, Balch et al. (1953) found that the weight of rumen contents had fallen in proportion to food intake and both the dry matter percentage of the rumen contents and the digestibility of the food were maintained. The constancy of the dry matter percentage of rumen contents is a concept of limited value in view of variation in the distribution of dry matter within the reticulorumen (Reid et al. 1967). When the rafting of dry matter is reduced by feeding finely ground roughage, the increased amount of dry matter in the ventral rumen and around the reticulo-omasal orifice may restrict the flow of material out of the rumen (Campling et al. 1963 ; Freer and Campling, 1965).

The importance of water in the transport of food residues along the gut is well recognised (Ash & Kay, 1963; Phillipson & Ash, 1965). Saliva has been estimated to supply 70-90% of all the fluid entering the reticulorumen (Bailey, 1961; Stacy & Warner, 1966) although appreciable amounts of fluid may enter through the rumen wall (Engelhardt, 1963a; Murray, Reid & Sutherland, 1962). The importance of saliva in regulating the volume and consistency of rumen contents has been discussed by Kay (1966).

Supplements of ash or various salts may improve food intake, feed efficiency and growth (Barnett & Reid, 1961, p. 174). The effects are particularly apparent when high starch, low roughage diets are fed

(Preston, 1963) where the concentration of dry matter in the reticulorumen is about 20% (Boyne, Campbell, Davidson & Cuthbertson, 1956; Rogerson, 1958) and ash content of the diet is low (Rogerson, 1958). In addition to the explanations discussed by Preston (1963), these effects may be an illustration of the importance of fluid in rumen function. Mineral supplements decrease the percentage of dry matter in the rumen contents and increase the volume of rumen fluid (Nicholson, Loosli & Warner, 1960; Nicholson, Cunningham & Friend, 1963), rate of flow of fluid to the omasum (Murray *et al*. 1962), rate of passage of chromic oxide through the alimentary tract and crude fibre digestibility (Elam, 1961; Nicholson, *et al*. 1960).

(c) Osmotic control of food intake.

While the results discussed above indicate a relationship between water, rumen function and food intake there is no direct evidence of osmometric control of food intake as has been suggested for non-ruminants. Certain aspects of ruminant digestion make this an attractive hypothesis.

Sheep are estimated to secrete 3-16l. of saliva daily and cattle 100-180 l. (Kay, 1966). The rapid secretion of alkaline saliva during feeding, together with other fluids associated with digestion, appears responsible for the phenomenon observed in sheep that about 30 min after the start of feeding, the normally alkaline urine becomes acid and the excretion of water and sodium diminishes sharply (Stacy & Brook, 1964). These reactions are apparently insufficient to prevent a decrease in plasma volume and an increase in plasma osmolality (Warner & Stacy, 1965). Passage of water through the rumen wall into the hypertonic rumen content may also be a contributing factor (Murray *et al*. 1962). Qualitative changes in the solute composition of the blood are to be expected in view of the composition of the secretions, differences in the rate of resorption of solutes from the gut contents (Kay & Hobson, 1963; Stacy & Warner, 1966) and presumably, renal tubular fluid.

The secretion of acid by the abomasum may partly counterbalance the changes in urinary pH during feeding although it has been claimed there is no abomasal response to feeding when feeding is frequent (Hill, 1965). After a feed of short duration, Ash (1961) observed that acid secretion by abomasal pouches increased in the subsequent 60 min, reached a peak between the second and third hour after feeding and thereafter declined. In contrast, McLeay (1967, ch. 2) found that acid secretion by abomasal pouches increased whenever sheep fed, whether food was continuously available or was offered after a period of fasting. Acid output reached a peak within 60 min of eating.

The sham feeding experiments of Campling & Balch (1961) suggest that dehydration consequent on the removal of secreted saliva is ineffective on its own in causing the cessation of eating. However, a combination of stimuli involving gut distension and the redistribution of fluid and electrolytes among the body compartments has the attraction of a close time relationship between the occurrence of these events and satiety.

It is of interest that experiments designed to test whether the intraruminal infusions of water inhibit food intake by distension of the reticulorumen have in fact, resulted in small increases in food intake (Campling & Balch, 1961; Davies, 1962).

4. <u>Chemostatic regulation of food intake</u>.

It has been proposed that ruminants eat to a constant digestible nutrient or energy intake when highly digestible rations are fed

(Conrad et al. 1964; Conrad, 1966; Montgomery & Baumgardt, 1965a,b), even when the highly digestible ration in diluted with inert material (Baile & Pfander, 1964). When access to a concentrate ration is restricted to 5 hr/day, nutrient intake may be lower than when roughages are fed but is increased when available continuously (Freer & Campling, 1963). Evidence that the amount of digesta in the reticulorumen of lactating cows did not limit their voluntary intake of highly digestible herbage has been found by Hutton, Hughes, Newth & Watanabe (1964). These workers observed a linear relationship between food intake and weight of dry matter in the reticulorumen.

A chemostatic form of regulation has been proposed by various groups of workers to account for these observations. Johns (1961, 1962) has considered it in relation to the consumption of ryegrass species by grazing sheep.

On theoretical grounds, Manning, Alexander, Krueger & Bogart (1959) have considered it unlikely that the regulatory metabolite is glucose. Short term infusions of glucose have been without effect on food intake (Manning et al. 1959; Dowden & Jacobson, 1960; Holder, 1963; Simkins, Suttie & Baumgardt, 1965b).

The importance of volatile fatty acids (VFA) in ruminant energy metabolism has led many workers to investigate their possible role in food intake regulation. Acetic acid has been suggested as the most likely of these acids (Ulyatt 1964, 1965). Kay & Hobson (1963) stated that "the concentration of VFA's in the rumen increase too slowly after a meal for these to be a likely stimulus to satiation". A similar belief is expressed by Balch & Campling (1962). In contrast, Simkins et al. (1965a) observed that satiation in cows fed for a 6 hr period occurred when blood and rumen concentrations of VFA were at a maximum.

The intraruminal infusion of VFA's into cattle (Rook, Balch, Campling & Fisher, 1963; Montgomery, Schultz & Baumgardt, 1963, Simkins et al. 1965a,b) and sheep (Ulyatt, 1965; Weston, 1966a; Egan, 1966; Baile & Pfander, 1966) have generally caused a depression in food intake. An exception to this generalisation has been reported by Ulyatt (1964, 1965). Intraruminal infusion of 200 kcal of propionic acid on alternate days to sheep significantly enhanced the intake of a low quality roughage during the first hour of feeding and caused small increases in the daily consumption of a concentrate ration and of a medium quality chaff. Infusion of acetic acid depressed food intake. The suggestion was made that acetic acid may be involved in satiation and the propionic acid facilitated its metabolism.

It has not been established whether intraruminal administration is necessary for the effects to occur. The intravenous infusion of sodium acetate, acetic and propionic acids produced highly significant depressions in the intake of lucerne hay by heifers during the 8 hr infusion period (Dowden & Jacobson, 1960). On the other hand, Holder (1963) found that the intravenous infusion of sodium acetate was without effect on the food intake of sheep. The abomasal infusion of 460 kcal/day of propionic acid for eight days depressed the intake of lucerne hay by sheep (Weston, 1966a). An 8 hr intraduodenal infusion of 22 g propionic acid was without effect (Egan & Moir, 1965).

Baile & Pfander (1966) have proposed that "ruminal acetic acid concentration change is perceived by the ruminant and, under some feeding regimens, acts as a regulatory feed intake mechanism" (sic). Their hypothesis is based in part on the observation that the intraruminal injection of 250 ml.of 1.0 M acetic acid twice daily immediately before feeding produced a 60% reduction in food intake and was usually accompanied by a partial inhibition of rumen motility.

Ash (1959) reported that buffered solutions of 0.1–0.2 M acetate, propionate and butyrate of pH 3.6–5.0 placed into emptied rumens of sheep inhibited reticulorumen motility. Since inhibition was produced by VFA vapour with only minor changes in blood composition, it was concluded that the inhibition was due to stimulation of acid-sensitive receptors in the reticulorumen wall. An extra-ruminal site of action is suggested by the similar effects obtained with intravenous injections as sodium salts of the VFA's (Le Bars, Lebrument, Nitescu & Simonnet, 1954) but in this case the effects were probably accompanied by changes in blood composition. Bell (1958) reported changes in the electroencephalogram of goats with the intravenous infusion of VFA's.

A continuation of food intake depression for 1–8 days after cessation of the intraruminal infusion of VFA has been observed by some workers (Holder, 1963; Montgomery et al. 1963; Ulyatt, 1964; Baile & Pfander, 1966; Weston, 1966a). Infusions of VFA for 4–24 hr were found on occasions by Egan (1966) to be without significant effect on food intake on the day of infusion but for up to seven days following, a depression occurred. It was most marked on the first and second days after the infusion.

Although it has often been concluded that chemostatic regulation of food intake by ruminants is possible, unequivocal evidence in support of this contention has yet to be provided. In the case of VFA, it is not certain whether the effects on food intake of infusions of these substances reflect the part they play in the normal regulatory processes, or whether the effects are non-specific. The published results, particularly those where persisting inhibitory effects were observed, would indicate that the apparent "satiation" induced by the VFA infusions is complex in nature. Weston (1966a) has commented that the effects may be related to an adverse effect on microbial digestion, osmotic pressure and cation changes, acid-base relations, heat dissipation limitations and an impairment of the rate of energy utilization. The importance of these and other effects must await the results of further research.

5. Protein and food intake in ruminants.

Food intake of sheep is increased by the addition of protein to low quality roughages (Williams, Nottle, Moir & Underwood, 1953; Blaxter & Wilson, 1963). As the energy content of the diet is increased, additional protein is required in order to maintain high food intake in sheep (Williams et al. 1953) and high intake and performance in cattle (Bond, Everson, Gutierrez & Warwick, 1962). The decline in the intake of roughages sometimes observed when concentrates are added to the diet depends on the type of roughage and the type of supplement (Campling, 1964, 1966; Murdoch, 1964).

When sixteen foods ranging in crude protein content from 2.6-10% were fed to sheep (Elliott & Topps, 1963), dry matter intake was closely related to nitrogen content but not dry matter digestibility. Moir & Harris (1962) observed that with sheep fed a semisynthetic diet in which only the nitrogen content was varied, the dry matter intake and rate of eating were greatly reduced when the nitrogen intake was below 6 g/day.

These effects are generally attributed to changes in the activity of the rumen microorganisms (Blaxter, 1962, p.287). Moir & Harris (1962) found that when a constant nitrogen intake was maintained by addition of casein via the duodenum, the dry matter intake of the sheep so supplemented did not decline as the nitrogen level in the diet was decreased. The suggestion was made that the effects of the duodenal supplementation were not wholly mediated through increased microbial activity. Evidence for this suggestion has been claimed by Egan & Moir (1965), Egan (1965a,b,c) and Egan (1966) working with sheep. An 8 hr intraduodenal infusion of casein resulted in a transitory increase in the intake of a low quality roughage which was not explicable in terms of an increase in the rate of digestion in the rumen (Egan & Moir, 1965). The increased intake was maintained when the infusions were continued for 18 days. Mean retention time of the food in the alimentary tract was not altered but dry matter digestibility and rate of cellulose digestion were depressed (Egan, 1965a). A smaller increase in the intake of wheaten straw supplemented with 3% urea was achieved under similar circumstances without alteration of dry matter digestibility, mean retention time and rate of cellulose digestion. Evidence suggested a greater degree of gut fill with the casein infusion.

Further evidence in support of this conclusion (Egan, 1965b) was that when either urea or casein were administered <u>per os</u> or <u>per duodenum</u>, the increase in dry matter and digestible energy intake was related to an increase in nitrogen retention. An increased rate of passage did not alone account for greater food consumption since at a given rate of passage, the intake was higher when the sheep were in higher nitrogen balance. This suggested a higher level of fill of the alimentary tract with improved nitrogen status.

See Egan on this

One effect of the casein supplements either <u>per os</u> or <u>per duodenum</u> was an improved ability to deal with imposed acetate and propionate loads given by intravenous injection (Egan, 1965c), suggesting a relationship between the ability to utilize energy and food intake. The decrease in food intake resulting from the imposition of an energy load in the form of intraduodenal infusions of casein to sheep receiving a low quality roughage to which casein had already been added or the intraruminal infusions of VFA (Egan, 1966) was interpreted as evidence in favour of this relationship.

It appears certain that casein supplements have an effect on food intake not explicable in terms of an increase in activity of the rumen microorganisms. The explanation is however, uncertain. The relationship to nitrogen status is not clear as a positive nitrogen balance indicates either increased growth or a process of replenishing depleted protein "stores" within the body and may thus be indicative of a changing physiological state. A high positive nitrogen balance may be achieved with widely different nitrogen intakes depending on whether the animal is protein depleted or replete and on the rate of nitrogen metabolism to which the body tissues are at that time adapted (Allison & Bird, 1964; Oser, 1965, p.815).

Caution should be exercised when considering the evidence claimed by Egan (1965c, 1966) in favour of a relationship between food intake, nitrogen status, and ability to utilise energy. The difference in tolerance to acetate and propionate injections may be explicable solely in terms of different intakes of digestible energy (Weston, 1966b), and the effects of intraruminal infusions of VFA may not be explicable in terms of energy per se as has been discussed above. It should also be pointed out that casein supplements per os resulted in higher food intake than did casein per duodenum (Egan, 1965c, 1966).

This review has emphasised the complexity of the manner whereby both ruminants and animals with a simpler form of stomach regulate their food intake. It is clear that a number of mechanisms are involved. It is also clear that the effects of supplements on the food intake of ruminants whether they be fatty acids or protein are unlikely to be explained in terms of any one mechanism. The effects of nitrogenous supplements on the food intake of sheep, the concern of the chapters that follow, proved to be no exception to this generalisation.

CHAPTER 2

SOME EFFECTS OF INTRADUODENAL INFUSIONS OF CASEIN ON FOOD INTAKE

INTRODUCTION

A supply of nitrogen inadequate to satisfy the growth requirements of the rumen microorganisms is a major limiting factor in the utilisation of low quality roughages by ruminants. The increased voluntary intake of these roughages when supplements of urea are added to the diet or directly to the rumen has in general been attributed to changes in the rate of cellulose digestion in the rumen (Clark & Quin, 1951; Blaxter, 1962, p.287; Coombe & Tribe, 1963).

The effects of an improved nitrogen status of the animal sometimes found when supplementation with urea is made (e.g., Coombe & Tribe, 1963) has recently attracted some attention.

Moir & Harris (1962) fed sheep a constant amount of a "semipurified" ration in which only the nitrogen content was varied. Three animals were given intraduodenal infusions of a casein preparation so as to keep the total nitrogen input constant as the dietary nitrogen intake was successively reduced from 12.2-2.2 g/day. Dry matter intake and rate of eating of the three sheep remained constant throughout the range of diets fed. In contrast, the dry matter intake and rate of eating of unsupplemented sheep were greatly reduced when the dietary nitrogen intake decreased below 6 g/day. It was suggested that the observed response in food intake by the supplemented sheep was not entirely due to increased activity of the rumen microorganisms.

In the present experiments, 8hr intraduodenal infusions of a casein preparation were administered to sheep fed a roughage low in nitrogen. A study was made of some factors affecting the change in food intake when the infusions were made each day, and, as in later experiments, at 5-7 day intervals.

During the course of the present study, the results of a similar investigation were reported (Egan & Moir, 1965; Egan, 1965a,b,c, 1966). These have been discussed in Chapter 1 of this thesis.

MATERIALS AND METHODS

Sheep and housing. Romney crossbred ewes, 16-28 months of age were used. Perspex or polypropylene cannulae were inserted in the duodenum about 10cm caudal to the pylorus. Both sub-abdominal (Phillipson, 1952) and rib-bed (McDonald, 1953) location of the cannulae were employed. The animals were housed indoors in individual stands in which they were restrained by head-stocks and a sling. Stainless steel mesh separators placed under the steel grille floors of the stands allowed the separate collection of faeces and urine. Maximum within day temperature variation in the room housing the animals was approximately 20°C.

Diet. The food was chaffed, threshed short rotation ryegrass hay (Table 1).

TABLE 1. Composition of the chaffed ryegrass straw. The variation was due in part, to differences between the batches of straw.

D.M. (%)	Nitrogen content (% dry weight)	D.M. digestibility %
85-88	0.90-1.05	39-55

The hay, obtained in approximately half-ton lots as required, was the seedless straw remaining after harvesting the seed from certified crops and hence almost free from contamination with other plant species. Water and salt lick (crude NaCl with trace amounts of KIO_3, $CoCl_2$ and Fe_2O_3 added; Summit Products Ltd, Palmerston North) were available at all times. The sheep were fed on the ryegrass chaff for a minimum of 21 days before an experiment commenced.

Infusions and apparatus. Initially, the apparatus used for intraduodenal infusions was a gravity feed system as described by Ulyatt (1964). In later experiments, DCL micropumps (F.A. Hughes and Co., Surrey, England) were used. Intravenous infusions were made using a gravity feed system.

The casein preparation, which on occasions was administered into the duodenum, was a water soluble calcium salt of milk protein ("Casilan", Glaxo Laboratories, (N.Z.) Ltd, Palmerston North). The composition of "Casilan", hereafter referred to as casein, as given by the manufacturer was:-

Protein	90.0%
Fat	1.8
Mineral Salts	3.8
Moisture	4.0
Glyceryl mono-oleate	0.4

Nitrogen content as estimated by the Kjeldahl method was 13.9-14.1% dry weight. Solutions of casein were made up the day before they were required by the addition of an appropriate volume of warm (c. 30°C) vehicle, shaken vigorously and allowed to stand overnight. Except where stated, the vehicle was a salt solution as described by Blaxter & Martin (1962), isotonic with duodenal contents and hereafter referred to as "Blaxter's Saline". A concentrated stock solution was stored at 5°C and diluted as required with warm tap water. Except where stated, all infusions were made at a constant rate for an 8hr period commencing with the presentation of fresh feed.

Feeding and Excreta. An amount of weighed food approximately 20% in excess of expected intake was offered at 8.30am or 9.00am. Residues were collected and weighed at the end of a 24hr feeding period. Dry matter (DM) intake for each sheep on each day was obtained by subtracting the DM refused from the DM offered.

Water intake was measured by determining the residue after 24hr when 4l. of fresh tap water had been offered at the time of feeding.

Urine and faeces were collected once daily immediately before the presentation of fresh food. Urine was collected in a polythene bottle containing 10 ml. of 5N H_2SO_4.

Samples of food, food residues and faeces for DM determination were taken daily and dried for 24hr in a forced-draught oven at 90-92°C.

Nitrogen balance estimation. Samples of food and food residues for nitrogen (N) estimation were taken from the oven dried material. For each sheep, a bulk sample of food residues was made by pooling subsamples taken from each day's refusals in proportion to the amount refused. N was estimated on each day's urinary and faecal samples or, when only the mean daily N output was required, on samples bulked as for food residues. Urinary and faecal samples were stored at -5°C. Preparation of faecal samples for analysis was as described by Reis & Schinckel (1961).

Blood sampling. Samples were collected into heparinised syringes from a catheter inserted into the jugular vein. Heparinised syringes containing 1-2 ml. of liquid paraffin B.P. were used when sampling blood for CO_2 estimation. The catheters (Sterivac, Size 2, Allen and Hanburys Ltd, London) were introduced into the jugular vein the day before required and kept filled with heparinised saline (0.9% NaCl) when not in use.

Plasma volume estimation. Estimations were made with Evans Blue (Warner-Chilcott, N.J., U.S.A.; one ampoule containing 5 ml. of 0.5% aqueous solution was made to 35 ml. with sterile 0.9% NaCl).

Immediately before feeding, 10 ml. of the dye was injected into the jugular vein through a catheter and 8 ml. blood samples were taken immediately before dye injection and at 10, 20 and 30 min after injection. Plasma was separated by centrifuging at 12,000 rev/min in a Servall RC2 centrifuge, rotor SS34 (18,500 g) for 15 min. Using a Beckman DU spectrophotometer zeroed against the sample taken immediately before dye injection, the optical density (O.D.) of each sample was compared with a standard made by adding 1.0 ml. of a 1 in 25 dilution of the injected dye to 4.0 ml. of plasma. The O.D. at zero time was found by extrapolation with the aid of semilog graph paper. The plasma volume was then calculated from this value, the O.D. of the standard and the dilution factors.

Packed cell volume. Blood samples were centrifuged for 10 min in a micro-capillary centrifuge (I.E.C., Model MB) and packed cell volume (PCV) determined with the aid of a micro-haematocrit reader (I.E.C.).

Chemical analysis

Nitrogen content of food, food residues, faeces and urine was estimated by a semi-micro Kjeldahl method using a Se-K_2SO_4 catalyst (Humphries, 1956).

Plasma protein concentration was estimated by the biuret reaction as described by Gornall, Bardawill & David (1949). Crystalline bovine serum albumin (Sigma Chemical Coy) was used as a standard.

Protein-free plasma for estimation of amino acid concentration was prepared by diluting plasma (1.0 ml.) with an equal volume of cold (1-5°C) distilled water and slowly adding with constant shaking 2.0 ml. of cold 10% trichloroacetic acid solution. After 10 min, the bulk of the precipitate was centrifuged down in a bench centrifuge and the supernatant filtered (Whatman's No.1).

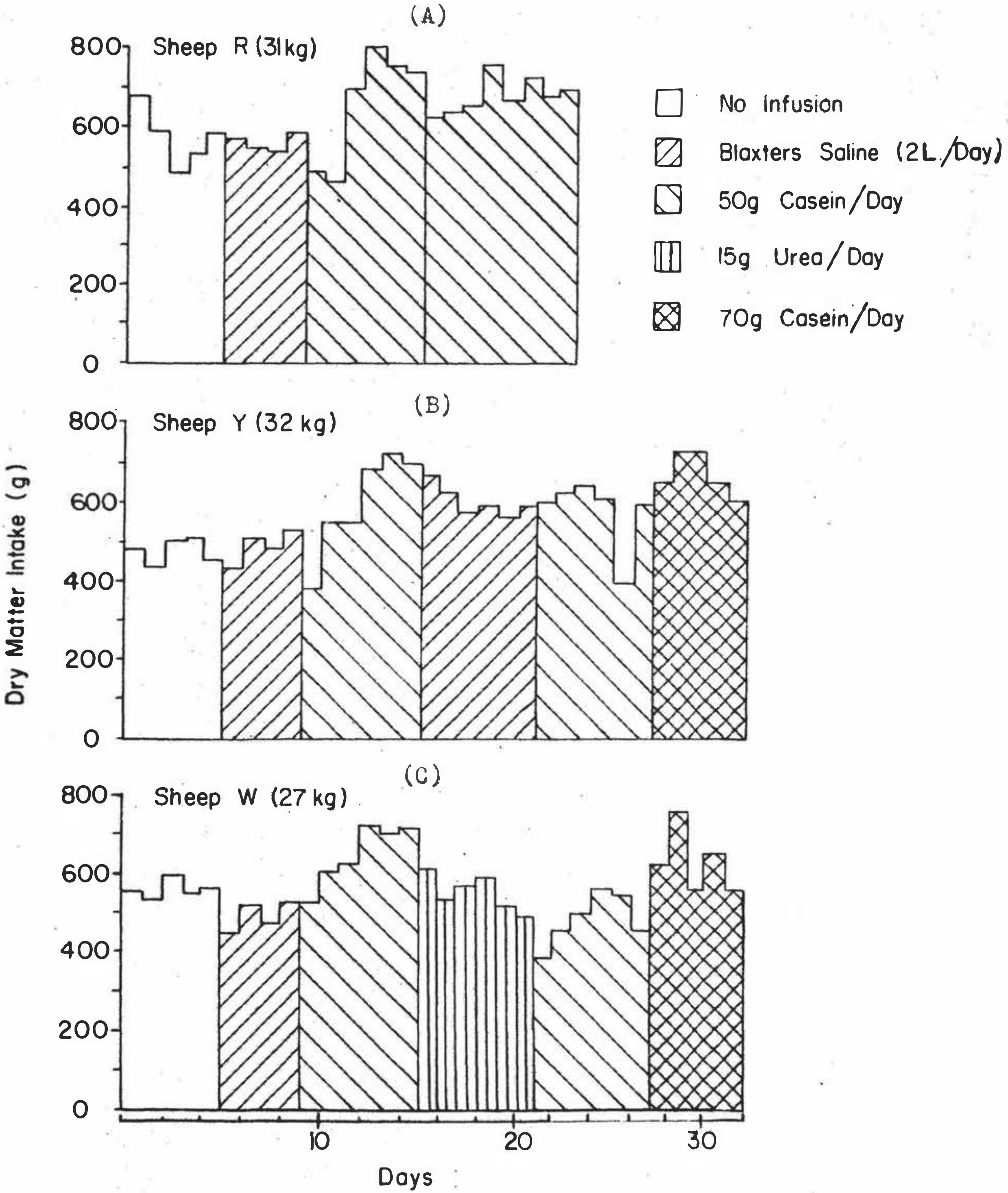

Fig.1. The food intake of the three sheep in Expt. 1. All infusions were into the duodenum and were for 8hr commencing at the time of presentation of fresh food. Blaxter's Saline (2 l./day) was the vehicle used to administer the casein and urea.

Plasma amino acid concentration was measured in suitable aliquots of the filtrate by the method of Rosen (1957) using leucine (Sigma) as a standard.

Blood CO_2 was estimated by the microdiffusion method of Conway (1957). The analysis commenced within 30 min of sampling.

Solute concentration was determined by the depression of freezing point method using an osmometer (Fiske Associates Inc., Connecticut, Model G62). In the case of duodenal contents, osmolality was estimated on the supernatant formed by centrifuging the contents as for plasma preparation.

EXPERIMENTAL AND RESULTS

A. SOME EFFECTS OF DAILY INTRADUODENAL INFUSIONS OF CASEIN.

The initial experiments were concerned with the effects of daily 8hr intraduodenal infusions of casein on voluntary food intake. The variety of responses obtained is illustrated by the following experiments.

Experiment 1. An experiment where the effect on food intake was variable (Fig. 1)

After five days of daily intraduodenal infusions of 2 l. of Blaxter's Saline to three sheep, the addition of casein (50 g/day) to the Saline caused an initial depression of food intake in two of the animals (Fig. 1a, b; Day 10). By the fourth day of infusion of the casein preparation, food intake had increased to approximately 140% of that when Blaxter's Saline alone was infused. Neither replacing the casein with urea (15 g/day, Fig. 1c) nor Blaxter's Saline alone (Fig. 1b) was effective in maintaining the increased level of intake. The effect of the continued infusion of casein was equivocal (Fig. 1a). No increase in intake comparable to that previously obtained occurred when 50 g/day of casein was again added to the Blaxter's Saline (Fig. 1b,c, Days 22-27). Increasing the amount of casein to 70 g/day caused both a transitory (Fig. 1b) and an irregular (Fig. 1c) increase in food intake.

Experiment 2. An experiment where there was little effect on food intake (Table 2)

During the first of two, 15 day periods, daily intraduodenal infusions of 1 l. of Blaxter's Saline were given to the two sheep surviving the previous experiment. During the second period, casein (50 g/day) was added to the Blaxter's Saline. N balance was estimated during the last 6 days of the first period and the last 8 days of the second. The casein preparation had little effect on food intake although it improved N balance and live weight gain (Table 2).

TABLE 2. Effect of daily intraduodenal infusions of casein on intake and apparent digestibility of dry matter, nitrogen balance and liveweight change of two sheep.

Treatment	DM intake (g/day)	DM digestibility %	N intake (g/day)	Faecal N (g/day)	Urinary N (g/day)	N balance (g/day)	Liveweight change (kg/day)
Sheep Y							
1 l.Blaxter's Saline/day	620	55.2	5.87	3.85	2.65	-0.63	-0.091
Casein (50g) in 1 l.Blaxter's Saline/day	657	54.7	12.97	3.68	5.68	+3.62	+0.155
Sheep W							
1 l.Blaxter's Saline/day	449	49.4	4.20	3.10	2.21	-1.11	-0.023
Casein (50g) in 1 l.Blaxter's Saline/day	442	50.3	11.04	3.22	4.57	+3.24	+0.096

Experiment 3. An effect on food intake associated with changes in the casein vehicle (Fig.2)

Three animals received daily intraduodenal infusions of 750 ml. of either Blaxter's Saline, water or 1% sodium phosphate ($Na_2HPO_4.12H_2O$, Egan & Moir, 1965). A mineral mix consisting of NaCl, $CaCO_3$, Ca_2HPO_4, $MgSO_4.7H_2O$, KCl and trace elements in the ratio 40:17:20:12:8:3 (Moir & Harris, 1962) was added to the chaff of the animal which received the phosphate vehicle. The sequence of treatments were as indicated below

Days	Experimental
1 - 5	No infusion
6 -18	Infusion of vehicle (750 ml./day) Sheep 52, water 51, Blaxter's Saline 4, Sodium phosphate
12 -18	First N balance collection period
19 -39	Casein (50g/day) added to each vehicle
23-29	Second N balance collection period
25	Sheep 52 removed from experiment because of leaking cannula

The data for N balance, DM intake, and digestibility for the two N balance collection periods are given in Table 3.

TABLE 3. Nitrogen balance, intake and apparent digestibility of dry matter when different vehicles were used to administer daily intraduodenal infusions of casein to three sheep.

Treatment	DM intake (g/day)	DM digestibility %	N intake (g/day)	Faecal N (g/day)	Urinary N (g/day)	N balance (g/day)
		Sheep No.4				
Na_2HPO_4(750ml./day)	657	50.1	6.80	4.13	2.79	-0.12
Casein (50g/day) in 750ml.Na_2HPO_4	664	50.4	13.53	4.91	5.88	+2.74
		Sheep No.51				
Blaxter's Saline (750ml./day)	639	47.3	6.81	4.22	2.67	-0.08
Casein (50g/day) in 750ml.Blaxter's Saline	669	49.4	13.76	5.08	5.65	+3.03
		Sheep No.52				
Water (750ml./day)	655	47.3	7.18	4.39	3.56	-0.77
Casein (50g/day) in 750ml. water	No data available					

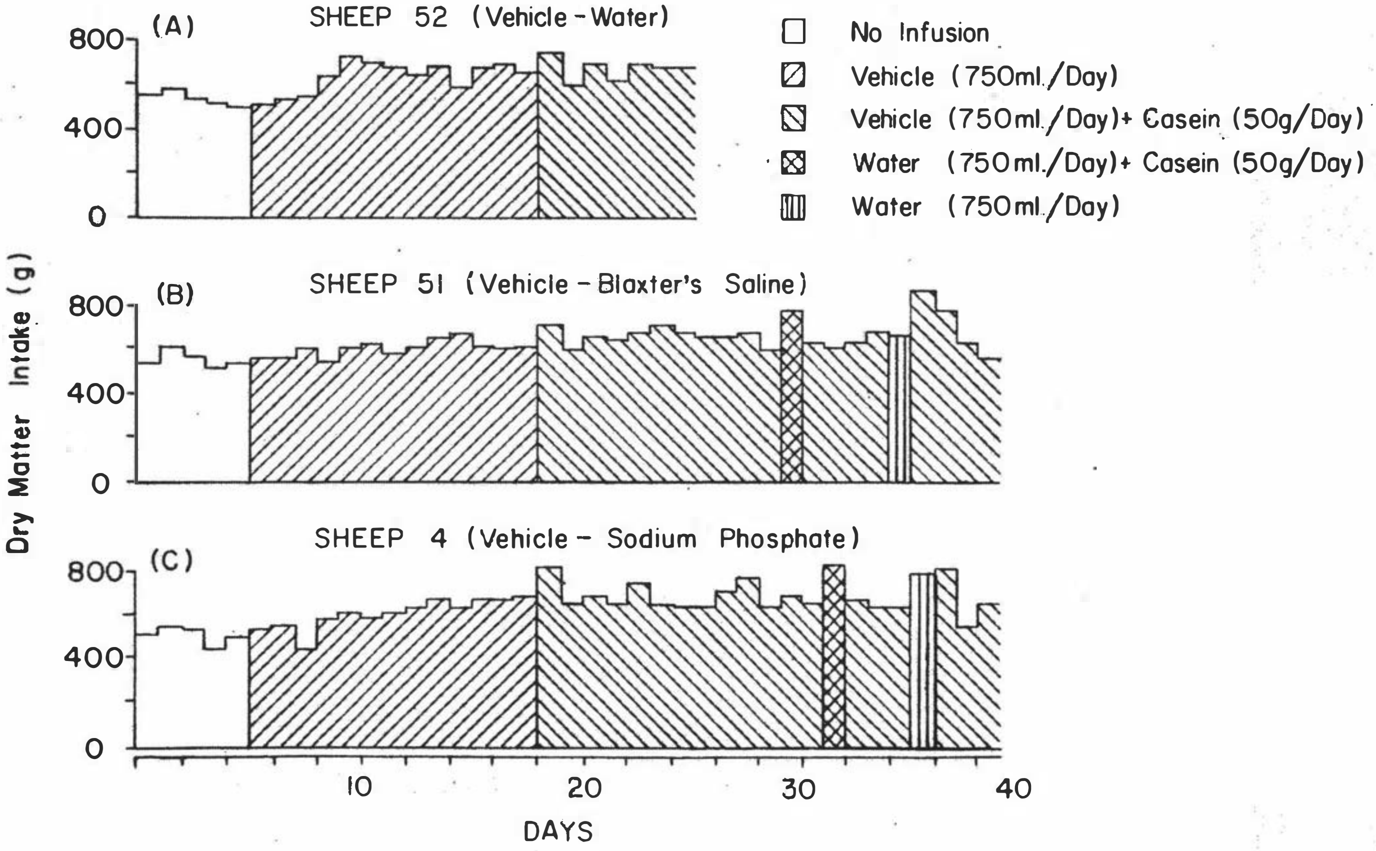

Fig.2. Expt. 3. A comparison of the effects on food intake when casein (50g/day) was infused in to the duodenum in three different vehicles. The vehicles were 750ml. of (a) water, (b) Blaxter's Saline and (c) 1% $Na_2HPO_4.12H_2O$. The infusions were for 8hr commencing at the time of presentation of fresh food. Little effect on food intake was apparent except on the first day that casein was added to the vehicles and in (b) and (c), when the vehicle was changed to water for one day and on the day following the intraduodenal infusion of 750ml. water.

There was no marked difference in DM intake between the two periods (see also Fig. 2) although the infusion of the casein preparation resulted in an improved N balance. Bodyweight measurements made on Days 1, 6, 10, 13 and 17 indicated little change during this time whereas measurements made towards the end of the casein infusion period (Days 27 and 29) indicated a gain as shown below.

Sheep	Liveweight (kg) Before casein (mean of 5 weighings)	During casein (mean of 2 weighings)
4	31.0	32.2
51	31.2	32.5
52	30.5	-
S.E.	± 0.14	-

In contrast to the previous experiment an increase in DM intake by the three sheep was evident on the first day of the addition of casein to the vehicles (Day 19, Fig. 2a,b,c). This was not evident on the first day of vehicle infusion (Day 6). In addition, when the casein had been infused for 11-13 days, a change in the casein vehicle from Blaxter's Saline or Phosphate to water for one day, was accompanied by an increase in DM intake on that day (Day 30, Fig. 2b; Day 32, Fig. 2c). The infusion of water alone had no effect in one animal (Day 35, Fig. 2b) but may have increased the food intake of the other (Day 36, Fig. 2c). In each instance, on the day following the infusion of water alone, a transient increase in DM intake occurred.

Experiment 4. Water metabolism during daily infusions of the casein preparation

On Days 2, 14, 18, 25 and 28 of the previous experiment, plasma volume, PCV and plasma protein concentration was estimated on blood samples taken immediately before the presentation of fresh feed. The results (Table 4) suggest a progressive haemodilution in Sheep 4 and 51;

TABLE 5. Voluntary water intake, faecal and urinary excretion during the intraduodenal infusion of 750ml. of vehicle alone or containing 50g/day of casein. Data from the two nitrogen balance collection periods, each of 7 days.

TREATMENT	SHEEP 51 Vehicle, Blaxter's Saline	SHEEP 4 Vehicle, Sodium phosphate
	Voluntary water intake (ml./day)	
Vehicle	729	1046
Vehicle + casein	1114	1139
S.E. of means	± 114	± 115
	Urine volume (ml./day)	
Vehicle	570	867
Vehicle + casein	733	986
S.E. of means	± 51	± 36
	Faecal dry matter output (g/day)	
Vehicle	337	329
Vehicle + casein	338	335
S.E. of means	± 8	± 9
	Faecal water output (g/day)	
Vehicle	831	783
Vehicle + casein	772	654
S.E. of means	± 29	± 32
	Faecal dry matter content (%)	
Vehicle	28.9	29.7
Vehicle + casein	30.6	34.0
S.E. of means	± 0.5	± 0.8

a similar change was not evident in sheep 52 from the available data which was obtained over a shorter period.

TABLE 4. Plasma volume (PV,ml.) packed cell volume (PCV,%) and plasma protein concentration (PP,g/100ml.) of the three sheep

Day	Nature of infusion	SHEEP 4 (Vehicle, Na_2HPO_4)			SHEEP 51 (Vehicle, Blaxter's Saline)			SHEEP 52 (Vehicle, water)		
		PV	PCV	PP	PV	PCV	PP	PV	PCV	PP
2	None	1400	34	7.68	1290	36	8.16	1480	36	8.85
14	Vehicle	1480	30	6.13	1400	35	7.36	1350	36	7.76
18		1480	28	6.00	1350	34	7.13	1210	36	7.50
25	Vehicle plus casein	1500	28	7.00	1550	31	7.51	No data available		
28		1700	26	6.30	1660	29	7.38			

Further evidence that a change with time had occurred in the water metabolism of sheep 4 and 51 is shown in Table 5. The infusion of casein tended to increase the amount of water drunk, the volume of urine excreted and reduce the daily output of water in the faeces. During the same period, the percentage of DM in the faeces increased with only a slight change in the daily output of faecal DM.

Whether these changes in water metabolism were caused by the infusion of casein or by the length of time the sheep were fed the low quality diet is not revealed by these results. They suggested that unless adequate facilities were available, it would be impossible to interpret the results of further studies of the effects of daily infusions of casein. The effect on food intake of changes in the casein vehicle (Experiment 3) also pointed to the complexity of the situation and provided a strong case for examining the effects of short term infusions. Subsequent work was therefore confined to investigating the effects of single 8hr intraduodenal infusions given at intervals of 5 - 7 days.

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TABLE 6. Effect of casein dose rate on food intake, faecal and urinary excretion of three sheep.

Treatment	Mean of no-infusion control days	Infusion day	Day after that of infusion
	Food intake (g DM/day)		
Blaxter's Saline	497 ± 10	529 ± 23	451 ± 23
50 g casein	527 ± 10	575 ± 23*	489 ± 23
75 g casein	513 ± 13	467 ± 29(*)	520 ± 29
	Voluntary water intake (ml./day)		
Blaxter's Saline	903 ± 49	500 ± 109**	733 ± 109
50 g casein	897 ± 65	883 ± 145	792 ± 145
75 g casein	932 ± 65	858 ± 145	750 ± 145
	Urinary volume (ml./day)		
Blaxter's Saline	227 ± 13	317 ± 29**	277 ± 229
50 g casein	232 ± 14	410 ± 31**	298 ± 31
75 g casein	234 ± 24	568 ± 53**	287 ± 53
	Faecal dry matter (g/day)		
Blaxter's Saline	270 ± 5	266 ± 11	260 ± 11
50 g casein	277 ± 4	270 ± 8	294 ± 8*
75 g casein	273 ± 4	230 ± 9**	267 ± 9
	Faecal water (g/day)		
Blaxter's Saline	510 ± 13	520 ± 30	491 ± 30
50 g casein	525 ± 13	545 ± 29	556 ± 30
75 g casein	488 ± 16	611 ± 36**	506 ± 36
	Faecal nitrogen (g/day)		
Blaxter's Saline	3.43 ± 0.07	3.33 ± 0.17	3.27 ± 0.17
50 g casein	3.54 ± 0.05	4.35 ± 0.12**	3.82 ± 0.12
75 g casein	3.44 ± 0.18	4.38 ± 0.39*	3.80 ± 0.39
	Urinary nitrogen (g/day)		
Blaxter's Saline	1.98 ± 0.12	2.18 ± 0.27	2.01 ± 0.27
50 g casein	1.97 ± 0.09	4.23 ± 0.20**	3.73 ± 0.20
75 g casein	2.00 ± 0.22	4.71 ± 0.50**	3.25 ± 0.50
	Faecal nitrogen concentration (mgN/100g DM)		
Blaxter's Saline	12.61 ± 0.19	12.50 ± 0.43	12.68 ± 0.43
50 g casein	12.54 ± 0.19	16.37 ± 0.42**	13.17 ± 0.42
75 g casein	12.36 ± 0.51	18.75 ± 1.14**	14.39 ± 1.14

* Significantly different from control value
(*) $p < 0.10$, * $p < 0.05$, ** $p < 0.01$

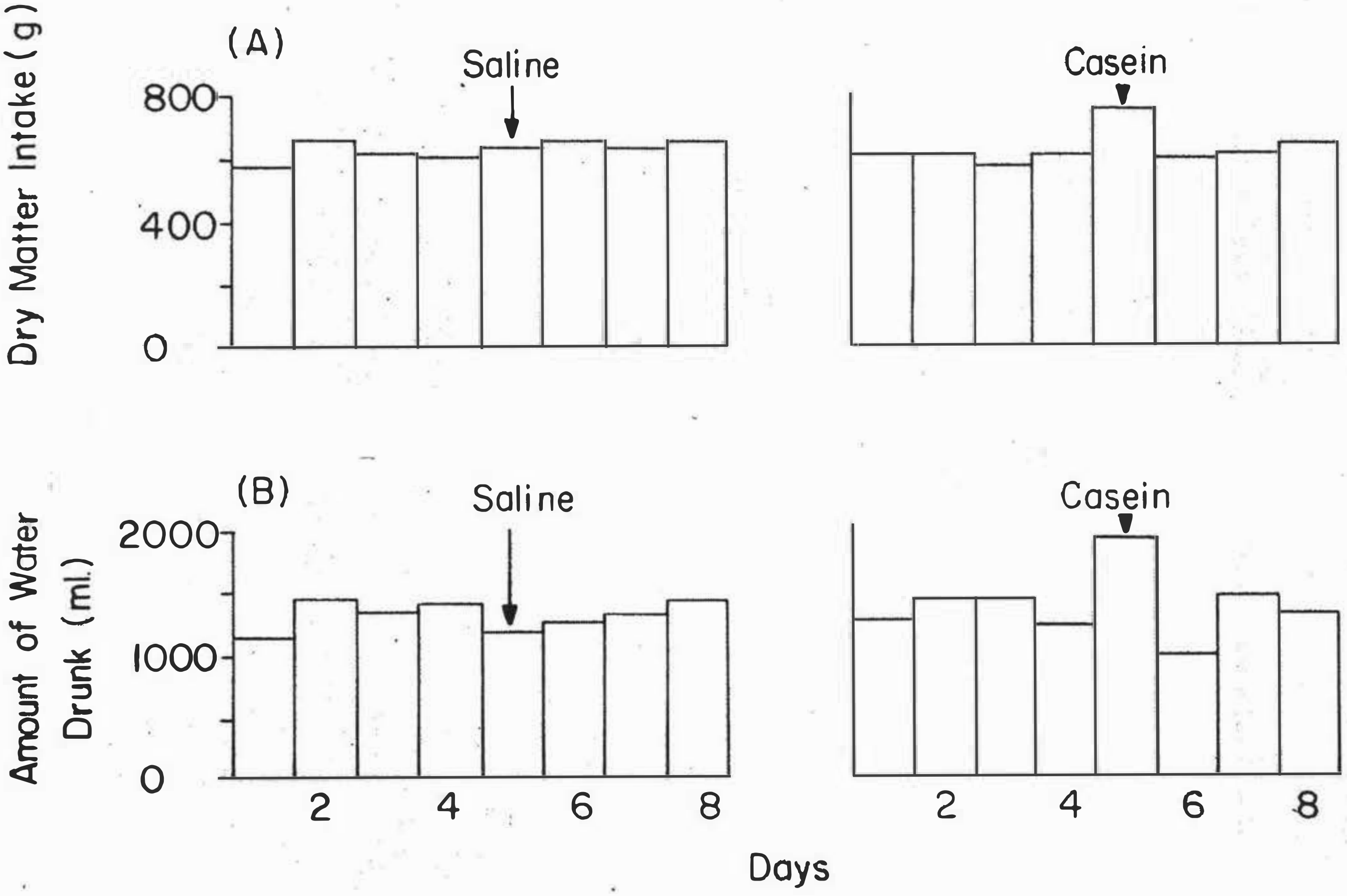

Fig.3. Expt. 5. Effect of intraduodenal infusions of 750ml. Blaxter's Saline alone (Saline) and containing 50g casein (Casein) on (a) food intake and (b) voluntary water intake. Mean of four sheep. Infusions were for 8hr commencing at the time of presentation of fresh food. On the first of two infusion days, two sheep were given Saline and the other two, Casein. On the second infusion day, six days later, the treatments were reversed.

B. SOME EFFECTS OF SINGLE INTRADUODENAL INFUSIONS OF CASEIN

Experiment 5. Intraduodenal infusions of casein over an 8hr period increased food and water intake on the day of infusion more than did a similar infusion of Blaxter's Saline ($p < 0.05$, $p < 0.01$ respectively; Fig. 3). The infusion of Blaxter's Saline was, in fact, accompanied by a mean decrease of 270 ml. in the amount of water drunk (statistically not significant). It was without obvious effect on food intake. No statistically significant effects were detected on the day after infusion although there was a mean decrease of 364 ml. in voluntary water intake on the day after the infusion of casein.

Experiment 6. The effect of dose rate of casein was examined using three sheep of similar liveweight, age and history. The treatments, applied at six day intervals in a 3 x 3 Latin Square arrangement, were intraduodenal infusions of:

1. 750 ml. of Blaxter's Saline,
2. Casein (50g) in 750 ml. of Blaxter's Saline,
3. Casein (75g) in 750 ml. of Blaxter's Saline.

On each infusion day, blood samples for estimation of packed cell volume, CO_2 and plasma amino acid content were taken immediately before the start of infusion and 4, 8 and 24hr later. Nitrogen balance was estimated during the six days preceding the first infusion day.

For each treatment, the data for food and water intake, faecal and urinary excretion on the day of and the day after that of infusion were compared with the same expressed as the mean of 5 days; the 3 days immediately preceding that of infusion and the second and third days following infusion (the "control days"; Table 6).

The treatments differed in their effect on food intake. Compared with the control days, food intake was increased on the day of infusion of 50g casein but was decreased when 75g casein was administered (Table 6).

Voluntary water intake was reduced on the day of infusion of Blaxter's Saline. This was in contrast to the effects of the two casein treatments, neither of which had obvious effects on that day. On the day after infusion, decreases in drinking were recorded with all treatments; they were not statistically significant.

All treatments were accompanied by an increase in urinary volume on the day of infusion; the greatest being recorded when 75g casein was administered.

Faecal dry matter output was reduced on the day of infusion of 75g casein. In contrast, a similar effect was not apparent when 50g casein was administered; instead, an increase was recorded on the day after infusion. Both casein treatments tended to increase the faecal excretion of water on the day of infusion; only that for 75g casein was statistically significant.

On the day of infusion, both casein treatments elicited an increase in the output of nitrogen in the faeces and urine. Faecal nitrogen concentration was also increased. Increases of lesser magnitude were observed on the day after infusion.

Nitrogen balance as estimated on each sheep at the start of the experiment indicated a daily net loss of 0.59, 0.63 and 0.59 g nitrogen.

Apparent water retention (Table 7). Compared with the control days, total water intake was increased on the day of infusion by all three treatments. It was most marked when casein was infused. Although the three treatments were accompanied by an increase in water output on the day of infusion, apparent retention increased on that day. It was particularly striking in the case of the 50g casein treatment, the only one to elicit an increase in food intake. Both total water intake and apparent water retention were reduced on the day after infusion.

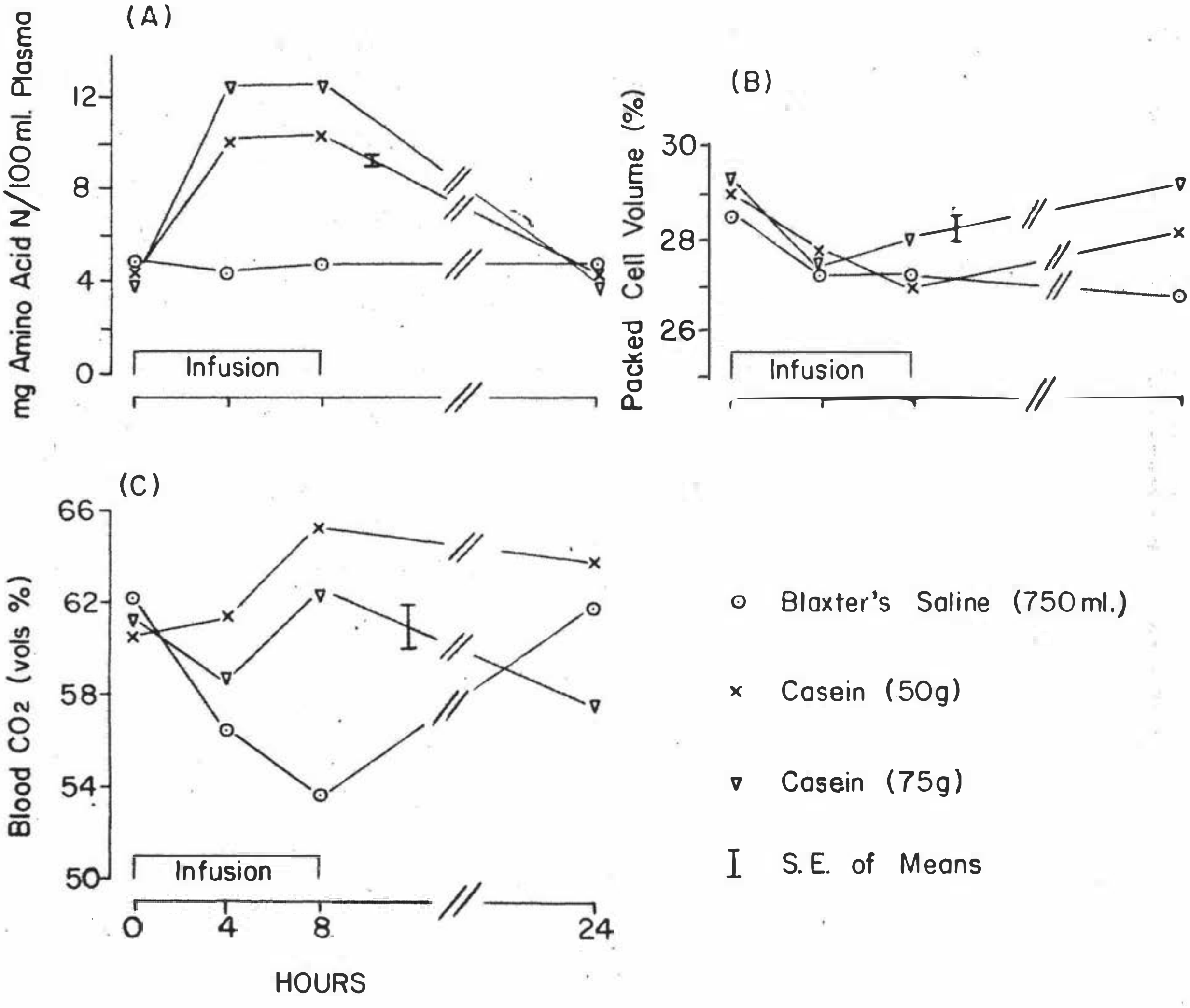

Fig.4. Expt. 6. Effect of intraduodenal infusions of 750ml. Blaxter's Saline containing 0, 50 and 75g casein on (a) plasma amino acid concentration, (b) packed cell volume and (c) blood CO_2 content. Mean of three sheep.

Blood characteristics. Plasma amino acid concentration rose more after the infusion of 75g casein than of 50g casein; no change was detected when Blaxter's Saline was infused (Fig. 4a).

There was no difference between treatments in their effect on packed cell volume (PCV, Fig. 4b). Blood CO_2 content decreased during the day of infusion of Blaxter's Saline to a minimum at the 8hr sampling whereas for the two casein treatments, it tended toward a maximum at that time (Fig. 4c). In contrast to samples taken immediately before the start of infusion (0hr), significant differences existed between treatments in the case of both PCV ($p < 0.01$) and blood CO_2 concentration ($p < 0.05$) at 24hr.

TABLE 7. The effects on apparent water retention of intraduodenal infusions of 0, 50 and 75g casein. Data from Table 6 and are the mean of three sheep.

Experimental period	Total water intake (water drunk, in feed & infused; ml./day)	Water output (faecal + urinary water, ml./day)	Apparent retention
		BLAXTER'S SALINE	
Mean of control days	991	737	+ 254
Infusion day	1343	837	+ 506
Day after infusion	813	768	+ 45
		50g CASEIN	
Mean of control days	990	757	+ 233
Infusion day	1735	955	+ 780
Day after infusion	878	854	+ 24
		75g CASEIN	
Mean of control days	1023	722	+ 301
Infusion day	1690	1179	+ 511
Day after infusion	842	793	+ 49

On completion of the above experiment, two of the sheep were used in an attempt to obtain additional evidence on the effects of casein dose rate on food intake. Intraduodenal infusions of 1, 2 and 4g casein/kg liveweight in 750ml. Blaxter's Saline were made at six day intervals. There were three 18 day periods during each of which, each treatment was administered to each animal. The sequence of treatments in each period was selected randomly.

In one sheep (No. 42, Table 8) the magnitude of the increase in food intake decreased with increasing casein dose rate whereas in the other, the 2 g/kg B.W. treatment elicited a greater increase than did the 1g/kg B.W. treatment.

TABLE 8. Effects on food intake of intraduodenal infusions of 1, 2 and 4g casein/kg body wei ht. Mean of three observations.

Casein dose rate (g/kg B.W.)	Mean intake on the 3 days preceding that of infusion	Intake on day of infusion	Mean intake on the 3 days following infusion.
		(g DM/day)	
		SHEEP 42	
1	638	755	615
2	648	708	600
4	615	632	632
S.E. of means	± 21	± 37	± 21
		SHEEP 56	
1	582	671	588
2	576	695	581
4	598	627	578
S.E. of means	± 11	± 19	± 11

<u>Experiment 7</u>. <u>The effect of an intraduodenal infusion of water immediately before that of casein</u>

It was observed in Experiment 3 that unless changes were made in the nature of the infusion, daily intraduodenal infusions of casein were apparently without obvious effects on food intake. One such change appeared to be an intraduodenal infusion of water; when this

TABLE 9. The effects of preceding 8hr intraduodenal infusions of casein and of Blaxter's Saline by 16hr intraduodenal infusions of water.

Experimental period *	Blaxter's Saline				Casein (2 g/kg B.W.)			
	Sheep No.				Sheep No.			
	75	94	116	117	75	94	116	117
				Food intake (g DM/day)				
No - infusion control	550	565	557	495	555	552	576	524
Day of infusion	+56	+64	+23	-18	+106	+145	+78	+177
Mean of 75, 94 & 116		+48				+110		
				Food intake (g DM/0-8hr)				
No - infusion control	414	472	440	278	507	451	433	256
Day of infusion	+42	-35	-44	-13	+69	+70	+45	-176
Mean of 75, 94 & 116		-12				+61		
				Voluntary water intake (ml./day)				
No - infusion control	1450	1450	1206	906	1410	1188	1412	816
	-410	-550	-306	-906	+144	+412	-212	-816
Mean of 75, 94 & 116		-422				+115		
				Faecal dry matter (g/day)				
No - infusion control	299	326	329	239	328	319	360	263
Day of infusion	+40	+32	+11	+64	-26	+11	-18	-56
Mean of 75, 94 & 116		+28				-11		

* No - infusion control is the mean of the four days preceding the water infusion.

Data for the day of infusion have had the no-infusion controls subtracted from them.

was followed by an intraduodenal infusion of casein, an increase in food intake resulted. In the present experiment, the effects of a preliminary infusion of water on those of an 8hr intraduodenal infusion of casein were examined.

To do this, a simple reversal experiment was carried out with four sheep in which previously an intraduodenal infusion of casein had been found to have a mildly inhibitory effect on food intake (see Experiment 12, Chapter 3). During the period -16 - 0hr, each of the four sheep were given an intraduodenal infusion of 1000 ml. water, then, in the period 0 - 8hr, two of the animals were given an intraduodenal infusion of 750 ml. Blaxter's Saline and the other two, 2g casein/kg B.W. in 750 ml. Blaxter's Saline. The treatments were reversed for the animals when the infusions were repeated 7 days later.

The results for food and voluntary water intake and faecal dry matter output for each animal are presented in Table 9. For three of the sheep, there was a small increase (of the order of 10%) in the 24hr food intake when Blaxter's Saline was infused, and a larger increase (of the order of 20%) when casein was infused. As judged by the amount of food eaten at the end of the 8hr casein infusion, the time course of these effects differed. Faecal dry matter output was increased on the day of infusion of Blaxter's Saline, whereas a decrease accompanied the casein infusion.

The results obtained from the fourth animal contrasted sharply with those obtained from the other three. This animal (No. 117) ate little during the 8hr of casein infusion and did not drink any water on the days of infusion. The effects of the two treatments on faecal dry matter output were more exaggerated than those detected with the other three animals.

It will be recalled that previously the intraduodenal infusion of casein had failed to increase the food intake of these animals. The prior infusion of water - or some factor associated with that procedure - apparently may modify this response.

Experiment 8. The influence of water on the effects of intraduodenal infusions of casein

It was reasoned that if the prior infusion of water could modify the response to casein infusions, the volume of water in which the casein was administered might be an important factor determining the effects of the infusion. An experiment was therefore carried out in which the same quantity of the same solutes was given with different amounts of water. The solutes consisted of casein (2 g/kg B.W.) plus the salts contained in 750 ml. of Blaxter's Saline. These solutes made up in 750 ml. of distilled water were regarded as isotonic with the duodenal contents. A comparison was made of the effects of an infusion of 750 ml. of the "isotonic" casein preparation with (1) the same treatment following an infusion of 1000 ml. of water, and (2) infusions of "hypertonic" and "hypotonic" casein preparations, made by dissolving the solutes in 400 ml. and 1500 ml. of distilled water respectively. In each case, the casein infusion was given over 8hr, starting at the time fresh feed was offered. The osmolalities and rates of infusion of the various preparations are given in Table 10.

TABLE 10. Osmolality, volume and rate of infusion of the casein vehicles and the preliminary infusion of water.

Treatment	Osmolality (mosm/kg H_2O)	Volume (ml.)	Infusion rate (ml./min)
HYPERTONIC (concentrated Blaxter's Saline)	398	400	0.83
ISOTONIC (unmodified Blaxter's Saline)	213	750	1.56
HYPOTONIC (dilute Blaxter's Saline)	106	1500	3.12
WATER PRIMING (As for Istonic with 1000 ml.H_2O infused during the preceding 16hr)	(Water) 0.02	1000	1.04
	(Blaxter's Saline) 213	750	1.56

The four treatments were applied to four sheep in a 4 x 4 Latin Square arrangement with seven day intervals between infusions. It was planned to use eight sheep so that concurrent infusions of vehicle only could be made. Four of these were rejected at the start of the experiment;, three because of leaking duodenal cannulae and one because it persistently voided faeces of low dry matter content (10 - 15%). The treatments were also administered to sheep No.117, the animal which in the previous experiment, reduced food intake when the casein preparation was given. The results for this animal are presented separately.

Blood samples for plasma amino acid concentration estimation were collected 6.5hr after the start of the infusion on the four infusion days.

Samples of duodenal contents (10 ml.) for osmolality estimation were collected from each sheep immediately before the presentation

TABLE 11. Effect on food intake when intraduodenal infusions of casein (2 g/kg B.W.) were either varied in volume and solute concentration or preceded by an intraduodenal infusion of water. Mean of 4 sheep.

Treatment	Mean control	Intake on day of infusion less control intake	Intake on day after infusion less control intake
	(a) Food intake (g DM/0 - 24hr)		
Hypertonic	671	+ 29	- 12
Isotonic	648	+ 48*	+ 61**
Hypotonic	676	+ 98**	- 20
Water priming	670	+ 96**	+ 28
S.E. of means +	± 11	± 19	± 14
	(b) Food intake (g DM/0 - 8hr)		
Hypertonic	539	+ 33	+ 23
Isotonic	529	+ 64*	+ 67**
Hypotonic	554	+ 70*	- 30
Water priming	539	+ 62*	+ 38
S.E. of means +	± 6	± 27	± 18
	(c) Food intake (g DM/8-24hr)		
Hypertonic	134	- 4	- 35*
Isotonic	122	- 16	- 6
Hypotonic	121	+ 28	+ 10
Water priming	129	+ 34	- 9
S.E. of means +	± 8	± 19	± 14

* Significantly different from control intake, p 0.05; ** p 0.01

\+ Required difference for significance between means;

S.E. x 3.2, $p < 0.05$
S.E. x 4.6, $p < 0.01$

of fresh feed and 4hr later on two days prior to the first infusion day. Samples were obtained by allowing the contents to flow out the opened cannulae.

Statistical analysis of results. To reduce the confounding effects of between animal differences and to enable an estimate to be made of the significance of differences between the treated and the untreated state, "no-infusion" controls were calculated for each parameter. These were the means of six days - the fourth, third and second days before infusion and the second, third and fourth days after infusion. Wherever an infusion was made, a separate no-infusion control was calculated for each sheep and each parameter. Analyses were made to ensure that there were no significant differences between the means of the three pre-infusion days and the three post-infusion days. The data for the day of infusion were converted to differences by subtracting from each the appropriate no-infusion control. This was repeated for the day after infusion and, in some cases, the day before the infusion. The converted data for the whole experiment were then subjected to analyses of variance and significant differences between treatment and between treatments and their controls were located by the "t" test. The treatment means and their standard errors are presented for each treatment. In the case of faecal dry matter output, the control was not valid; significant differences occurred between the pre- and post-infusion days. Separate analyses of variance were performed on each day's data.

Food intake on the day of infusion was greater for the hypotonic and water priming treatments than for the hypertonic treatment (Table 11a). The increase recorded for the hypertonic treatment was not statistically significant. The trend towards increased food intake with decreasing vehicle tonicity (or increasing vehicle volume) was significant ($p = 0.024$). The majority of the increase in food intake occurred during the 8hr infusion period (Table 11b); there were however, increases which approached significance during the 16hr following the infusion of the hypotonic and water priming treatments (Table 11c). The isotonic treatment was the only one to elicit a statistically significant increase in intake on the day after infusion (Table 11a).

<u>Voluntary water intake</u> on the day of infusion was decreased by the hypotonic treatment but increased by the hypertonic treatment (Table 12). The trend towards decreased drinking with decreasing vehicle tonicity (or increasing volume) was highly significant ($p = 0.002$).

TABLE 12. Effect on voluntary water intake when intraduodenal infusions of casein (2 g/kg B.W.) were either varied in volume and solute concentration or preceded by an intraduodenal infusion of water. Mean of 4 sheep.

Treatment	Mean control intake	Day before infusion less control	Day of infusion less control	Day after infusion less control
			(ml./day)	
Hypertonic	1819	+ 69	+ 469**	- 106
Isotonic	1796	- 77	+ 136	- 43
Hypotonic	1810	-278	- 535**	- 435*
Water priming	1792	-104	- 298*	- 167
S.E. of means	± 43	±192	± 137	± 184

See Table 11 for requirements for significance

All treatments reduced the amount of water drunk on the day after infusion but only that for the hypotonic treatment was statistically significant. The infusion of water that preceded the casein infusion in the water priming treatment had no obvious effect on voluntary water intake during the day before casein infusion.

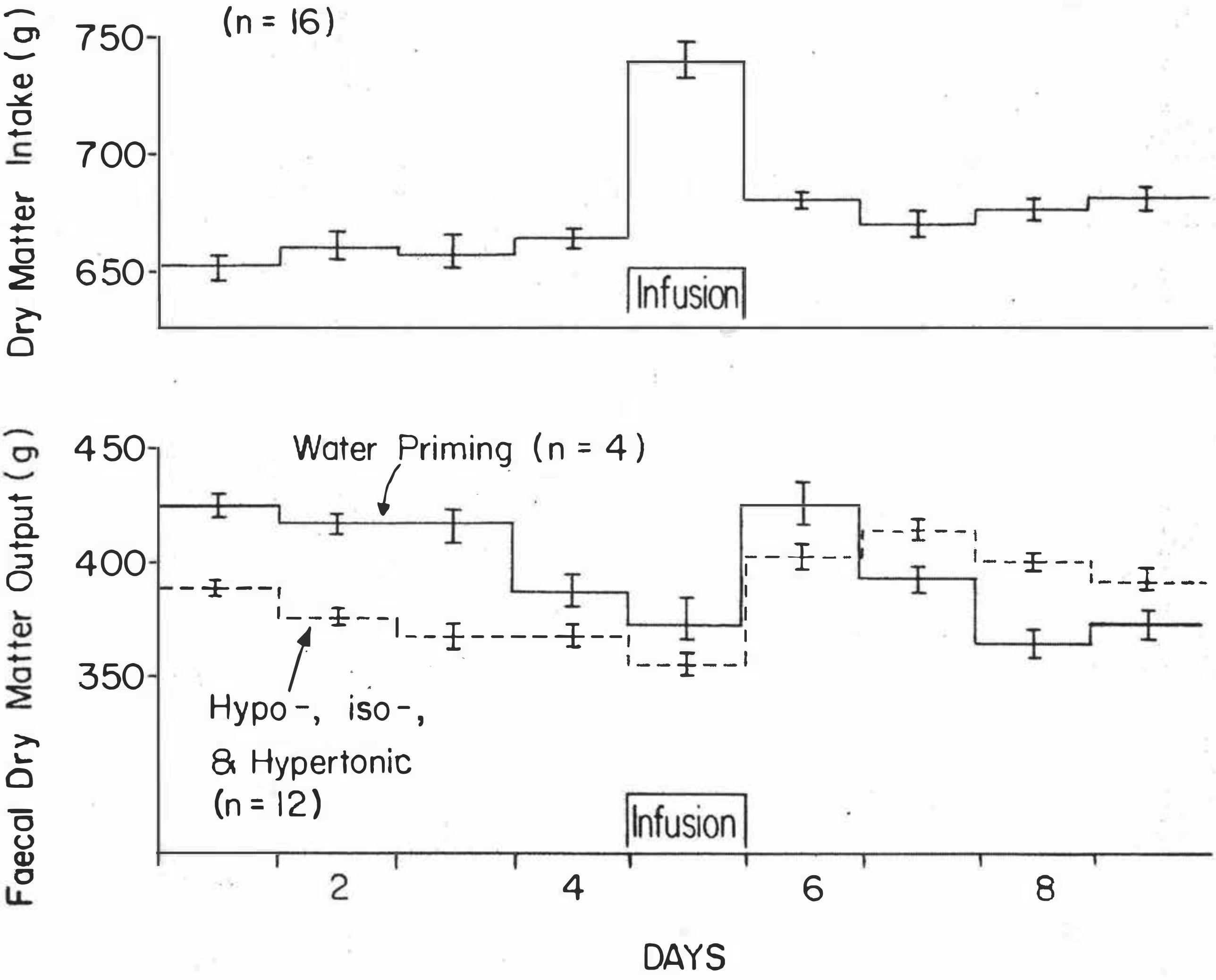

Fig.5. Expt. 8. The mean effects of the treatments on food intake compared with those on faecal dry matter output. The effects of the water priming treatment on faecal dry matter output differed from the mean of the hypo-, iso- and hypertonic treatments and are therefore shown separately. The number of observations making up each mean is indicated by n and the S.E. of each mean by the vertical bars.

Faecal excretion (Table 13). Less faeces were voided on the day of infusion. The consequent reduction of faecal water output was significant (compared with the output during the control days) in the case of the water priming, isotonic and hypotonic treatments (Table 13).

TABLE 13. Excretion of water in the faeces when intraduodenal infusions of casein (2 g/kg B.W.) were either varied in volume and tonicity or preceded by an intraduodenal infusion of water. Mean of 4 sheep.

Treatment	Mean of controls	Day of infusion less control	Day after infusion less control
		(a) Faecal dry matter (%)	
Hypertonic	31.6	- 0.5	- 1.3
Isotonic	29.9	+ 1.1	+ 2.2
Hypotonic	32.1	+ 1.5	+ 1.1
Water priming	30.9	+ 1.8	+ 1.4
S.E. of means	±0.6	± 1.4	± 1.4
		(b) Faecal water output (g/day)	
Hypertonic	844	- 55	+ 56
Isotonic	917	-185**	- 33
Hypotonic	848	-165**	- 44
Water priming	906	-168**	- 10
S.E. of means	±30	± 32	± 57

See Table 11 for requirements for significance

Effects on the output of faecal dry matter were less marked (Fig.5). The hypertonic, isotonic and hypotonic treatments elicited a small decrease in output on the day of infusion; this was followed by an increase during the subsequent two days. The effects of the water priming treatment differed from this pattern in that dry matter output was also reduced on the day before casein infusion; a more transient increase followed infusion.

Urinary volume was increased by all treatments on the day of infusion. The increase observed for the hypertonic treatment was greater than

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TABLE 15. Changes in apparent water retention when intraduodenal infusions of casein (2g/kg B.W.) were either varied in volume and tonicity or preceded by an intraduodenal infusion of water. Data obtained from Tables 11 - 14 and are the mean of four sheep.

Experimental period	Water input (water drunk, in feed & infused)	Water output (faecal plus urinary water) (ml./day)	Apparent retention (input-output)
		Hypertonic treatment	
Mean of controls	1937	1454	+ 483
Infusion day	2811	2025	+ 786
Day after infusion	1829	1472	+ 357
		Isotonic treatment	
Mean of controls	1910	1459	+ 451
Infusion day	2805	1636	+1169
Day after infusion	1878	1309	+ 569
		Hypotonic treatment	
Mean of controls	1929	1427	+ 502
Infusion day	2911	1574	+1337
Day after infusion	1491	1415	+ 76
		Water priming treatment	
Mean of controls	1910	1454	+ 456
Infusion day	2379	1553	+ 826
Day after infusion	1748	1457	+ 291

those of the other three treatments ($p < 0.10$). A marked increase in urine volume was observed on the day before casein infusion when an infusion of water preceded that of casein (water priming treatment).

TABLE 14. Urinary volume when intraduodenal infusions of casein (2 g/kg B.W.) were either varied in volume and tonicity or preceded by an intraduodenal infusion of water. Mean of 4 sheep.

Treatment	Mean control volume	Day before infusion less control	Day of infusion less control (ml./day)	Day after infusion less control
Hypertonic	610	+ 148	+ 626**	- 38
Isotonic	542	+ 54	+ 362*	- 117
Hypotonic	579	0	+ 312*	+ 32
Water priming	548	+ 535**	+ 267*	+ 13
S.E. of means	± 25	± 90	± 123	± 58

See Table 11 for requirements for significance.

Apparent water retention on the day of infusion of the hypertonic, isotonic and hypotonic treatments tended to increase with increasing volume (or decreasing tonicity) of the casein vehicle; it was greatest for the hypotonic treatment, the one that elicited the greatest increase in food intake (Table 15). These differences appeared to arise through changes in water output rather than changes in water input. In contrast, the reduced apparent retention on the day after the infusion of the hypotonic treatment was a reflection of reduced input on that day. Although the water priming treatment had an effect on food intake similar to that of the hypotonic treatment, it differed in its effect on apparent water retention. However, the volume of water infused on the day before that of casein infusion and not accounted for in the urine was 465 ml. (1000 - 535, Table 14). Inclusion of this in the water input for the day of casein infusion results in an apparent retention value which resembles that of the hypotonic treatment.

Nitrogen excretion (Table 16). The treatments did not differ significantly in their effect on urinary nitrogen output or faecal nitrogen concentration. Small increases were observed in urinary nitrogen output on the day after the infusion of the hypotonic and water priming treatments. They were not statistically significant.

TABLE 16. Urinary nitrogen output and faecal nitrogen concentration when intraduodenal infusions of casein were either varied in volume and tonicity or preceded by an intraduodenal infusion of water. Mean of 4 sheep.

Treatment	Mean of controls	Day of infusion less control	Day after infusion less control
	Urinary nitrogen (g/day)		
Hypertonic	2.92	+ 2.43**	- 0.14
Isotonic	2.81	+ 2.00**	- 0.36
Hypotonic	2.97	+ 2.42**	+ 0.45
Water priming	2.86	+ 2.03**	+ 0.41
S.E. of means	± 0.1	± 0.56	± 0.29
	Faecal nitrogen (mgN/g DM)		
Hypertonic	14.13	+ 1.10	
Isotonic	14.34	+ 1.46	
Hypotonic	13.67	+ 1.20	
Water priming	13.77	+ 0.88	
S.E. of means	±0.20	± 0.68	

See Table 11 for requirements for significance.

Plasma amino acid concentration 6.5hr after the start of the hypertonic and isotonic infusions was lower than that observed with the other infusions. The difference was not statistically significant. The data, shown below, are expressed as mg amino acid N/100 ml. plasma.

Treatment	Amino acid concentration
Hypertonic	9.60
Isotonic	9.90
Hypotonic	10.26
Water priming	10.00
S.E. of means	± 0.48

Osmolality of duodenal contents did not differ significantly either between sheep or time of day (Table 17). The overall means for each day were significantly different ($p < 0.01$).

TABLE 17. Osmolality of duodenal contents (mosm/kg H_2O). Mean of duplicate estimations.

Sheep	Day and time of sampling			
	Day 1		Day 2	
	0hr	4hr	0hr	4hr
75	214	222	218	213
87	228	223	218	215
94	225	224	215	208
112	221	218	212	206
117	223	228	214	215
Daily mean (± S.E.)	223 ± 1.2		213 ± 1.2	

The effects of the treatments on faecal dry matter output, food and voluntary water intake of sheep 117 are given below. The data are expressed as that for the day of infusion less the mean of the six control days.

Treatment	Food intake (g DM)			Voluntary Water intake (ml.)	Faecal DM output (g)
	0-24hr	0-8hr	8-24hr		
Mean of control days	578	314	264	998	321
Hypertonic	+ 29	+ 82	- 52	+ 967	- 29
Isotonic	+ 92	+209	-117	+ 154	- 22
Hypotonic	+122	+173	- 51	- 167	- 57
Water priming	+125	+ 68	+ 57	- 546	- 55

In contrast to the results of Experiment 7, the effects of casein infusions were comparable to those observed with the other four sheep.

DISCUSSION

A characteristic feature of the present experiments was the variable effect of the daily intraduodenal infusion of casein on food intake. This contrasts sharply with the results of Egan (1965a). Using an unspecified casein preparation and a diet of low quality similar to that used here, he apparently found no difficulty in either maintaining or repeating an increase in food intake during prolonged periods of daily infusions of casein: at least there is no reference in his published papers (Egan, 1965a, b, c) to such difficulty.

The source of variability did not appear attributable to poor retention of the infused N. Even though the daily casein infusions resulted in an improved N status, an increase in intake of the order subsequently elicited by single infusions was not observed (Experiment 2 and 3). A positive relationship between N status (g N retained/kg B.W.$^{0.73}$/day) and energy intake (Egan, 1965b) evidently did not apply in the experiments discussed here.

The increases in food intake observed on the first day of casein infusion or when the vehicle which contained the casein was changed from either sodium phosphate or Blaxter's Saline to water for one day (Experiment 3), suggests that the diet was not the primary cause of the lack of response. They do suggest that the increase in food intake may be dependent on short term changes occurring within the animal, induced by changes in the nature of the intraduodenal infusion.

Single infusions of casein resulted in more consistent increases in food intake. The response was modified by several factors:-

(a) Dose rate. The tendency for a decreased response with the infusion of increasing amounts of casein suggests the likelihood of an inhibitory effect at the higher dose rates. The results suggest at least three possible causes:-

(i) A change in the osmotic conditions normally prevailing in the lower gut, brought about by a failure of the sheep to absorb completely the casein infused at the higher dose rates. Support for this is indicated by changes in faecal water, N output and concentration on the day of infusion (Table 6).

(ii) Some degree of inhibition of gut motility: there was a decrease in faecal DM output on the day of infusion (Tables 6, 9, Fig.5) even when an increase in food intake occurred. Alternatively it is possible that this may have resulted from a temporary increase in the digestive capacity of the lower gut.

(iii) Changes in blood composition: In Experiment 6, the levels of blood CO_2 and PCV recorded 24hr after the start of infusion were different from those observed at 0hr, indicative perhaps, of a substantial load imposed by the casein infusions on the homeostatic mechanisms.

A positive linear relationship between dose rate and food intake observed by Egan (personal communication, see also Egan, 1965b) was not obtained in the present experiments.

(b) Nature of the infusion. The magnitude of the increase in food intake depended on the nature of the vehicle in which the casein was contained. The design of Experiment 8 does not permit a distinction to be made between the effects of tonicity and volume of vehicle, the vehicle itself and the interaction between casein and vehicle. It appears more certain that a preliminary infusion of water facilitated an increase in food intake during and shortly after the subsequent infusion of casein. The results of Experiment 7 suggest that the increase depended on the casein component of the infusion and not the vehicle. A comparison of the effects of the isotonic and water priming treatments in Experiment 8 indicate that the water infusion did in fact, enhance the effects of the isotonic treatment.

(c) State of the animal. The performance of sheep 117 in Experiment 7 and 8 suggests that conditions prevailing within the animal is of importance in determining the effects of casein on food intake and that this may change quite rapidly. The increases in intake for the other three animals when the casein was infused (Experiment 7) rather than a decrease as previously observed may also be partly attributable to a change of conditions within the sheep as well as the effects of the preliminary infusion of water. The nature of the changes involved are unknown.

The present experiments do not provide unequivocal evidence as to the mechanism whereby increased food intake was elicited by intraduodenal infusions of casein. There are however, at least three observations which seem to be of some moment.

(1) Site of action. When single infusions of casein were made, the increase in food intake occurred mainly during the 8hr infusion period Tables 9 and 11). The decrease in intake by sheep 117 (Experiment 7) occurred entirely during the period of infusion. It would seem that in contrast to the conclusion of Egan & Moir (1965), the possibility of casein acting directly within the duodenum to influence food intake cannot be discounted.

(2) Plasma amino acids. There was no clear relationship between plasma amino acid concentration and food intake. In Experiment 6, the infusion of 50g casein resulted in a greater increase of food intake and a smaller elevation of plasma amino acid concentration than did 75g casein. In contrast, those treatments in Experiment 8 which elicited the greatest increase of food intake, also increased plasma amino acid levels the most. It is to be remembered however, that no statistically significant differences were observed between these treatments in their effect on plasma amino acid levels.

(3) <u>Water metabolism</u>. Increased voluntary water intake, reduced faecal water output and possibly a haemodilution, suggests that daily intraduodenal infusions of casein had an effect on water metabolism. Single infusions had a marked effect on the amount of water drunk, the route of water excretion and apparent water retention. The increased requirement for water may be partly attributable to increased urinary N excretion and food intake, but the effects on food intake of an infusion of water prior to the infusion of casein (Experiment 7 and 8), suggest an intimate relationship between water metabolism and increased food intake.

It is evident that the effects of the casein infusions on food intake are complex: the net effect appears to be the result of several interacting factors. Further consideration is given to their nature and importance in subsequent chapters of this thesis.

CHAPTER 3

OBSERVATIONS ON THE NATURE AND LOCATION OF THE MECHANISMS INVOLVED IN THE EFFECT OF PROTEINACEOUS INFUSIONS ON FOOD INTAKE

INTRODUCTION

The experiments described in the previous chapter established that single 8hr intraduodenal infusions of casein may result in an alteration of food intake. The effects were variable. On some occasions, an increase in food intake occurred; on others, obvious effects were absent, whereas on others again, an apparent inhibition of intake was recorded.

Identification of the site or sites of action of casein is likely to provide an important clue as to the mechanisms involved in bringing about these effects.

In studies with sheep fed a low quality roughage, Egan and Moir concluded that the increase in food intake observed when casein was administered either orally (Egan, 1965b) or into the duodenum (Egan & Moir, 1965; Egan, 1965a) was not due solely to increased activity of the rumen microorganisms. In the present work, the same conclusion is suggested by the observations that the magnitude of the increase in food intake could be modified by changes in the nature of vehicle in which the casein was administered and by intraduodenal infusions of water immediately prior to that of casein.

It might be logical to propose that the increase in food intake results from the presence of casein or its degradation products either in the gut caudal to the stomach or in the body tissues, respectively before or after absorption. Some indication as to the site and/or mechanisms involved may therefore be given by the temporal relationships between infusion and effects. In the experiments reported in Chapter 2, when an increase in food intake occurred, it appeared to take place mainly during the period of casein infusion. In contrast, in their experiments with casein infusions, Egan & Moir (1965) found the increase in food intake to occur not during the period of infusion itself but in the succeeding 40hr. Clearly, a more precise definition of the temporal relationships under the present experimental conditions is required.

Additional information as to the sites and mechanisms of action may be obtained by examining the effects of different amino acid-containing materials.

In the first instance, these may be administered to different regions of the lower gut - the duodenum, the jejunum, the ileum or even the most cranial regions of the hind gut. In the present investigation, emphasis has been directed towards the intraduodenal infusion of protein material at different stages of hydrolysis - protein, a peptide-containing hydrolysate and a mixture of amino acids.

In the second instance, a comparison of the effects elicited by administering similar amino acid-containing preparations into the duodenum and into the jugular vein may be a useful analytical procedure. Infusions into the jugular vein may represent an artificial situation only achieved under experimental conditions because in it, the material is presented directly into the general circulation. This contrasts with the normal events of absorption where the majority of protein digestion products pass directly to the liver by way of the portal vein. Jugular vein infusions may therefore emphasize mechanisms other than those associated with the gut, the portal vein or the liver.

The four experiments reported in this chapter are:

(a) Expt 9. An examination of the effects of intraduodenal infusions of casein on the circadian pattern of food and water intake.

(b) Expt 10. A comparison of the effects of intraduodenal infusions of casein and casein hydrolysate.

(c) Expt 11. An examination of the effects of intraduodenal infusions of an amino acid mixture.

(d) Expt 12. A comparison of the effects of intraduodenal infusions of casein and intravenous infusions of casein hydrolysate.

The results of these experiments suggest that several mechanisms are likely to be involved in the effects on food intake of infusions of proteinaceous materials. In particular they point to the importance of water and electrolyte metabolism.

MATERIALS AND METHODS

Experimental design

Experiment 9. Three treatments were applied to three sheep (28 - 35kg B.W.) in a 3 x 3 Latin Square arrangement. The treatments were applied four times at seven day intervals and involved the following intraduodenal infusions:

Treatment A.	50g casein in 500ml. Blaxter's Saline infused over a 6hr period commencing at the time of presentation of fresh food (i.e. 0 - 6hr infusion).
Treatment B.	As in A but infusion 6hr later (i.e. 6 - 12hr infusion).
Treatment C.	As in A but given in equal parts during the periods 3 - 6 and 9 - 12hr. In addition, water (250ml.) was infused during the period 0 - 3hr and again at 6 - 9hr.

Experiment 10. One sheep was given an intraduodenal infusion of casein and another, an intraduodenal infusion of casein hydrolysate. When repeated eight days later, the treatments were reversed between the two sheep.

Experiment 11. Intraduodenal infusions of a solution containing amino acids and, as a control, a salt solution, were given to four sheep (25 - 31kg). They were given at five day intervals in the order salt solution, amino acids and were repeated so that each sheep received each treatment twice. Two sheep received the infusions one day later than did the other two.

Experiment 12. Four treatments, applied to four sheep at seven day intervals in a 4 x 4 Latin Square arrangement were:

A. Intravenous infusions of

(1) 0.9% NaCl

(2) Casein hydrolysate, and

B. Intraduodenal infusions of

(3) Blaxter's Saline

(4) Casein in Blaxter's Saline.

TABLE 18. Composition and details of infusion of the solutions used in Experiments 9, 10, 11 and 12.

Treatment	Expt	Route of infusion	Solutes	pH	Nitrogen content (mgN/100ml.)	Volume infused (ml.)	Infusion rate (ml./min.)	Nitrogen dose rate (gN/kg B.W.)
Casein	9	i.d.	As for Blaxter's Saline plus casein- 10g/100ml. in Expt 9; 2g/kg B.W. in Expt 10 & 11.	5.9	1400	500	1.39	0.20-0.25
	10 & 12	i.d.			890-1050	750	1.56	0.28
Casein * hydrolysate	10	i.d.	%w/v Ash 0.15 Na$^+$ 0.03 Cl' 0.01 Amino acids 60g/l.	5.5	820	890-1000	1.85-2.08	0.28
	12	i.v.						
Amino acid mixture *	11	i.d.	As for Salt Solution (see below) plus - Cl' 25m-equiv/l. Amino acids 33.3g/l.	5.4	530	1500	3.13	0.26-0.32
Blaxter's Saline	12	i.d.	m-equiv/l. Na$^+$ 105.5 K$^+$ 5.4 Ca^{++} 3.6 Mg^{++} 4.2 Cl' 115.8 PO_4''' 8.7	5.0	-	750	1.56	-
Isotonic saline *	12	i.v.	m-equiv/l Na$^+$ 154.0 Cl' 154.0	-	-	890-1000	1.85-2.08	-
Salt Solution	11	i.d.	m-equiv/l. Na$^+$ 11.7 K$^+$ 8.3 Mg^{++} 1.7 Cl' 10.0 CH_3COO' 11.7 Sorbitol 33.3g/l.	5.5	-	1500	3.13	-

* Solute composition as given by the manufacturers.

Solutions and their infusion. Except in Experiment 9, all infusions were for an 8hr period commencing at the time fresh food was presented (9.00am). Intravenous infusions were made into the jugular vein using a gravity feed system. Details of the infusion solutions, their volume and rates of infusion are given in Table 18.

Casein, its nature and preparation for infusion were as described previously (Methods, Chapter 2). This was infused into the duodenum in Experiments 9, 10 and 12.

Casein hydrolysate was a sterile 6% solution of amino acids and peptides derived from casein by acid hydrolysis, fortified with dl methionine, dl tryptophan and glycine ("Parenamine", F.Stearns and Coy, Sydney). This was used as an intraduodenal infusion in Experiment 10 and as an intravenous infusion in Experiment 12.

Amino acid mixture was prepared from "Aminofusin forte" (J.Pfrimmer and Co., Erlangen, W.Germany); a sterile solution containing 100 g/l. of crystalline amino acids in proportions claimed by the manufacturer to be optimal for human parenteral nutrition. Immediately prior to the start of an infusion, 500ml. "Aminofusin forte" were diluted to 1500ml. with distilled water. The resulting solution, which was infused into the duodenum in Experiment 11, is referred to as the "amino acid mixture".

Blaxter's Saline, as described previously (Methods, Chapter 2), served as the vehicle for intraduodenal casein infusions (Experiments 9, 10 and 12) and as a control infusion in Experiment 12.

Isotonic NaCl (Sodium chloride injection, U.S.P.; Abbot Laboratories N.Z. Ltd, Wellington) served as a control for the intravenous infusion of casein hydrolysate (Experiment 12).

Salt solution. Inorganic salts and sorbitol contained in 500ml. of "Aminofusin forte" were made to 1500 ml. with distilled water and the pH adjusted to 5.5 with N/1 HCl. The resulting solution, used in Experiment 11 as a control for the intraduodenal infusion of the amino acid mixture, is referred to as the "Salt Solution".

Rate of dry matter and voluntary water intake were measured in Experiment 9. Measurements commenced seven days prior to the first infusion day and ended on the day after the last infusion; a total of thirty days. On those days intake was measured at 3hr intervals commencing at the presentation of fresh food (0hr) until 12hr later and then again at 24hr. The DM content of the food offered at 0hr and that of the residue remaining at 24hr was estimated by oven-drying samples as described previously in Chapter 2. DM content of the food residues remaining at other times was calculated by assuming that the decrease in DM content was in direct proportion to the wet weight of food eaten. 4 l. of water was offered at 0hr and drinking was measured by determining the residue each time the food was weighed.

Blood samples were collected in the manner described in Chapter 2, immediately before the start of an infusion and at 4, 8, 11 and 24hr later (Experiments 10 and 12). Where intravenous infusions were made, both jugular veins were catheterised, one catheter being used for blood sampling and the other for infusion.

Jaw movements were recorded on some occasions in Experiments 11 and 12. Records were obtained with a balloon - tambour system, kymographically. The sheep were accustomed to wearing the leather halter by which the balloon was kept lightly pressed against the lower jaw.

TABLE 19. Food intake (gDM/day) on the day of infusion of 50g casein in 500ml. Blaxter's Saline. Mean of 4 observations on each of 3 sheep.

Treatment	Mean control intake	Intake on day of infusion	Intake on day of infusion less control intake
A (Casein infusion 0 - 6hr)	713	820	+ 107 **
B (Casein infusion 6 - 12hr)	735	874	+ 139 **
C (Casein infusion 3-6 and 9-12hr; water infusion 0-3 and 6-9hr)	746	837	+ 91 *
S.E. of means +	± 8	± 25	± 27
Overall mean ± S.E.	731 ± 5	844 ± 15	+ 113 ± 16 **

\+ Required difference for significance between means:

S.E. x 3.3 $p < 0.05$
S.E. x 5.6 $p < 0.01$

* Significantly different from the control, $p < 0.05$; **$p < 0.01$

Statistical analysis of the results of Experiments 9, 11 and 12 was essentially as described for Experiment 8. In Experiment 9 the form of the analysis of variance was as for a Youden Square in which one block has an extra replication (Cochran & Cox, 1957). On some occasions, when no significant differences were detected between treatment means, an overall mean for all treatments and its standard error were calculated.

The "no-infusion" controls for each experiment were as follows:

Experiment	No-infusion control
9	The five days immediately preceding that of infusion
10	The four days immediately preceding that of infusion
11	The two days immediately preceding and the 3rd and 4th days following that of infusion
12	The three days immediately preceding and the 3rd and 4th days following that of infusion

Other materials and methods used were as described in Chapter 2.

RESULTS

In all experiments, a relationship appeared between the infusion of amino acid containing materials and the intake of food and water. The results of the experiments are considered separately.

Experiment 9. Effects of intraduodenal infusions of casein on the circadian pattern of food and water intake

All three procedures led to an increase of food intake on the day of infusion (Treatments A and B, $p < 0.01$; C, $p < 0.05$; Table 19). The differences between treatments were not statistically significant.

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TABLE 20. Relationship between the time of casein infusion and the increase in food intake on the day of infusion. Individual observations on three sheep.

	Intake during the hours corresponding to those of infusion (g DM)		Intake during the remainder of the day (g DM)	
	Mean of 4 controls, each of 5 days (± S.E.)	Day of infusion less mean control	Mean of 4 controls, each of 5 days (± S.E.)	Day of infusion less mean control
	Treatment A (casein infusion, 0-6hr)			
4	437	+ 122	265	+ 18
24	401	- 54	235	+ 137
51 (a)*	623	+ 94	232	- 9
(b)	623	+ 154	232	- 3
	(± 25)		(± 30)	
	Treatment B (casein infusion, 6 - 12hr)			
4	212	+ 52	491	+ 91
24 (a)*	212	+ 92	424	- 36
(b)	212	+ 23	424	+ 68
51	209	+ 168	623	+ 43
	(± 14)		(± 22)	
	Treatment C (casein infusion, 3 - 6 and 9 - 12hr; water infusion 0 - 3 and 6 - 9hr)			
4 (a)*	223	+ 226	479	+ 19
(b)	223	+ 48	479	+ 43
24	230	+ 14	406	+ 3
51	282	+ 137	572	- 4
	(± 13)		(± 20)	

* Indicates that two observations were made on the same animal.

There was considerable variation between sheep in the time of occurrence of the increase in food intake (Table 20). In only one sheep (No.51) did the increase occur every time during the hours of infusion. The other sheep showed increases during the hours of infusion on one occasion for No.24 and on two occasions for No.4. The magnitude of the increase in food intake during the hours of infusion did not reflect the amount of food normally eaten at that time.

On three of the four occasions when casein was administered 6 - 12hr after the presentation of fresh food (Treatment B), an increase was observed during the period 0 - 6hr (Table 21). There appeared to be no appreciable increase of food intake in the post infusion period.

TABLE 21. Partition of the increase in food intake between the infusion and non-infusion periods when casein was infused during the period 6-12hr after the start of feeding (Treatment B). Individual observations on three sheep. Note the repeat observations for Sheep 24.

Time of day (hr)	Intake on day of infusion less the mean intake during the same period on the five control days (g DM)			
	Sheep 4	Sheep 24		Sheep 51
0 - 6	+ 78	- 34	+ 71	+ 60
6 - 12 (infusion period)	+ 52	+ 92	+ 23	+ 168
12 - 24	+ 21	- 2	- 3	- 17

<u>Voluntary water intake on the day of infusion</u> differed for each treatment (Table 22). A marked increase in water intake was evident when casein was infused during the period 6 - 12hr (Treatment B). In contrast, a reduction in drinking ($p < 0.10$) was recorded when two casein infusions were preceded by an infusion of water as in Treatment C.

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TABLE 23. Relationship between the time of casein infusion and the increase in voluntary water intake on the day of infusion. Individual observations on three sheep.

	Water drunk during the hours corresponding to those of infusion (ml.)		Water drunk during the remainder of the day (ml.)	
	Mean of 4 controls, each of 5 days(±S.E.)	Day of Infusion less mean control	Mean of 4 controls, each of 5 days(±S.E.)	Day of infusion less mean control
	Treatment A (casein infusion, 0 - 6hr)			
4	1538	+ 455	559	- 610
24	1061	- 60	396	+ 230
51(a)	1805	+ 255	225	+ 130
(b)	1805	+ 640	225	- 280
	(± 90)		(± 128)	
	Treatment B (casein infusion, 6 - 12hr)			
4	260	+ 765	1836	+ 125
24(a)	229	+ 310	1229	+ 60
(b)	229	+ 230	1229	+ 70
51	100	+ 400	1930	- 130
	(± 60)		(± 69)	
	Treatment C (casein infusion, 3-6 and 9-12hr; water infusion, 0-3 and 6-9hr)			
4(a)	470	+ 590	1626	- 330
(b)	470	+ 550	1626	- 585
24	465	+ 340	992	-1085
51	390	+ 615	1640	- 330
	(± 81)		(± 82)	

TABLE 22. Voluntary water intake (ml./day) on the day of infusion of 50g casein in 500ml. of Blaxter's Saline. Mean of four observations on three sheep.

Treatment	Mean control intake	Intake on day of infusion	Intake on day of infusion less control intake
A (Casein infusion 0 - 6hr)	1899	2067	+ 168 *
B (Casein infusion 6 - 12hr)	1789	2310	+ 521 **
C (Casein infusion 3-6 and 9-12hr; water infusion 0-3 and 6-9hr)	1896	1804	- 92
S.E. of means	± 108	± 83	± 44

See Table 19 for requirements for significance

With one exception (Sheep 24, Treatment A), the increased drinking indicated in Table 22 occurred mainly during the hours of casein infusion (Table 23). It was evident even though 500ml.water were contained in the casein vehicle. In Treatment C also, although there was a reduction of water intake over the whole day, consistent increases in drinking were elicited during the actual infusion period.

Food and voluntary water intake on the day after infusion were less than during the control days (Table 24). The decreases, recorded for all treatments were not statistically significant.

TABLE 24. Food and voluntary water intake for the 24hr of the day after infusion. Mean of 4 observations on three sheep.

Treatment (Time of casein infusion in parenthesis)	Intake on the day after infusion	Intake on day after infusion less mean intake during control days
	Food intake (gDM/day)	
A (0-6hr)	707	-5
B (6-12hr)	729	-7
C (3-6 & 9-12hr)	722	-24
S.E. of means	± 15	± 16
Overall mean ± S.E.	718 ± 8	-12 ± 9
	Voluntary water intake (ml./day)	
A (0-6hr)	1709	-193
B (6-12hr)	1686	-103
C (3-6 & 9-12hr)	1462	-434
S.E. of means	± 192	± 238
Overall mean ± S.E.	1619 ± 111	-243 ± 137

During the first 3hr of the day after infusion however, less dry matter was eaten ($p < 0.05$) and less water was drunk ($p < 0.01$) than in the corresponding times during the control days (Table 25). There were no significant differences between treatments.

TABLE 25. Food and voluntary water intake for the three hours commencing at the presentation of fresh food on the day after infusion. Mean of 4 observations on three sheep.

Treatment (Time of casein infusion in parenthesis)	Mean control intake	Intake on day after infusion	Intake on day after infusion less control intake
	<u>Food intake (g DM/0-3hr)</u>		
A (0-6hr)	323	284	- 39
B (6-12hr)	340	303	- 37
C (3-6 & 9-12hr)	351	320	- 31
S.E. of means	± 10	± 20	± 23
Overall mean ± S.E.	338 ± 6	302 ± 12	-36 ± 13 *
	<u>Voluntary water intake (ml./0-3hr)</u>		
A (0-6hr)	937	605	- 332
B (6-12hr)	1247	825	- 422
C (3-6 & 9-12hr)	1126	615	- 512
S.E. of means	± 68	± 155	± 95
Overall mean ± S.E.	1103 ± 39	681 ± 81	-422 ± 55 **

See Table 19 for requirements for significance

That there was a relatively greater depression of water than of food intake is shown by the amount of water drunk per unit of food eaten (Table 26). Less water was drunk ($p < 0.05$) for each gram of dry matter eaten during the first 3hr of the day after infusion than in the corresponding time during the control days. No significant differences were detected for the remainder of the day or for the day as a whole.

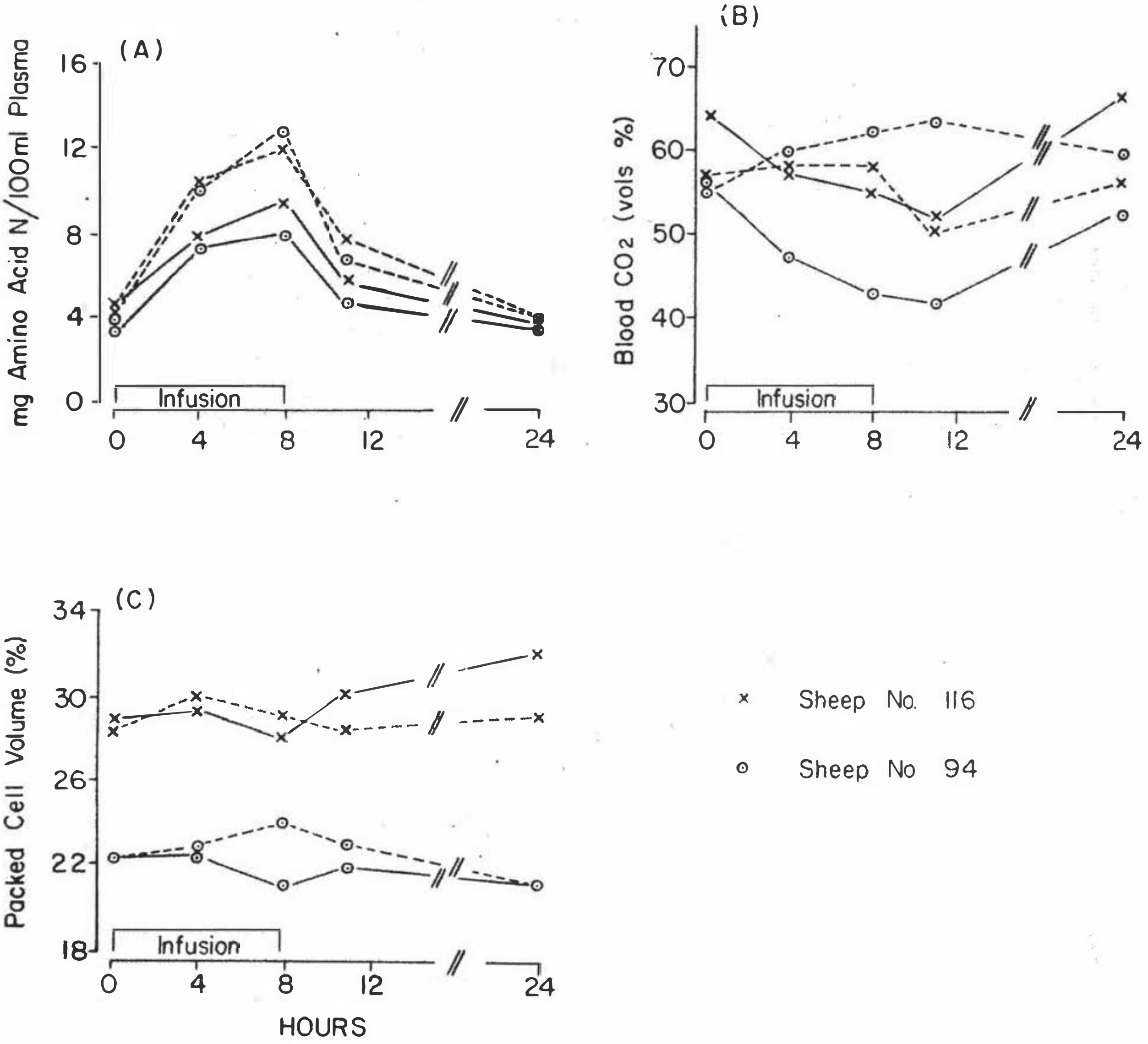

Fig.6. Expt. 10. A comparison of the effects of intraduodenal infusions of casein (solid line) and casein hydrolysate (broken line) on (a) plasma amino acid concentration, (b) blood CO_2 content and (c) packed cell volume. Infusions (0.28g nitrogen/kg B.W.) were for 8hr commencing at the time of presentation of fresh food.

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TABLE 27. Effect of intraduodenal infusions of casein and casein hydrolysate. Individual observations on two sheep. For each item, data expressed as day of infusion less the value (in parenthesis) which is the mean of the four days immediately preceding that of infusion.

ITEM	CASEIN (0.28gN/kg BW) in 750ml. Blaxter's Saline		CASEIN HYDROLYSATE (0.28gN/kg BW)	
	Infusions over 8hr starting at the time of presentation of fresh food			
	Sheep 116	94	116	94
Food intake (g DM/0-8hr)	(482) + 65	(462) - 12	(522) + 47	(448) + 28
(g DM/0-24hr)	(577) + 46	(539) - 9	(610) + 60	(566) + 49
Water intake (ml./day)	(1225) + 325	(1188) + 237	(1250) + 400	(1431) + 444
Urinary volume (ml./day)	(353) + 287	(359) + 166	(294) + 101	(595) + 255
Urinary nitrogen (g/day)	(2.28) + 2.27	(2.78) + 3.90	(2.48) + 2.59	(2.57) + 2.29
Faecal DM output (g/day)	(345) - 19	(321) - 37	(358) - 58	(335) - 58
Faecal water (g/day)	(616) - 126	(729) - 224	(704) - 112	(695) - 19

TABLE 26. The ratios of water drunk to food eaten on the day after infusion. In the absence of significant differences between treatment means, only the overall means and their standard errors are presented. Each is the mean of 12 observations.

Time of day (hr)	Ratio of voluntary water intake (ml.) to food intake (g DM) for: A Day after infusion	B Corresponding time during control days	Significance of differences between A & B
0 - 3	2.19 ± 0.30	3.21 ± 0.08	$p < 0.05$
3 - 24	2.26 ± 0.21	1.93 ± 0.14	$p > 0.05$
0 - 24	2.24 ± 0.16	2.55 ± 0.10	$p > 0.05$

Experiment 10. A comparison of the effects of intraduodenal infusions of casein and casein hydrolysate

Food and water intake, faecal and urinary excretion data are presented for each sheep in Table 27. The intraduodenal infusion of casein increased the food intake of one sheep but not the other; the infusion of casein hydrolysate resulted in small increases by both sheep. An increase in the amount of water drunk and a decrease in faecal dry matter output were apparent each time an infusion was made. There was no obvious difference between treatments in their effect on either urinary volume or nitrogen output. The reduction in faecal water output, observed each time an infusion was made, was particularly marked when casein was administered to Sheep 94. On that occasion, no increase of food intake was observed.

Blood (Fig.6). Compared with the infusion of casein, the infusion of casein hydrolysate resulted in an increase in plasma amino acid content, packed cell volume and blood CO_2 levels.

Experiment 11. Effects of intraduodenal infusions of an amino acid mixture.

Food intake on the day of infusion as a whole, was increased by infusion of the Salt Solution and decreased by infusion of the amino acid mixture (Table 28a). The difference between treatments was significant ($p<0.01$).

TABLE 28. Effect on food intake of intraduodenal infusions of Salt Solution and an amino acid mixture. The solutions (1500ml.) were infused over an 8hr period starting at the time of presentation of fresh feed. Mean of 2 observations on each of 4 sheep.

Treatment	Mean control intake	Intake on day of infusion	Intake on day after infusion
	(a) DM intake (g/0 - 24hr)		
Salt solution	710	812**	698
Amino acid mixture	680	438**	573**
S.E. of means+		± 28	± 17
Significance of difference between means		$p<0.01$	$p<0.01$
	(b) DM intake (g/0-8hr)		
Salt solution	525	586**	487
Amino acid mixture	484	228**	362**
S.E. of means+		± 17	± 25
Significance of difference between means		$p<0.01$	$p<0.01$
	(c) DM intake (g/8-24hr)		
Salt solution	185	227	213
Amino acid mixture	196	210	212
S.E. of means+		± 17	± 12
Significance of difference between treatments		N.S.	N.S.

** Significantly different from control intake, $p<0.01$

\+ Standard errors given also apply to mean control intake

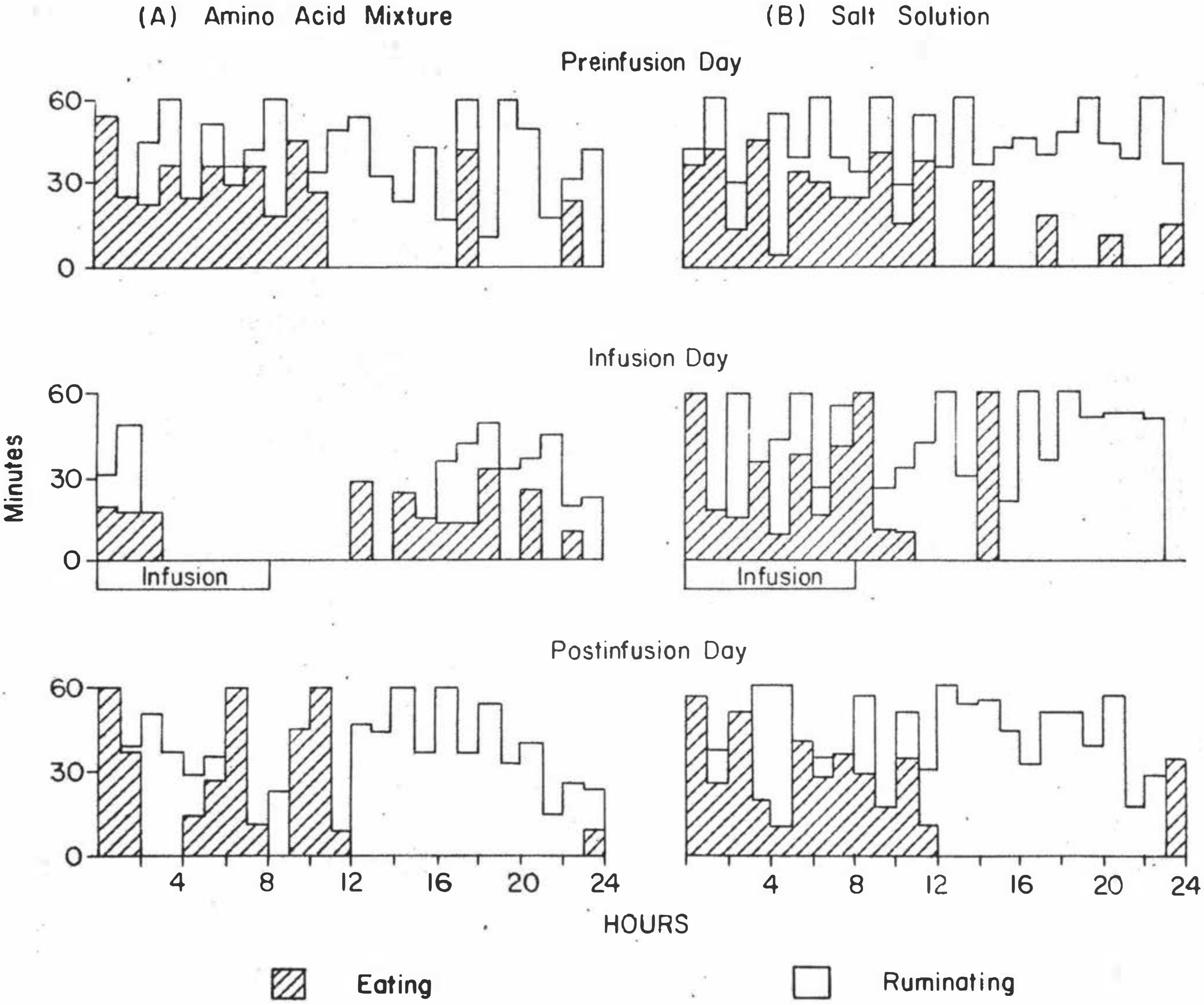

Fig.7. Expt. 11. The effects of intraduodenal infusions of (a) an amino acid mixture and (b) Salt Solution on the circadian pattern of eating and ruminating of one sheep. Note the marked reduction of the time spent eating and ruminating on the day of infusion of the amino acid mixture.

With both treatments, the effect on food intake occurred mainly during the 8hr infusion period (Table 28b). On the day following the infusion of the amino acid mixture, food intake for the first 8hr and for the day as a whole was less than on the control days ($p < 0.01$) and on the day after the infusion of the Salt Solution ($p < 0.01$; Table 28a).

Jaw movements recorded from a single sheep, indicated that this sheep neither fed nor ruminated during the period 3 - 13hr after the start of infusion. There followed a period of infrequent eating not evident on either the pre- or post-infusion days (Fig. 7a). The infusion of the Salt Solution had no obvious effect on the circadian pattern of eating (Fig.7b). On completion of the experiment, jaw movements of another sheep were recorded under conditions similar to those described here. On the day of infusion of the amino acid mixture to this animal, eating and rumination were absent until the thirteenth hour after the start of the infusion. Some eating occurred during the first 80min. of infusion.

Voluntary water intake was reduced on the day of and the day after the infusion of the amino acid mixture. A small decrease was recorded on the day of infusion of the Salt Solution. None of these effects were statistically significant. The data are shown below.

	Mean control intake	Intake on day of infusion (ml./day)	Intake on day after infusion
Salt Solution	2103	1813	2019
Amino acid mixture	2014	1669	1681
S.E. of means		± 157	± 182

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TABLE 29. Effect on faecal dry matter and water excretion of intraduodenal infusions of Salt Solution and an amino acid mixture. The solutions (1500ml.) were infused over an 8hr period starting at the time of presentation of fresh feed. Mean of 2 observations on each of 4 sheep.

Treatment	Mean of control periods	Day of infusion	Day after infusion
(a)	Faecal dry matter (g/day)		
Salt Solution	405	401	405
Amino acid mixture	392	189 **	359 **
S.E. of means +		± 6	± 6
Significance of difference between means		$p < 0.01$	$p < 0.01$
(b)	Faecal dry matter %		
Salt Solution	31.6	20.7 **	31.1
Amino acid mixture	31.1	23.1 **	27.8
S.E. of means +		± 0.77	± 1.08
Significance of difference between means		N.S.	N.S.
(c)	Faecal water (g/day)		
Salt Solution	878	1528 **	899
Amino acid mixture	880	771	970
S.E. of means +		± 58	± 55
Significance of difference between means		$p < 0.01$	N.S.

** Significantly different from the control, $p < 0.01$

+ Standard errors given also apply to control means

Urinary volume was not obviously affected by the infusion of Salt Solution. In contrast, a large increase was observed on the day that the amino acid mixture was infused ($p < 0.01$).

	Mean control urine volume	Urine volume on the day of infusion (ml./day)	Urine volume on the day after infusion
Salt Solution	821	825	837
Amino acid mixture	970	1608**	969
S.E. of means		± 140	± 99

** Significantly different from control; $p < 0.01$

Faecal excretion (Table 29). The infusion of Salt Solution was without obvious effect on faecal dry matter output. In contrast, infusion of the amino acid mixture elicited a marked decrease on the day of infusion ($p < 0.01$); a lesser decrease was apparent on the day after infusion (Table 29a). The percentage of dry matter in the faeces voided on the day of infusion was reduced by both treatments (Table 29b). The treatments differed in their effects on faecal water excretion; a marked increase was observed on the day that the Salt Solution was infused whereas a small decrease (not statistically significant) was apparent on infusion of the amino acid mixture. (Table 29c).

Experiment 12. Effects of intraduodenal infusions of casein and intravenous infusions of a casein hydrolysate.

Food intake (Table 30). In contrast to what was observed in Experiment 9, the intraduodenal infusion of casein reduced food intake. The decrease was significant at the end of the 8hr infusion period (p 0.05, Table 30a) but not for the day as a whole (i.e. 16hr later,

Table 30c). The intravenous infusion of casein hydrolysate elicited a more marked decrease, significant at the end of the 8hr infusion period ($p < 0.01$, Table 30a) and for the day as a whole ($p < 0.01$, Table 30c). In the 16hr following the end of infusion of the hydrolysate, an increase of food intake was observed (Table 30b).

TABLE 30. Effect on food intake of intraduodenal (i.d.) infusions of casein and intravenous (i.v.) infusions of casein hydrolysate. Infusions (Table 18) were for 8hr starting at the time of presentation of fresh feed. Mean of 4 sheep.

Treatment	Mean control intake	Day of infusion less control	Day after infusion less control
(a) Food intake (g DM/0-8hr)			
Blaxter's Saline (i.d.)	391	- 25	- 3
Casein (i.d.)	409	- 64 *	- 60 *
Isotonic Saline (i.v.)	376	+ 22	- 30
Casein hydrolysate (i.v.)	370	-195 **	- 61 *
S.E. of means [+]	± 16	± 26	± 20
(b) Food intake (g DM/8-24hr)			
Blaxter's Saline (i.d.)	106	- 3	- 17
Casein (i.d.)	124	+ 40	+ 18
Isotonic Saline (i.v.)	107	- 9	- 14
Casein hydrolysate (i.v.)	106	+ 124 **	+ 27
S.E. of means [+]	± 9	± 20	± 24
(c) Food intake (g DM/0-24hr)			
Blaxter's Saline (i.d.)	497	- 28	- 19
Casein (i.d.)	532	- 24	- 42 *
Isotonic Saline (i.v.)	483	+ 13	- 44 *
Casein hydrolysate (i.v.)	476	- 72 **	- 34 *
S.E. of means [+]	± 11	± 17	± 15

* Significantly different from no-infusion control, $p < 0.05$
** $p < 0.01$

+ Required difference for significance between means,
S.E. x 3.1 $p < 0.05$
S.E. x 4.3 $p < 0.01$

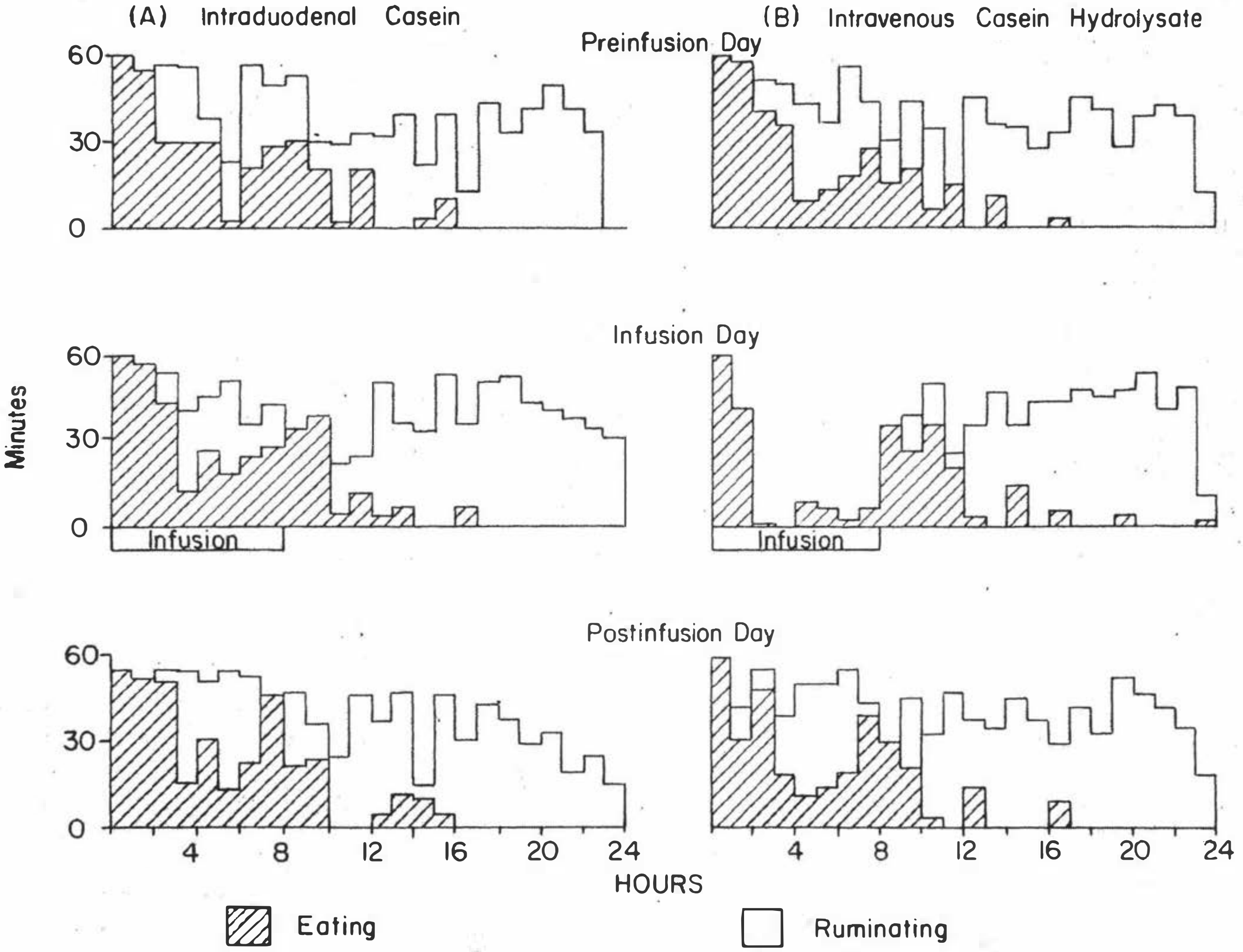

Fig. 8. Expt. 12. The effects of (a) intraduodenal infusion of casein and (b) intravenous infusion of casein hydrolysate on the circadian pattern of eating and ruminating. Mean of three sheep. Infusions (0.28g nitrogen/kg B.W.) were for 8hr commencing at the time of presentation of fresh food. The intravenous infusion of the casein hydrolysate but not the intraduodenal infusion of casein reduced the time spent eating and ruminating on the day of infusion.

The inhibitory effects of the casein and casein hydrolysate infusions may have been present on the day after infusion for total dry matter intake on that day was reduced (Table 30c). A reduction of similar magnitude was however, also observed on the day after the infusion of the isotonic saline. Food intake was reduced in the period 0-8hr on the day after the infusion of the two nitrogen treatments (Table 30a); this may have been a consequence of the increased eating during the previous 16hr (Table 30b).

Jaw movement recordings indicated that the intraduodenal infusion of casein had no marked effect on the circadian pattern of feeding or rumination (Fig.8). In contrast, time spent eating was greatly reduced during the period 2-8hr after the start of the intravenous infusion of casein hydrolysate; rumination was absent for a further 2hr.

Voluntary water intake on the day of infusion was reduced by all treatments except the intraduodenal infusion of casein (Table 31). Small but consistent reductions in drinking were recorded on the day after the infusion for all treatments. They were not statistically significant.

TABLE 31. Effect on voluntary water intake of intraduodenal infusions of casein and intravenous infusions of casein hydrolysate. Infusions (Table 18) were for 8hr starting at the time of presentation of fresh feed. Mean of 4 sheep.

Treatment	Mean control intake	Intake on day of infusion less control	Intake on day after infusion less control
	(ml./day)		
Blaxter's Saline (i.d.)	1106	- 644 **	- 209
Casein (i.d.)	1061	+ 15	- 255
Isotonic saline (i.v.)	1064	- 376 *	- 224
Casein hydrolysate(i.v.)	1026	- 789 **	- 145
S.E. of means	± 54	± 133	± 138

For significance, see Table 30

Faecal dry matter output on the day of infusion was reduced by the intraduodenal infusion of casein (Table 32a). A greater reduction was elicited by the casein hydrolysate infusion. Faecal water output was also reduced by the two nitrogen treatments but in neither case was the effect statistically significant (Table 32b).

TABLE 32. Effect of faecal excretion of dry matter and water of intraduodenal infusions of casein and intravenous infusions of casein hydrolysate. Infusions(Table 18) were for 8hr starting at the time of presentation of fresh feed. Mean of 4 sheep.

Treatment	Mean control Output	Output on the day of infusion less control	Output on the day after infusion less control
(a) Faecal dry matter (g/day)			
Blaxter's Saline (i.d.)	285	+ 5	- 2
Casein (i.d.)	300	- 27 *	+ 3
Isotonic Saline (i.v.)	286	- 11	- 5
Casein hydrolysate (i.v.)	295	- 62 **	+ 25
S.E. of means	± 13	± 10	± 14
(b) Faecal water (g/day)			
Blaxter's Saline (i.d.)	469	- 75	+ 15
Casein (i.d.)	502	- 47	- 4
Isotonic Saline (i.v.)	493	+163	+ 190 *
Casein hydrolysate (i.v.)	452	-100	+ 71
S.E. of means	± 15	± 91	± 66

For significance, see Table 30.

Urinary excretion (Table 33). Both nitrogen treatments elicited an increase in urinary volume on the day of infusion, the greatest increase being that for the intravenous infusion of casein hydrolysate (Table 33a). Both saline treatments also increased urinary volume but that for the intraduodenal infusion of Blaxter's Saline was not statistically significant. Of the 6.7 - 7.9g nitrogen administered,

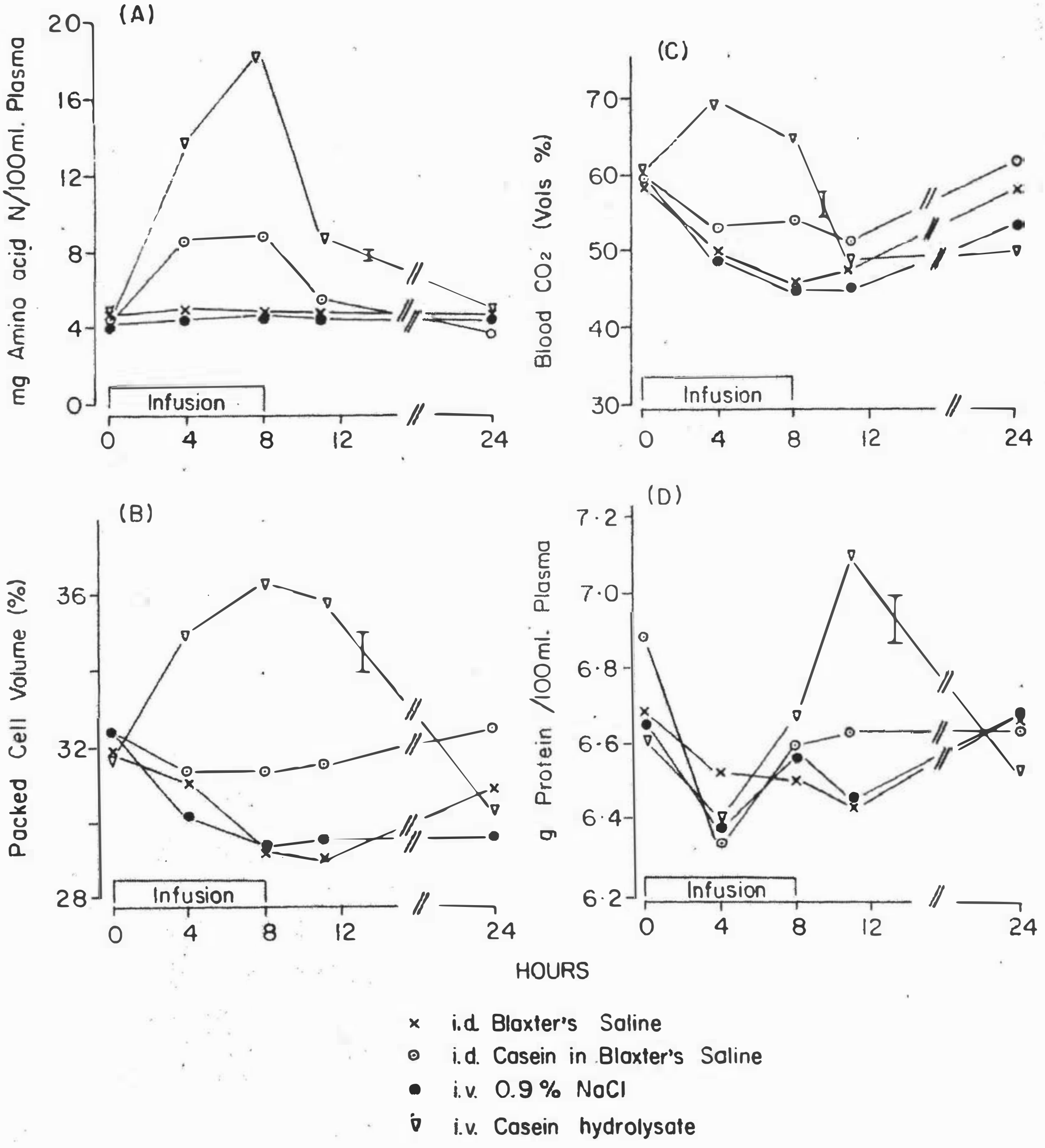

Fig.9. Expt. 12. Effect of intraduodenal infusion of Blaxter's Saline alone (750ml., 1.56ml./min) and containing casein (0.28g nitrogen/kg B.W.) and intravenous infusion of 0.9% NaCl (1.85-2.08ml./min) and casein hydrolysate (0.28g nitrogen/kg B.W., 1.85-2.08ml./min) on some blood parameters. Mean of four sheep. S.E. of means indicated by vertical bars.

more appeared in the urine on the day of and that after the intravenous infusion of casein hydrolysate (6.2g) than was observed when casein was infused into the duodenum (3.2g; Table 33b).

TABLE 33. Effect on urinary volume and nitrogen excretion of intraduodenal infusions of casein and intravenous infusions of casein hydrolysate. Infusions (Table 18) were for 8hr starting at the time of presentation of fresh feed. Mean of 4 sheep.

Treatment	Mean control output	Output on day of infusion less control	Output on day after infusion less control
	(a) Urinary volume (ml./day)		
Blaxter's Saline (i.d.)	370	+ 89	+ 24
Casein (i.d.)	295	+375 *	+ 20
Isotonic saline (i.v.)	325	+294 *	+117 *
Casein hydrolysate (i.v.)	300	+792 **	+ 2
S.E. of means	+33	+104	± 57
	(b) Urinary nitrogen (g/day)		
Blaxter's Saline (i.d.)	3.06	+ 0.48	- 0.11
Casein (i.d.)	2.66	+ 3.02 **	+ 0.22
Isotonic saline (i.v.)	3.02	+ 0.78	- 0.03
Casein hydrolysate (i.v.)	2.79	+ 5.17 **	+ 1.06 *
S.E. of means	± 0.17	± 0.45	± 0.39

For significance, see Table 30.

Blood (Fig. 9). The intravenous infusion of casein hydrolysate elicited significant increases ($p < 0.01$) in plasma amino acid concentration, packed cell volume and CO_2 levels during the infusion period. Values approximating those observed at the start of infusion were reached at the 24hr sampling. Increases of smaller magnitude were observed when casein was infused into the duodenum. Plasma protein concentration was variable.

Bodyweight of the sheep showed no consistent change over the experimental period. Mean weights of each sheep calculated from eight measurements made at 3 - 5 day intervals were 24.1, 27.6, 24.8 and 24.9 kg (S.E. of means, ± 0.2kg).

Nitrogen balance as estimated during the six days immediately preceding the day of the first infusion ranged from - 1.34 to - 2.50 g N/day for the four sheep. Digestibility of feed dry matter during the same period was 42 - 48%.

DISCUSSION

Both the nature of the amino acid containing material and the route of administration modified the effect of the infusions on food intake. An excitation of food intake was observed only when infusions were made into the duodenum, clearly shown when casein (Experiment 9) and less clearly when casein hydrolysate (Experiment 10) were the amino acid containing materials administered. In contrast, intraduodenal infusion of the amino acid mixture and intravenous infusion of the casein hydrolysate resulted in a profound inhibition of food intake.

The possibility that receptors involved in the excitation of food intake may be located in, or associated with, the intestinal walls or the portal vascular field is suggested by the demonstration that food intake was increased by the intraduodenal infusion of casein or casein hydrolysate but reduced by the intravenous infusion of casein hydrolysate. The triggering stimuli for these receptors may be events associated with the presence of the infused material in the intestines and/or their digestion and absorption. The time course of the increase in food intake is consistent with this view. It is difficult to explain the relationship between the time of infusion and the occurrence of increased food intake established in Experiment 9 in terms of changes in activity of the microorganisms in the reticulorumen or metabolism of digestion products once absorption has taken place; processes which may be considered slow to take effect and of relatively long duration. Egan & Moir (1965) found that intraduodenal infusions of urea over an 8 hr period led to an increase in the rate at which cellulose was digested in the reticulorumen of sheep. This was thought to be the main factor responsible for the increased food intake that was observed on the day after infusion. The delay was considered due to "the time taken for the recycled nitrogen to influence microbial activity in the rumen." Campling <u>et al</u>. (1962) found that continuous intraruminal infusion of urea (150g/day) did not increase the cow's consumption of oaten straw until 3 - 4 days after the start of infusion.

The variability of the effects on food intake of intraduodenal infusions of casein was again demonstrated in the present series of experiments. On occasions intake was stimulated (Experiment 9 and sheep No.116, Table 27), whereas at other times an inhibition was observed (Experiment 12, Table 30). This emphasises the complex nature of the effects on food intake of infusions of proteinaceous materials. It appears unlikely that a single mechanism operates in mediating these effects; rather a number of mechanisms should be envisaged which act separately, additively or synergistically. Whether a single experimental procedure, the infusion of casein into the duodenum, elicits an increase or a decrease of food intake will depend on the summation of the stimuli arising from the various mechanisms, a process which appears to be influenced or modified by various factors including dose rate, the previous experimental history and the physiological status of the animal.

The infusion of different amino acid-containing materials into the duodenum failed to reveal either their site of action or the nature of the mechanisms involved. Because no further supplies of casein hydrolysate were available, only a limited number of observations could be made on its effect when administered into the duodenum. The small increases in food intake observed on those occasions may be therefore regarded as equivocal. The relative importance of the physiological status of the animal, and the dose rate, nitrogenous components, inorganic solutes, and the physical properties of the infusate in determining the observed effects are not known. The marked inhibitory effects on food intake of intraduodenal infusions of the amino acid mixture occurred even though (a) the dose rate expressed in terms of nitrogen was slightly smaller than those of either casein or casein hydrolysate (b) the vehicle (Salt Solution) was hypotonic and of relatively large volume, conditions which may facilitate the excitatory effects on food intake of intraduodenal infusions of casein (Experiment 8), and (c) infusions of the vehicle alone increased food intake. Although a marked reduction in food intake was recorded here, the possibility cannot be excluded that an infusion of the amino acid mixture at an appropriate dose rate may have resulted in an increase of food intake.

The effect on food intake of intravenous infusions of casein hydrolysate were predominantly one of inhibition. This contrasts sharply with the mildly excitatory effects observed when a similar infusion was made into the duodenum. These differing effects suggest that the intravenous route enabled the casein hydrolysate to act at sites or on mechanisms not immediately accessible when the same material was infused into the duodenum. There may have been a specific stimulation of a satiety mechanism, in some way related to circulating amino acids or alternatively, the inhibitory effects may have been non-specific, possibly associated with the presence in the infusate of physiologically active compounds formed during the preparation or storage of the hydrolysate.

It seems logical to propose that the metabolic activity of the intestinal walls and the liver were of some importance in modifying the effects of the infusions on food intake. Irrespective of the nature of the inhibitory mechanisms involved, their effects were reduced when the hydrolysate was infused into the duodenum. Their complete elimination seems unlikely for that procedure elicited a reduction in faecal dry matter output on the day of infusion (Table 27) as well as an increase in food intake. Metabolism of amino acids by the intestinal walls and liver may have also contributed to the different effects of intravenous and intraduodenal infusions of casein hydrolysate on plasma amino acid content (Fig.6 & 9) and renal response (as judged by urinary volume and nitrogen output,(Tables 27 & 33). Indirect support for these observations is to be found in the unequivocal demonstration that the intravenous infusion of a protein hydrolysate results in less efficient utilisation of nitrogen than when the same material is administered into the gastrointestinal tract (Silber, Seeler & Howe, 1946; Alper, Chow & Debiase, 1950; Allison & Bird, 1964).

It also seems logical to suggest that intraduodenal infusions of the amino acid mixture may have partially overwhelmed any ameliorating effects of the liver. This may have resulted in a more effective stimulation of extraintestinal mechanisms capable of inhibiting food intake than when less readily absorbed materials such as casein or a peptide containing casein hydrolysate were infused into the duodenum. As no observations were made on the effects on blood composition of infusions of the amino acid mixture, this suggestion must remain speculative. It has been shown however, that the rate of absorption of nitrogen from the gut is faster and plasma amino acids attain a higher concentration when amino acid solutions rather than whole proteins are fed (Free & Leonards, 1944; Crane & Neuberger, 1960). On the other hand, Fisher (1967), on reviewing protein absorption, has stated that "all one can say in conclusion is that the form in which the products of protein digestion enter the body is still not established. No one has been able to show that more than a minority of it enters the bloodstream as amino acids." It is possible therefore, that the infusion of large amounts of free amino acids is an unphysiological process which could readily lead to disturbances of homeostasis. The occurrence and magnitude of disturbances of this nature is suggested by the marked renal response and the persisting effects of the amino acid mixture on feeding behaviour, food intake, faecal and urinary excretion. In contrast, the inhibitory effects of intravenous infusions of casein hydrolysate were not obvious after the completion of the 8hr infusion (Fig.9).

It seems unlikely that the blood constituents investigated were those determining either the excitatory or the inhibitory effects of the infusions on food intake. Although marked changes in the parameters measured were observed when food intake was decreased (Experiment 12) similar changes were apparent when food intake was increased (Experiment 10). In addition, when feeding recommenced at the completion of the intravenous infusion of casein hydrolysate, the magnitude of the changes in blood composition were at or near their maximum (Fig.8 & 9). Nonetheless, the observed blood changes might be involved under the following circumstances:

(a) if the receptors involved were sensitive to the rate of change in concentration of the metabolites;

(b) if their action(s) were indirect, with the observed changes being only part of the triggering mechanisms;

(c) if the effect of the infusions on food intake was the summation of both inhibitory and excitatory stimuli; or

(d) if changes in the relative proportions of the free amino acids of plasma were of importance.

As discussed in Chapter 1, the work of Harper and associates suggests that the pattern of amino acids in the blood may be of importance in the regulation of food intake by rats and mice. The presence of either an excess or deficiency of certain essential amino acids in the diet may lead to reduced food intake (Harper, 1964), sometimes within a few hours of adding the imbalanced amino acid mixture to the low protein basal diet (Kumta & Harper, 1962). It seems unlikely however, that any of the amino acid preparations used here were sufficiently imbalanced in their amino acid content for this to account for the inhibitory effects. The casein hydrolysate and amino acid mixture were commercial preparations specifically designed for human parenteral nutrition and the casein for supplementing the diet of children and convalescent or otherwise debilitated adults. Even so, there is no certainty that the requirements of sheep in this respect are the same as those of humans; and individual variation, both in the proportions of amino acids in the plasma associated with homeostasis (Williams, 1963) and the interconversion occurring during digestion and absorption (Gitler, 1964) may have resulted in an imbalance.

The present experiments have not revealed the mechanisms whereby infusions of amino acid containing materials modified food intake. Their major contribution has been the demonstration that the situation is of considerable complexity, unlikely to be resolved by simple experiments such as those undertaken here. They have indicated that there may be a number of mechanisms involved with receptors located in a number of sites in the body: intraduodenal infusions of casein and casein hydrolysate appeared to act on mechanisms or at sites that were different from those reached by either intravenous infusions of casein hydrolysate or intraduodenal infusions of the amino acid mixture.

TABLE 34. Apparent water retention on the day of infusion for all treatments in Experiments 10, 11 and 12.

Treatment	Water Intake (ml./day)				Water Excretion (ml./day)			
	Contained in food	Infused	Drunk	TOTAL INTAKE (a)	Urinary	Faecal	TOTAL EXCRETION (b)	APPARENT RETENTION (a - b)
Experiment 10								
Casein	90	750	1487	2327	583	498	1081	+ 1246
Casein hydrolysate	95	950	1763	2808	623	634	1257	+ 1551
Experiment 11								
Salt Solution	143	1500	1813	3456	825	1528	2353	+ 1103
Amino acid mixture	77	1500	1669	3246	1608	771	2379	+ 867
Experiment 12								
Casein Hydrolysate	71	950	237	1258	1092	352	1444	- 186
Casein	90	750	1076	1916	652	455	1107	+ 809
Isotonic Saline	83	950	688	1721	619	656	1275	+ 446
Blaxter's saline	88	750	462	1300	459	544	1003	+ 279
Mean of all no-infusion controls	98	-	1411	1509	504	683	1187	+ 322

Note: Calculations based on data presented in Table 27, Experiment 10; Tables 28, 29 and text, Experiment 11; Tables 30, 31, 32 and 33, Experiment 12. 1.0g faecal water has been assumed equivalent to 1.0ml. The estimates of apparent water retention during the no-infusion control periods in Experiments 10, 11 and 12 were + 277, + 364 and + 325ml. water respectively.

It is apparent that the net effect of the infusions on food intake resulted from the summation of excitatory and inhibitory stimuli. Reduced faecal output on the day of treatment was a feature common to all infusions of amino acid containing materials, irrespective of their effect on food intake. This suggests that a reduced rate of passage of digesta along the gastrointestinal tract may be one of the inhibitory components, present even when an increase in food intake was observed. Further consideration is given to these ideas in subsequent chapters. A possible clue to the nature of the excitatory component is suggested by the effects of the infusions on water metabolism. In Experiment 9, increased voluntary water intake consistently occurred during the period of casein infusion even though in one treatment, this was of 3hr duration and preceded by an infusion of water. Reduced drinking in the first 3hr of the day following that of infusion suggests that the water was retained in the body, becoming available during that period and partially satisfying normal water requirements. In Experiments 10, 11 and 12, infusions which resulted in increased food intake (casein hydrolysate, Experiment 10; Salt Solution, Experiment 11) or which previously had been shown to do so (casein, Experiments 10 and 12) also markedly increased apparent water retention (Table 34). In contrast, these infusions which reduced food intake (intravenous casein hydrolysate and intraduodenal amino acid mixture), greatly increased urinary water excretion with a lesser effect on apparent water retention. As neither respiratory nor body surface losses were measured, the estimates of water retention may be regarded as approximate. There is however, consistency between the estimates for the no-infusion controls in the three experiments and between those values and the estimates for the saline infusions where there was little cause to expect disturbances in water metabolism. The estimate of retention when the amino acid mixture was infused into the duodenum appears anomalous but it is to be remembered that with this treatment, the effects were not confined to the day of infusion.

While the nature of the compensatory mechanisms eliciting the changes in apparent water retention remains uncertain, the possibility arises that these changes may be linked to the excitation of food intake. An osmometric mechanism, whereby the sensations of hunger and satiety arise at least in part from changes in water distribution may be of some importance in monogastric animals (Brobeck, 1955; Lepkovsky _et al_., 1957; Schwartsbaum & Ward, 1958; Smith, 1966). Such a concept is attractive in that it may eventually provide an explanation as to why the intraduodenal infusion of the Salt Solution increased food intake. Irrespective of the cause of the increased apparent water retention when the Salt Solution was infused - whether it was due to its effects on the absorption of nutrients already in the gut lumen, or to its physical or chemical properties - the similarity in this respect to the effects of those amino acid containing infusions which increased food intake is striking.

CHAPTER 4

THE EFFECT OF INTRADUODENAL INFUSIONS OF CASEIN ON THE URINARY EXCRETION OF SODIUM AND POTASSIUM

INTRODUCTION

There are a number of reports suggesting that an intimate relationship exists between amino acid and electrolyte metabolism.

Christensen & Riggs (1952) found that the uptake of neutral amino acids by Ehrlich ascites tumour cells was accompanied by a somewhat less than equivalent net loss of potassium from the cell. This and subsequent observations have led to the suggestion that entry of neutral amino acids into the cell is in some way coupled to, and dependent on, the outward migration of the potassium ion (Christensen, Riggs & Coyne, 1954; Riggs, Walker & Christensen, 1958; Christensen, 1960). The effect of amino acid uptake on potassium migration appears to be dependent in part on the amino acid being concentrated. Levinsky, Tyson, Miller & Relman (1962) found that incubation of rat skeletal muscle in a media rich in the cationic amino acid, lysine, resulted in a loss from the muscle of up to one third of the cellular potassium. The potassium lost from the cells was substituted with an equivalent amount of lysine. The inclusion of neutral amino acids in the media resulted in less marked effects while anionic amino acids had no effect. In some cases however, it has been observed that an increase in intracellular potassium accompanies the uptake of anionic amino acids (Terner, Eggleston & Krebs, 1950; Christensen & Riggs, 1952; Ames, 1956).

Subsequent to the above observations, Heinz (1962) suggested that sodium facilitates the transfer of amino acids across cellular membranes. In support of this, Fox, Thier, Rosenberg & Segal (1964) found that when rat kidney cortex slices were incubated in a sodium-free media, the active transport of the neutral amino acids $\propto$ aminoisobutyric acid and glycerine was completely inhibited and that of lysine and histidine reduced. Intracellular accumulation of these amino acids increased as the concentration of sodium in the media was increased. Using isolated rabbit ileum, Schultz & Zalusky (1965) showed that the transport of amino acids not only required sodium but also increased the rate at which sodium was transferred from the solution bathing the mucosa to the interior of the mucosal cells. Eddy, Mulcahy & Thomson (1967) found that the kinetics of glycine uptake by mouse ascites-tumour cells were consistent with the view that glycine entered the cells as a ternary complex with a carrier and sodium. That sodium is required for the transport of both amino acids and sugars is now generally accepted and the observation has been extended to include many tissues (Crane, 1965; Csáky, 1965; Quastel, 1965).

The above results have been obtained using in vitro techniques and their significance in the intact animal is uncertain. It is reasonable to expect that in some tissues at least a form of internal circulation is involved (Crane, 1965) and there may be no net loss electrolytes from the body.

The necessity of adequate potassium for effective utilization of amino acids has been demonstrated (Cannon, Frazier & Hughes, 1952; Frost, Smith & Felts, 1953). It is for this reason that a relatively large amount (25m-equiv./l.) of potassium is incorporated in the amino acid solution, "Aminofusin", which is designed for parenteral alimentation.

Iacobellis, Muntwyler & Dodgen (1956) and Eckel, Norris & Pope (1958a,b) have shown that during potassium depletion in the rat, lysine and other cationic amino acids accumulate in the skeletal musclature. Eckel and co-workers designated these amino acids as "mendicant cations" since they could replace the lost intracellular potassium and maintain internal neutrality. The reverse may also occur, for Gershoff, Coutino-Abath, Antonowicz, Mayer, Shen & Andrus (1959) found that increased potassium intake by rats fed a lysine deficient diet partially prevented the occurrence of lysine deficiency.

Although Eckel et al. (1958b) found that the feeding of diets rich in lysine to normal rats did not lead to displacement of muscle potassium, Dickerman & Walker (1964) showed that the intravenous infusion of the cationic amino acids, lysine, arginine or ornithine to dogs increased the urinary excretion of potassium to levels that exceeded the filtered load of potassium. It was postulated (Walker, Dickerman & Jost, 1964) that displacement of hydrogen ion by lysine produced an extracellular acidosis and an intracellular alkalosis. This in turn suppressed the ability of the kidney to secrete hydrogen ions and facilitated potassium for sodium exchange with consequent excretion of potassium. These effects may not be confined to potassium for Lindeman, Alder, Yiengst & Beard (1967) found that ingestion of casein or various carbohydrates by humans led to an increase in the urinary excretion of the divalent cations, calcium and magnesium.

It has been suggested in previous chapters of this thesis that infusions of amino acid containing materials effected changes in water metabolism and acid-base balance that may be explicable in terms of a disturbance in electrolyte balance. The experiments reported in the present chapter represent a preliminary investigation into the effects of intradsodenal infusions of casein on the sodium and potassium excretion of fasted sheep maintained on two contrasting diets.

TABLE 35. Details of the four experimental sheep.

Item	Sheep			
	A	B	C	D
Weight (kg)	25	22	20	21
Age (months)	16	16	18	18
Diet	Chaffed Red Clover hay (*Trifolium pratense* L., 3.5% nitrogen)		Chaffed ryegrass straw as described in Chapter 2 (0.95% nitrogen)	
History	Sheep A. Fed clover chaff for 8-9 weeks preceding the experiment. No previous infusions. Sheep B. Fed ryegrass straw diet for a 6 week period ending 3 weeks before the start of the experiment. During that time three intraduodenal infusions of casein had been given. Subsequently fed on clover chaff.		Both animals had been fed the ryegrass straw diet for 5 months prior to the experiment. During that time intravenous and intraduodenal infusions of amino acid containing materials had been given. No infusions were given in the 2 weeks preceding the present experiment	

MATERIALS AND METHODS

Animals. Four sheep fitted with duodenal cannulae and housed indoors in crates as described previously (Chapter 2) were used. Details concerning these animals and their experimental diets are given in Table 35. The animals had been accustomed to being fed at 9.00am each day and having food continuously available. Two weeks before the experiment, the sheep were placed on a regime in which they had unrestricted access to food for 15hr out of 24hr commencing 5.00pm on one day and ending at 8.00am on the next. Three days before the first infusion day, feeding was restricted to the period 6.00 - 10.00pm. This latter routine was continued until the end of the experiment. Water and salt lick, normally freely available, were removed with the food the day before an infusion.

Infusions. Casein (40g, 5.6g nitrogen) in Blaxter's Saline (750ml.) and, as a control, Blaxter's Saline (750ml.) alone were used. The method of preparation was as described previously except that the solutions were made to volume with glass distilled water. The electrolyte composition of the Blaxter's Saline and the sodium and potassium content of the casein were as follows:

Electrolyte	Amount present in 750ml. Blaxter's Saline (m-equiv)	Amount present in 40g casein (m-equiv)
Na^+	79.20	0.40
K^+	4.02	0.10
Ca^{++}	2.70	)
Mg^{++}	3.15	) Not
Cl'	87.00	) determined
PO_4'''	6.49	)

The solutions were infused into the duodenum by means of a DCL micropump for a 4hr period commencing either 10.00 or 10.30am. The sequence of infusion of the solutions was:

Day of experiment	Sheep identification A	B	C	D
1	Casein			
3		Casein		
5	Blaxter's Saline	Blaxter's Saline		
7			Blaxter's Saline	Blaxter's Saline
9			Casein	Casein

Collection and preparation of samples

Urine. Indwelling Foley catheters, inserted the previous day, and tared polythene vaccine bags were used to collect the urine quantitatively and with minimum exposure to air. The polythene bags (Tasman Vaccine Laboratories Ltd, Wellington) were collapsed before use and their necks fitted tightly into the catheter. Collection was for 8.5hr commencing 90min before the start of infusion. At 30min intervals, the bags were replaced, tightly stoppered and weighed. Samples of urine for pH estimation were obtained through the bag wall with the aid of a syringe and needle. The remaining urine was then transferred to a measuring cylinder.

Blood. Venous blood samples were collected with minimum exposure to air into heparinised syringes from a catheter inserted into the jugular vein the previous day. pH was estimated within 5min of sampling on blood collected into a separate syringe. On the day of infusion of the casein preparation, blood was sampled at -45, -15, +45min from the start of infusion and thereafter every hour for 6hr. On the day of infusion of Blaxter's Saline, samples were collected at -45, -15, +105, +225 and +345min from the start of infusion.

Plasma and red blood cells were separated under paraffin in serum agglutination tubes (i.d.8mm) by centrifugation for 30min at 3500 rev/min in a B.T.L. Bench Centrifuge. Plasma was removed with a transfer pipette and suitable aliquots were diluted immediately for sodium and potassium determination. From a further aliquot, a protein free filtrate for amino acid estimation was prepared as described in Chapter 2 and stored at -5°C. Aliquots of the red blood cells were taken and suitably diluted for sodium and potassium estimation after removal of the paraffin, remaining plasma and top one third of the sediment. Blechner (1961) centrifuged sheep's blood for 30min at 2600g in tubes of i.d. 12mm and found that the plasma content of the bottom two thirds of the red blood cell sediment was 3 - 4%. Centrifuging for 120min resulted in a sodium and potassium content of the red blood cell sediment that was similar to that found when centrifugation was for 30min.

Analytical. Sodium and potassium were estimated by flame photometry (E.E.L. Mark II) and the specific gravity of urine using a Clay-Adams urinometer. Volume of urine was calculated from its weight and specific gravity. Packed cell volume and plasma amino acid concentration were estimated as outlined in Chapter 2. Haemoglobin was estimated as cyanmethaemoglobin (Drabkin powder, Diagnostic Reagents Ltd.) with the aid of an E.E.L. haemoglobin meter calibrated with the supplied standards.

The pH of blood and urine was determined at 38°C with the aid of a Beckman Model 76 expanded scale pH meter and a Beckman micro-blood 46850 assembly mounted in a Beckman constant temperature block. Immediately before a sample was injected, the electrodes were standardised against a pH 6.84 buffer consisting of equal volumes of 0.025M KH_2PO_4 and Na_2HPO_4. Each sample was examined repeatedly until the reading 2min after injection was consistent for two consecutive injections.

All dilutions were made with double glass distilled water. The glassware used in the analyses was washed in a cleaning solution and

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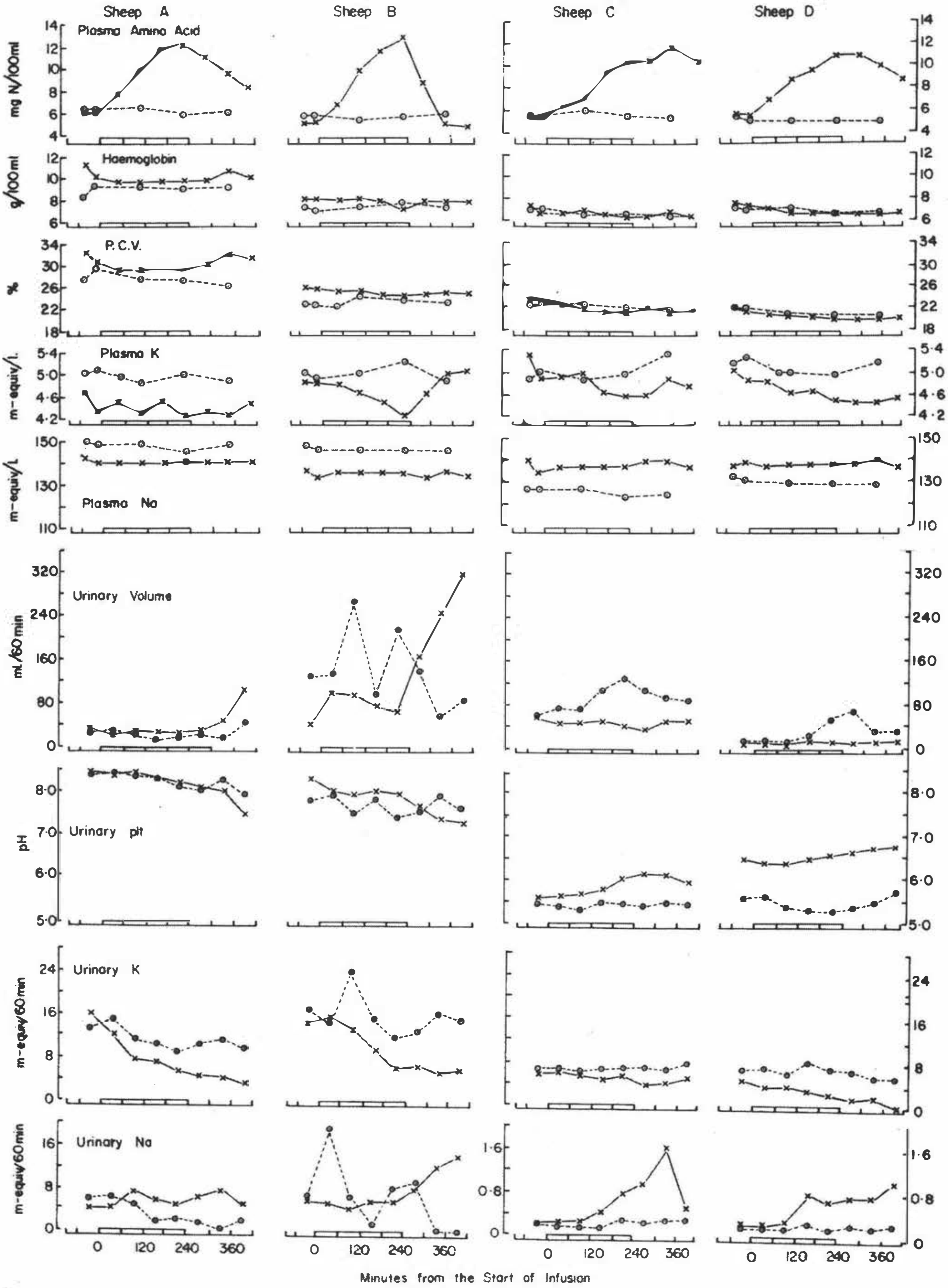
Sheep A
Sheep B
Sheep C
Sheep D
Plasma Amino Acid
mg N/100ml
14
12
10
8
6
4
Haemoglobin
g/100ml
12
10
8
6
P.C.V.
%
34
30
26
22
18
Plasma K
m-equiv/l.
5·4
5·0
4·6
4·2
m-equiv/L
150
130
110
Plasma Na
Urinary Volume
ml/60 min
320
240
160
80
0
Urinary pH
pH
8·0
7·0
6·0
5·0
Urinary K
m-equiv/60 min
24
16
8
0
Urinary Na
m-equiv/60 min
16
8
0
1·6
0·8
0
0
120
240
360
Minutes from the Start of Infusion

Fig.10. Changes in the composition of urine and venous blood when intraduodenal infusions of 750ml. Blaxter's Saline alone (broken line) and containing 40g casein (solid line) were made to four sheep. The infusions were for 4hr (0 - 240min) and commenced after a fast of either 12 or 12.5hr. The diet of Sheep A and B was red clover chaff and that of Sheep C and D was chaffed ryegrass straw. Mid-points of 60min collection periods plotted for urinary volume, pH, sodium and potassium excretion.
Note change in scale between the pairs of sheep for urinary sodium excretion.

rinsed in the sequence of hot tap water, metal distilled water and double glass distilled water before drying in an electrically heated oven.

RESULTS

The effect of casein infusion on plasma amino acid concentration appeared to be related to the diet of the sheep (Fig.10). The maximum levels observed in t e ryegrass fed sheep (C and D) were lower and more persistent than those observed in the pair fed clover (A and B). Changes in haemoglobin concentration and packed cell volume suggest that with Sheep B, a contraction of plasma volume occurred on infusion of Blaxter's Saline. In contrast, an expansion was observed on infusion of casein. Similar but less obvious changes may have occurred with the other three sheep. Sheep A appeared to be the only animal not anaemic or, alternatively, without an expanded plasma volume.

A decrease in potassium content of plasma occurred on infusion of casein. Some variation was evident in that it was particularly marked for Sheep B but less obvious with Sheep A. There was no appreciable change in plasma sodium content on any one day but a relatively large between day difference was apparent. The day on which the lowest values were recorded was also the first infusion day. The sodium and potassium content of the red blood cells did not appreciably differ either between days or between sampling times within days (Table 36). There was no consistent difference in blood pH either between sheep or sampling times on infusion of casein (Table 36).

TABLE 36. Effect of intraduodenal infusions of Blaxter's Saline and casein on blood pH and sodium and potassium content of red blood cells. Mean of 5 duplicate observations in the case of Blaxter's Saline, 10 in the case of casein.

Treatment	Sheep A	B	C	D	S.E. of mean
	Sodium content of red blood cells (m-equiv/l.)				
Blaxter's Saline	97.1	76.3	84.7	83.2	1.02
Casein in Blaxter's Saline	93.7	74.5	85.6	87.6	0.90
	Potassium content of red blood cells (m-equiv/l.)				
Blaxter's Saline	14.8	25.2	12.2	17.9	0.12
Casein in Blaxter's Saline	14.2	25.4	13.1	18.0	0.06
	Blood pH				
Blaxter's Saline	No data collected				
Casein in Blaxter's Saline	7.38	7.38	7.41	7.39	0.01

The infusions differed in their effect on urinary volume (Fig.10 and Table 37). With the exception of Sheep A, urinary volume increased on infusion of Blaxter's Saline. Little change was evident when casein was administered to the ryegrass fed animals (C and D). The increases in volume of urine excreted by Sheep A and B toward the end of the collection period may have been due to the excitement exhibited on approach of feeding time rather than to the infusions.

A striking feature of urinary pH was the acid reaction of the urine excreted by the ryegrass fed sheep (C and D, Fig.10). The pH was not appreciably affected by either the Blaxter's Saline or the casein infusions. There was however, some difference between days, particularly marked in the case of Sheep D.

TABLE 37. The amounts of sodium and potassium excreted in the urine and the volume of urine excreted by each sheep during the 510min collection period.

Treatment	Sheep			
	A	B	C	D
	Sodium excreted in the urine (m-equiv)			
(a) Blaxter's Saline	27.60	52.89	1.88	1.71
(b) Casein in Blaxter's Saline	46.00	64.10	5.10	4.73
Difference (b - a)	+18.40	+11.21	+3.22	+3.02
	Potassium excreted in the urine (m-equiv)			
(a) Blaxter's Saline	100.0	138.3	57.0	57.2
(b) Casein in Blaxter's Saline	65.3	87.0	43.1	24.3
Difference (b - a)	-34.7	-51.3	-13.9	-32.9
	Urinary volume (ml.)			
(a) Blaxter's Saline	203.6	1146.3	773.1	257.9
(b) Casein in Blaxter's Saline	313.8	1122.8	422.2	96.5
Difference (b - a)	+110.2	-23.5	-350.9	-161.4

Without exception, urinary excretion of potassium decreased and that of sodium increased on infusion of casein. The net effect at the end of the collection period (Table 37) may be regarded as minimal for the lowest rate of potassium excretion (3 sheep) and the highest rate of sodium excretion (2 sheep) were observed in the last 60min of collection. In the case of sodium excretion, there were two additional striking features. Firstly, the differences between the two infusions in the amount of sodium exereted in the urine were small relative to the 80m-equiv of this ion infused, the majority of which was unaccounted for. This contrasts to the differences observed between the infusions in the excretion of potassium; they were large relative to the 4m-equiv that were infused. Secondly, sodium excretion by the ryegrass fed sheep (C and D) was approximately a tenth of that excreted by the pair fed clover (Sheep A and B).

DISCUSSION

The present work indicates that the intraduodenal infusion of casein may evoke a potassium retention of considerable magnitude. This was so in fasted sheep maintained on two diets. The full extent of the retention could not be assessed as potassium excretion in the urine was minimal, and in some instances still declining at the end of the collection period. The differences in urinary sodium excretion between the casein infusions and those of the vehicle alone were less striking; in each case a large proportion of the administered sodium was retained.

The decline in plasma potassium concentration on infusion of the casein is consistent with the view that the location of the retained potassium was intracellular.

These observations appear inconsistent with those of Christensen & Biggs (1952), Levinsky et al. (1964) and others. These workers have shown that accumulation of cationic, and to lesser extent, neutral, amino acids within cells is accompanied by a depletion of intracellular potassium, even to the extent of eliciting an increase in the urinary excretion of that ion (Dickerman & Walker, 1964; Walker et al., 1964). It does not follow however, that the results of studies on the uptake of single amino acids applies to a situation involving the range of amino acids which constitute a protein such as casein. The net effect of a mixture of amino acids on electrolyte metabolism may depend on to what extent each of the various transport systems implicated in amino acid transport (Christensen, 1960; Benson & Rampone, 1966) are used, competition between amino acids for these systems (Webber, 1962; Gitler, 1964) and the species and quantity of the individual amino acids contained in the mixture. Thus it might be argued that since anionic amino acids have been shown to stimulate the intracellular accumulation of potassium (Terner et al., 1950; Christensen & Riggs, 1952; Ames, 1956), and as anionic glutamic acid is the most abundant amino acid in casein (Hipp, Basch & Gordon, 1961), the net effect of infusion of casein may be one of intracellular potassium accumulation. It is pertinent to recall here that Lindeman et al., (1967) found that the ingestion of 50g casein by humans resulted in decreased urinary excretion of potassium as well as the increase in divalent cation excretion mentioned previously. The effects were less marked than those observed when an equal weight of glucose was ingested.

In the absence of a more comprehensive analysis of changes in blood and urine composition elicited by infusion of casein, the mechanisms responsible for the observed changes are uncertain. The previous observation that intraduodenal infusions of casein may elicit an increase in the CO_2 content of the blood is consistent with the view that migration of potassium into the cells results in an extracellular alkalosis and an intracellular acidosis. This is the reverse of what Walker et al., (1964) have postulated. These workers suggested that the increase in urinary excretion of potassium on infusing cationic lysine into dogs resulted from substitution of intracellular hydrogen ions with lysine. The resulting intracellular alkalosis led to the kidney secreting potassium ions rather than hydrogen ions. As the ingestion of carbohydrates modifies the urinary excretion of cations (Lindeman et al., 1967), it appears that mechanisms other than those dependent on amino acid metabolism may be involved.

The retention of the water component of the casein vehicle may be regarded as osmotic obligation by the sodium retained in the body. Additional factors may be involved when the casein was infused as the apparent retention of water was greater than that observed on infusion of the Blaxter's Saline alone. This was particularly marked with the ryegrass fed sheep. The difference between the infusions in their effect on urinary volume may be due in part to the water accompanying the amino acids into the cell interior (Christensen & Riggs, 1952; Ames, 1956; Heinz, 1957). There may also have been an increase in the secretion of antidiuretic hormone. Little & Radford (1964) suggested that the consumption of high protein diets by rats elicits an increase in plasma antidiuretic hormone activity which may be maintained for 19hr after feeding.

A feature of the present experiment has been the demonstration that the ryegrass fed sheep were physiologically abnormal. That this was so is indicated by the low values observed for packed cell volume, haemoglobin, plasma osmolality (as indicated by sodium content) and a low excretion rate of an acid urine. The low sodium content of plasma, the small amount of sodium excreted in an acid urine and the response of plasma sodium concentration on administration of the first infusion are all consistent with a state of partial sodium depletion. Both the composition of the ryegrass and the previous infusions of amino acid containing materials may have been contributing factors.

This work also suggests that changes in the amount of amino acids reaching the duodenum may be involved in the changes in electrolyte excretion observed when sheep are changed from a diet of hay and meal to one of grass (Dobson & McDonald, 1963; Dobson, 1965; Dobson, Scott & Bruce, 1966). It was shown that the sodium retained by sheep during a period of hay and meal feeding was rapidly lost in the urine on changing to a grass diet. Reverse changes in potassium retention were observed. The similarity between the results of Dobson and co-workers and those reported here is emphasised by the report (Annison, Lewis & Lindsay, 1959) that such a dietary change is accompanied by a marked peak in α amino nitrogen concentration in the rumen on the first day of grass feeding.

While results obtained under conditions of fasting and water restriction may not be applicable to a situation where both feed and water are freely available, the present observations support the previously advanced contention that infusion of amino acid containing materials elicits an alteration in electrolyte metabolism. Rewarding though this field of investigation appears to be, it seemed necessary in this thesis to examine the effects of major end products of nitrogen metabolism on gut function and food intake. The results of such a study are presented in the following chapter.

CHAPTER 5

THE EFFECTS OF SOME NITROGENOUS COMPOUNDS ON RETICULORUMEN MOTILITY

INTRODUCTION

Urea is the major product of protein metabolism. It has additional significance in the ruminant in that appreciable quantities enter the rumen by way of the saliva and by diffusion from the blood through the rumen wall (Phillipson, 1964). This recycled urea provides a significant portion of the nitrogen requirements of the rumen micro-organisms when the sole diet of the host animal is one of low nitrogen content (Egan, 1964).

It has been unequivocally established that supplements of urea added to the diet or administered directly to the rumen are effective in increasing the consumption of low quality roughages (Campling et al., 1962; Coombe & Tribe, 1963). It has also been established that there is a limit to the amount of urea that can be safely added to the rumen. Excessive amounts may result in reduced food intake (Coombe, Tribe & Morrison, 1960; Coombe & Tribe, 1963; Phillips & Dyck, 1964), impaired reticulorumen motility (Clark & Lombard, 1951, Weiss, 1953; Coombe et al., 1960), and eventually, the appearance of the more pronounced signs of urea toxicity which have usually been attributed to the production of excessive amounts of ammonia in the rumen (Clark, Oyeart & Quin, 1951; Coombe et al., 1960; Lewis, 1960).

It has been previously reported in this thesis that infusions of amino acid containing materials may reduce food intake and the excretion of faecal dry matter. The absence of rumination on administering intravenous infusions of casein hydrolysate and intraduodenal infusions of an amino acid mixture was also reported. It was suggested that these observations indicated an alteration of gut motility. Such an alteration might be caused by an increase in the amount of urea recycled to the rumen consequent on an enhancement of blood urea levels. For this reason it was thought desirable to examine the effects of urea administered at different points along the digestive tract on reticulorumen motility and food intake. In view of its unpleasant taste, additions of urea to the diet were avoided.

The psychic, tactile, chemical and other stimuli experienced by the feeding or replete animal contribute to the effective stimulation of contractions of the reticulum and rumen (Titchen & Reid, 1965). This suggests that the fasted animal may constitute a sensitive preparation for assessing the effects of urea on gut motility. In part, this was the reason for undertaking the initial experiments described in the present chapter, which were concerned with the effects on reticulorumen motility of administering urea to fasted sheep by the intraruminal and intraduodenal routes. Subsequently, the effects on food intake and reticulorumen motility of intraruminal infusions of urea and intraduodenal infusions of casein and an amino acid mixture were examined. A discussion has been undertaken of a number of possible sites and mechanisms of action of the stimuli accorded by both urea and the amino acid preparations. It is concluded that the effects of these infusions may be due in part to disturbances of electrolyte and water metabolism.

MATERIALS AND METHODS

Animals. Of the ten sheep used, five were fitted with duodenal cannulae as described in Chapter 2, and six with rumen cannulae (Jarrett, 1948) while seven had partial exteriorisations of the reticulum and rumen (Titchen, 1958; Reid, 1963).

Diet, feeding and housing were as described in Chapter 2 except that sheep used in a fasted condition were maintained on a medium quality meadow hay (2.5% of D.M. as nitrogen). The sheep had free access to water but were fasted 20 -24hr before an experiment.

Infusions. Apparatus and solutions were as described in Chapter 3. In addition, urea (B.D.H., AR grade), in aqueous solution was administered into the rumen as a single dose with the aid of a syringe, the nozzle of which was fitted with a length of polythene tubing (60cm long, 2mm i.d.). The tubing was passed through the cannula and guided to different regions of the rumen during the short (1 - 2min) injection.

Recording. Kymographic records of the motility of the reticulum and rumen were obtained by attaching to the exteriorisations threads connected to the writing pens. A system of jockey pulleys was used to reduce artefacts caused by body movements (Reid & Titchen, 1959). On some occasions rumen motility was recorded with the aid of a small partially inflated balloon placed in the dorsal sac of the rumen and connected by rubber tubing to a recording tambour. The times spent eating, idling and ruminating were obtained from tracings of jaw movements recorded as described in Chapter 3. The times spent lying and standing were by observation or from kymographic records obtained with the aid of a balloon-tambour system arranged so that pressure in a balloon, occasioned by the animal lying in its sling, was recorded.

Rumen liquor samples (10-20ml.) were obtained by removing ingesta from various regions of the rumen with a pair of forceps or by gentle suction through a rubber tube. The liquor was expressed through muslin. Sub-samples for the estimation of ammonia were treated with an equal volume of 0.1N HCl.

Analytical. pH of rumen liquor was determined within 2min of sampling with the aid of a Beckman model 76 or a Radiometer model 23 pH meter.

Ammonia content of rumen liquor was estimated with the method outlined by Johns (1955) and blood CO_2, urea and ammonia content with those of Conway (1957). Urease (0.5ml.), prepared by crushing two tablets of a urease preparation (B.D.H.) in 10ml. of a 1 in 10 dilution of the buffer described by Conway (1957), was used to liberate the ammonia from the urea.

RESULTS

Experiments with fasted animals

Gross signs of urea toxicity may be readily induced by the intraruminal administration of relatively large amounts (10-40g) of urea (Clark et al., 1951; Lewis, 1960). The main objective of the present experiments was to study the effects on reticulorumen motility of administering urea in amounts that were insufficient to induce these signs. In all experiments undertaken, observations on various blood and rumen liquor parameters were made in addition to those on reticulorumen motility.

(a) Effects of single doses of urea administered into the rumen

On five occasions, water (100ml.) containing 2-6g urea was administered as a single intraruminal dose to four sheep. The effect of the procedure on reticulorumen motility is summarised below:

TABLE 38. The effect of infusing urea into the rumen on frequency of reticulum contractions and some blood and rumen parameters.

Sheep No.	Urea infusion rate (mg/kg B.W./min)	No. of contractions/min during 60min commencing A 30min before start of infusion	B 300min after start of infusion	Difference (A - B)	Levels at 360min after start of infusion of Rumen liquor pH	NH$_3$ (mg NH$_3$-N /100ml.)	Blood CO$_2$ (vols %)	Urea (mg/100ml.)
58	0.76	0.72	0.63	0.09	8.05	50.0	51.0	63.7
43	0.70	0.91	0.73	0.18	8.70	74.7	59.0	60.2
H	0.71	0.64	0.39	0.25	8.25	-	-	-
50	0.81	0.57	0.25	0.32	8.30	-	-	-
58	1.14	0.77	0.40	0.37	8.50	64.1	54.2	72.0
H	0.68	0.67	0.22	0.45	8.20	55.2	65.0	60.0
55	1.02	0.75	0.28	0.47	8.20	66.7	57.9	56.8
50	0.77	0.66	0.13	0.53	8.30	45.3	63.0	51.9
50	0.62	0.73	0.12	0.61	8.20	50.5	62.6	49.1

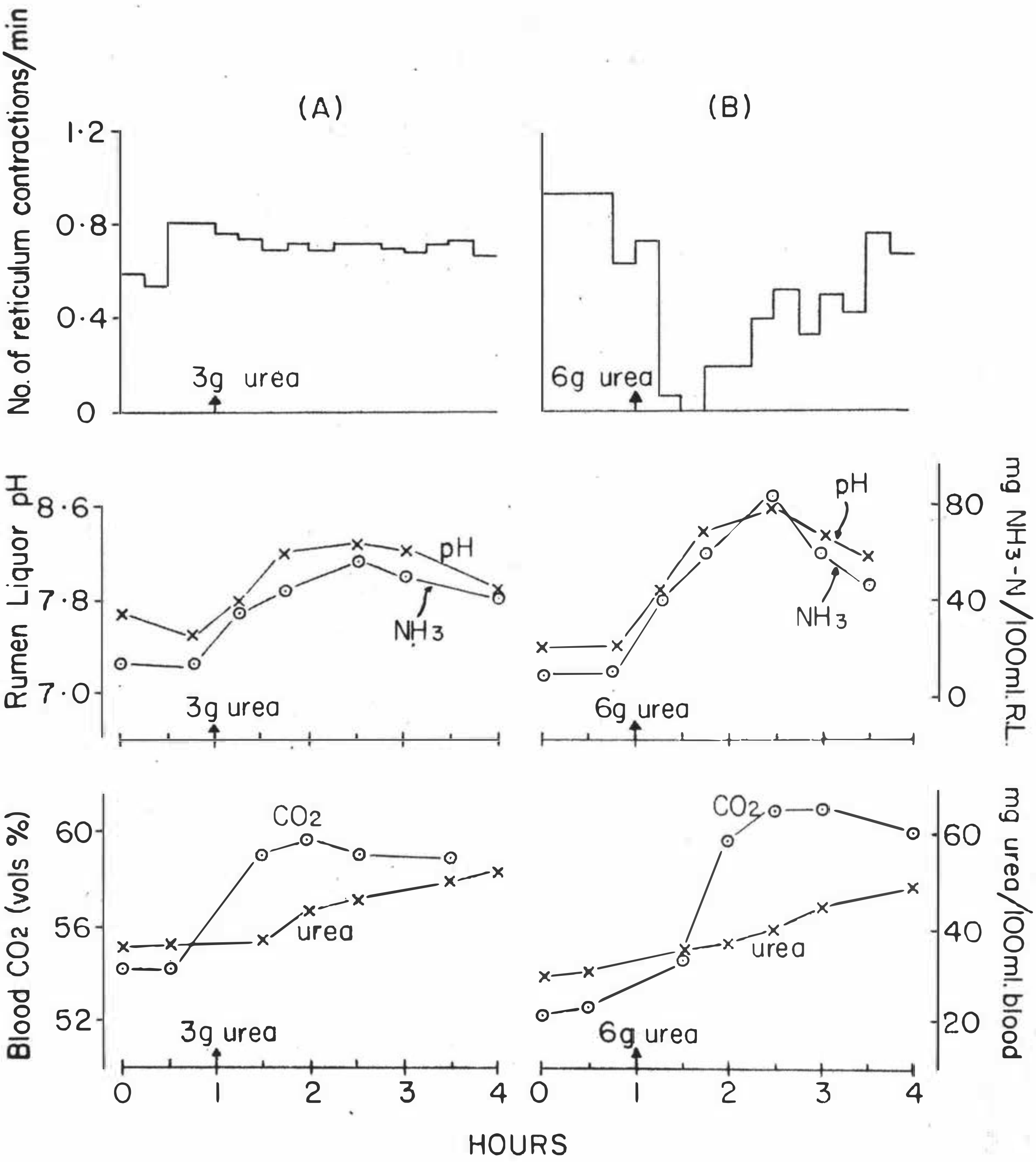

Fig.11. Sheep 55, 23.5kg, fasted 24hr. The effect of injecting 100ml. water containing (a) 3g and (b) 6g urea into the rumen (as at arrow) on the frequency of reticulum contraction, pH and ammonia levels of rumen liquor, CO_2 and urea content of venous blood. Blood ammonia content remained less than 75 µg NH_3-N/100ml. throughout. Contractions of the reticulum which occurred during any attempted periods of rumination (which totalled less than 5min) are not included. Note the marked reduction of motility that occurred when 6 but not 3g urea were injected.

Sheep No.	Bodyweight (kg)	Amount of urea dosed (g)	Time after dosing until onset of inhibition (min)	Time after onset of inhibition until occurrence of a contraction (min)
43	24.5	2.0	26	48
55	23.5	3.0	No inhibition	-
58	21.0	4.0	17	12
50	26.0	5.0	42	55
55	23.5	6.0	16	30

With one exception, there was a sudden inhibition (latency, 16-42min) of the major contractions of the reticulum and rumen which persisted for 12-55min before the reappearance of a reticulum contraction. Thereafter, the frequency of contractions increased in an irregular manner towards an apparent normal motility. During recovery, abnormal forms of motility were seen; on one occasion a series of reticulum contractions not accompanied by rumen contractions was observed.

In Sheep No.55, 6 but not 3 g urea resulted in an inhibition of motility. The results of those two experiments are presented in detail in Fig.11. As with the other sheep, the maximum effect on motility did not coincide with the maxima of any of the blood and rumen liquor parameters measured. The effects of the 3g dose on those parameters were similar in nature but lesser in magnitude than those of the larger dose.

(b) Effects of infusions of urea into the rumen

In nine experiments, 6-10g urea were infused into the rumen over a 6hr period. In each instance, a priming dose of 1g urea in 50ml. of water was injected through the rumen cannula immediately before the start of the continuous infusion (1-2ml./min of a 2% urea solution). The results are summarised in Table 38; those of four consecutive experiments (two control (no-infusion) and two infusion days) with one sheep are presented in more detail in Fig.12 and 13.

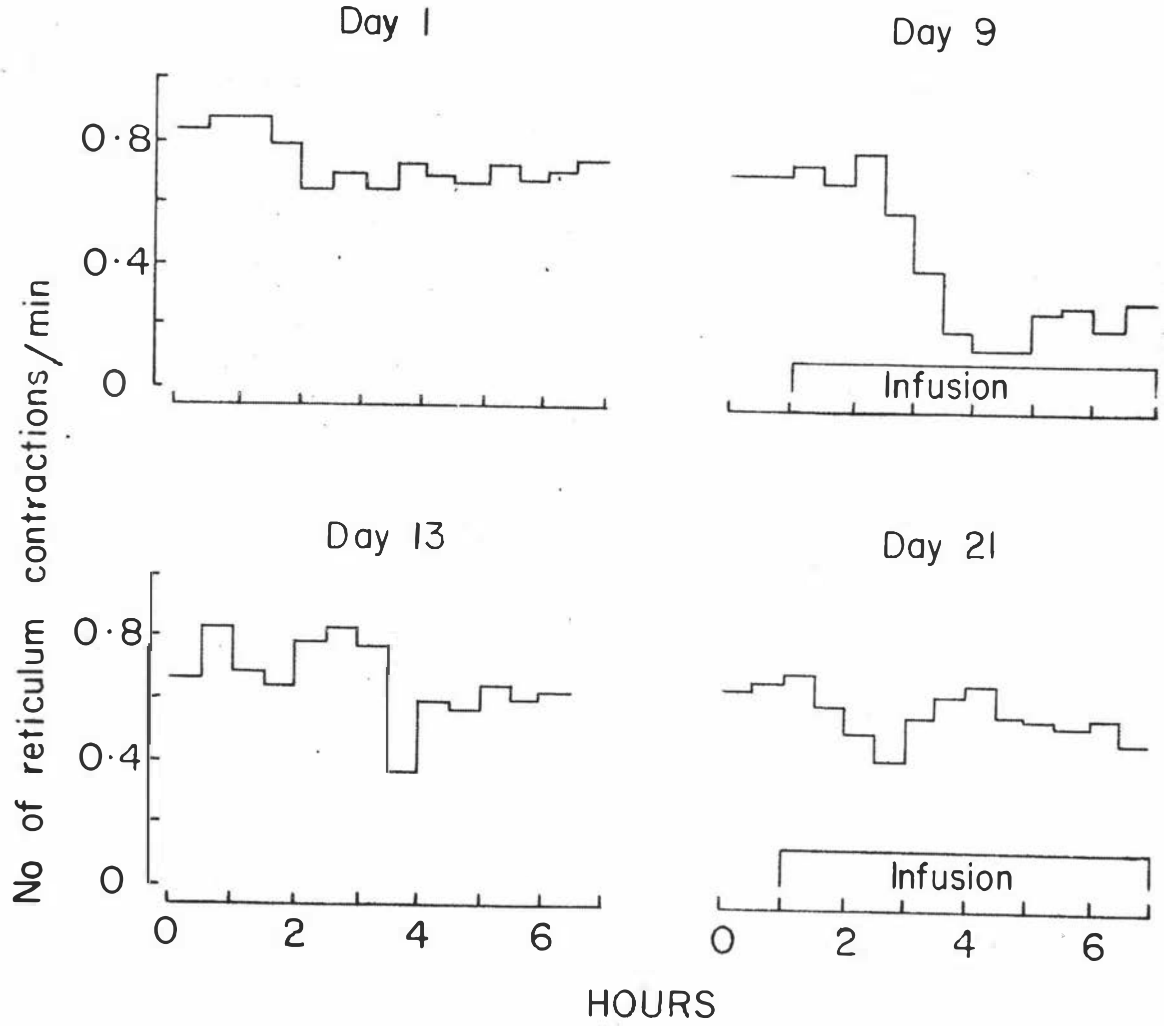

Fig.12. Sheep 58, 21kg, fasted 24hr. The effect on frequency of reticulum contraction of a continuous 6hr infusion of a 2% urea solution into the rumen. Infusion rate on Day 9, 23.8 and on Day 21, 15.8mg urea/min. Each infusion preceded by an intraruminal injection of 50ml. water containing 1g urea at the start of the infusion. No infusion on Days 1 and 13. Contractions of the reticulum which occurred during any attempted periods of rumination (which totalled less than 5min on any one day) are not included. Note the gradual reduction of motility that was occasioned by the higher dose rate.

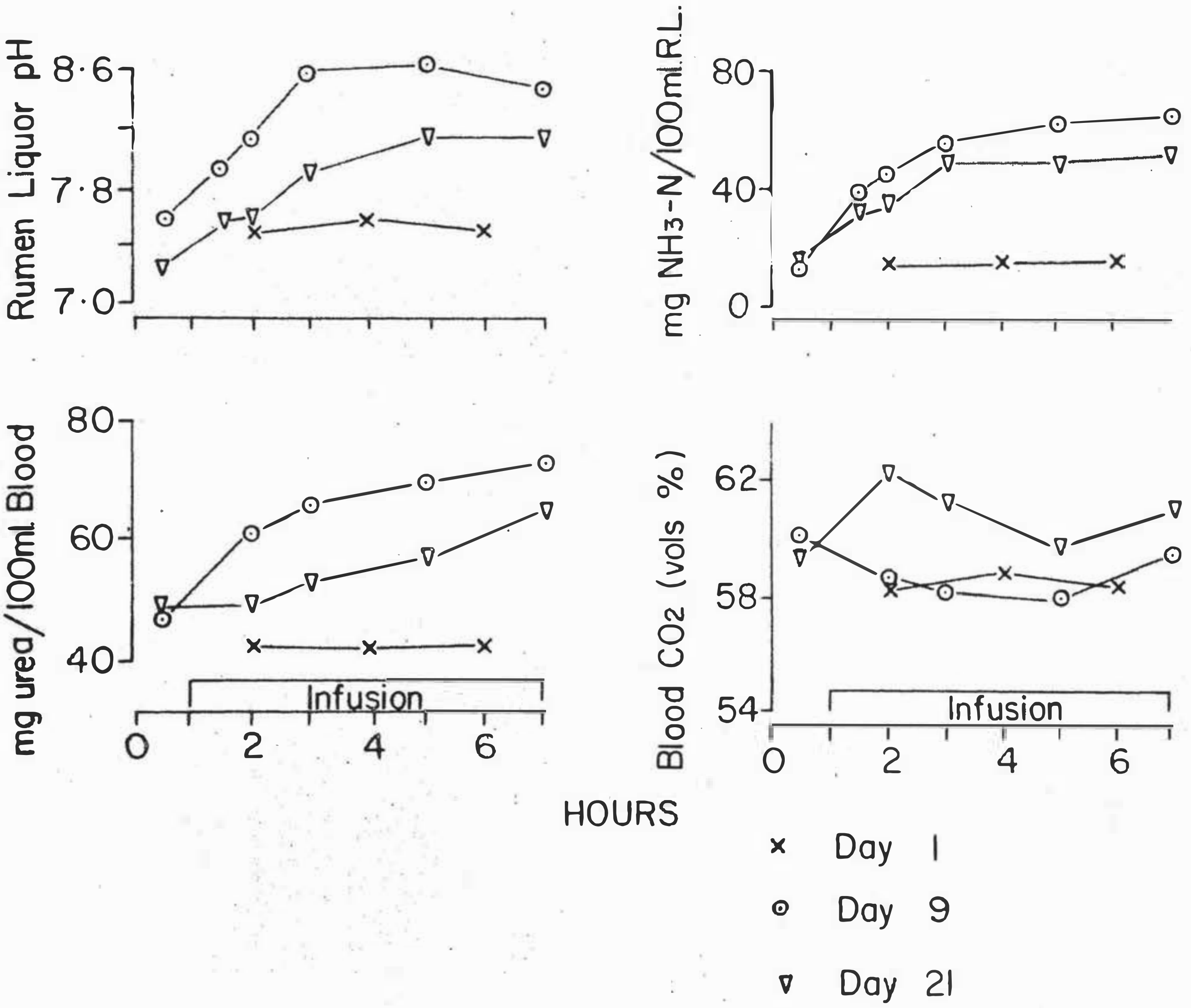

Fig.13. Changes in ammonia content and pH of rumen liquor, CO_2 and urea content of venous blood during the experiments shown in Fig.12. Results from only one control day (Day 1) are shown.

As judged by the effect on reticulorumen motility, there was a marked difference between sheep in their susceptibility to urea. In addition, the susceptibility of those sheep given more than one infusion of urea may not have been constant. When a change of motility was observed, it consisted of a gradual reduction in the frequency of contraction from an initial level of 0.5-0.9 contractions/min to 0.1-0.4 contractions/min 4-6hr after the start of the infusion. Periods of varied frequency were often observed at that stage.

There was no obvious relationship between the magnitude of the reduction in motility and any of the blood or rumen liquor parameters measured. Blood ammonia content did not rise above 100μg NH_3-N/100ml.

The frequency of reticulum contraction was generally greater when the sheep were standing than when they were voluntarily suspended or lying in their sling, a decrease in height of approximately 10cm from the standing position. This is apparent when the data of Fig.12 are represented in tabular form:

Day	Rate of urea infusion into the rumen (mg/min)	Recording time (min)	Time spent standing as per cent of recording time	No. of reticulum contractions/min during: Standing	Lying
1	0	402	80.4	0.79	0.54
9	23.8	403	69.2	0.58	0.21
13	0	334	68.3	0.75	0.55
21	15.8	418	66.7	0.66	0.56

These differences were often accentuated during the infusion of urea, most noticeable as in the above instance, at the higher dose rates of urea.

Irrespective of any effects on reticulorumen motility the urea may have had during its infusion, the presentation of fresh food at the end of the infusion was immediately followed by vigorous feeding and a

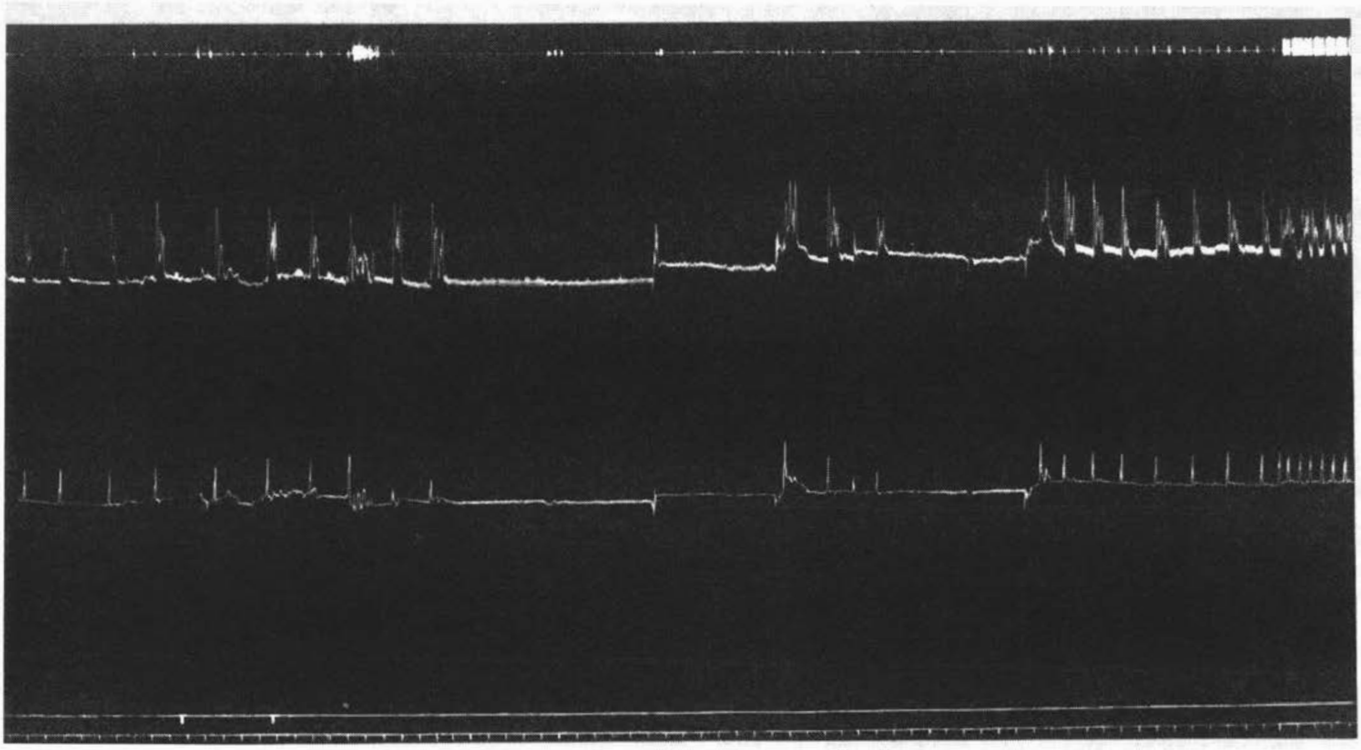

Fig. 14. Sheep 29.5kg, fasted 20hr. Inhibition of reticulum and rumen contractions on injection as at signal of 200ml. isotonic $NaHCO_3$ (14.0g/l.) into the jugular vein. Sequence of records from above downwards: jaw movements, anterior dorsal rumen, reticulum, signal, time marker 1 min.

frequency of contraction of the reticulum at a rate of up to 2.5 contractions/min. However, when feeding followed an infusion that had depressed reticulorumen motility, an abnormally low frequency of contraction was often seen when the sheep was idle in the 10-16hr after the food was offered.

(c) Effects of intraduodenal infusions of urea

A continuous intraduodenal infusion of a 4% urea solution (0.04g urea/min, equivalent to 1.63-1.90mg urea/kg B.W./min) for 6-8hr was without effect on motility. The levels of urea in the blood at the end of the three experiments performed were 102, 106 and 112mg urea/100ml. There was no appreciable difference as regards the pH or ammonia content of rumen liquor or the CO_2 content of venous blood between samples taken before and at the end of the infusions.

(d) Effect on motility of an intravenous injection of $NaHCO_3$

Three sheep were each given an injection of isotonic $NaHCO_3$ through a catheter inserted in the jugular vein. An inhibition was observed on one occasion (Fig.14). The sheep concerned had been previously used for a number of experiments involving the administration of urea into the rumen, the last of which was eight days previous to the present experiment. Similar injections had no effect on the reticulorumen motility of the other two sheep, neither of which had been previously given urea.

Experiments with feeding animals

(a) Effects on food intake, faecal dry matter output and reticulum motility of intraruminal infusions of urea

Intraruminal infusions of urea reduced both food intake and faecal dry matter output (Fig.15). The reduction of food intake

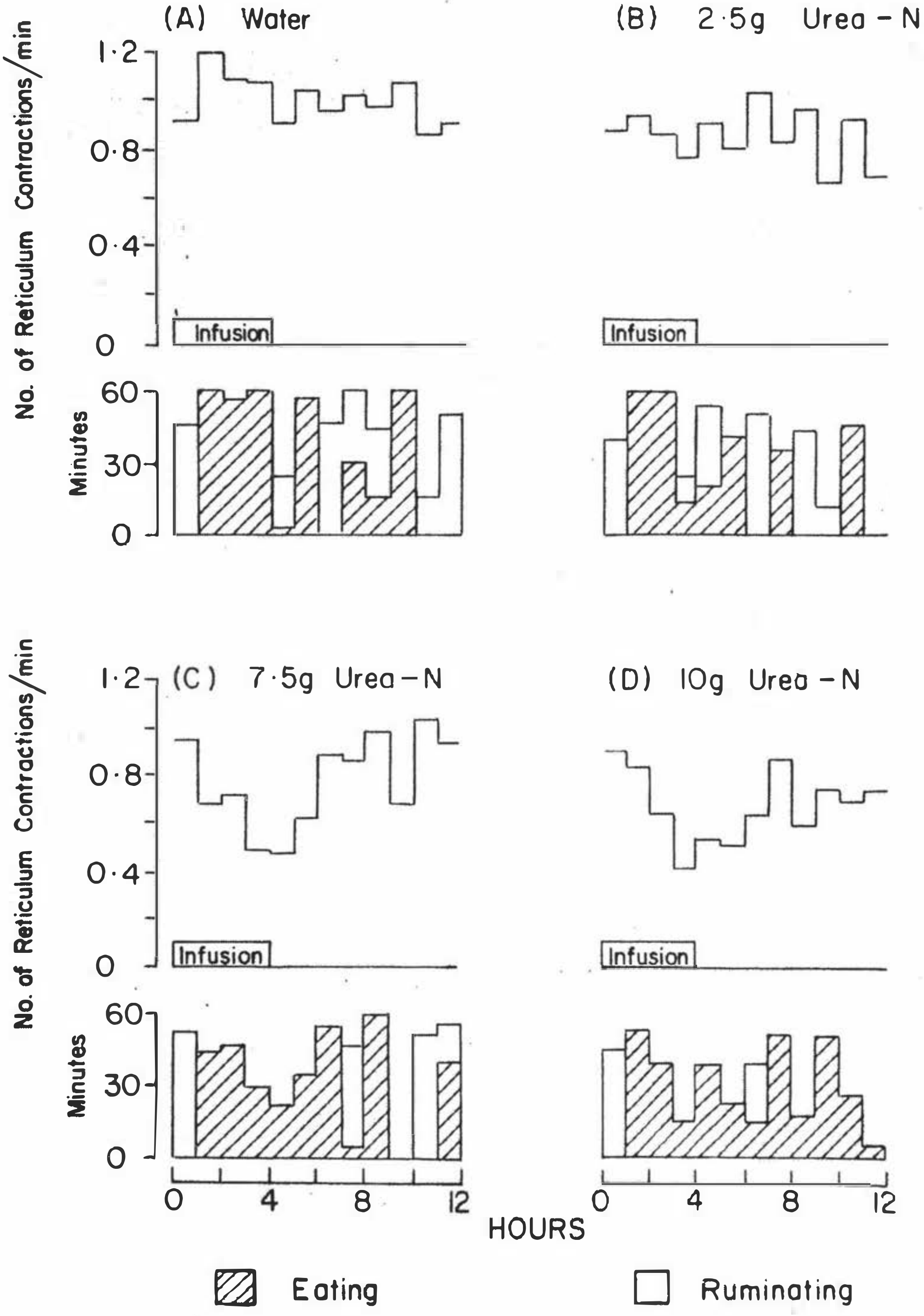

Fig.17. The frequency of reticulum contraction and the time spent each hour in eating, ruminating and idling during the four infusion days of the experiment shown in Fig.16. The data are for the 12hr commencing at the start of infusion.

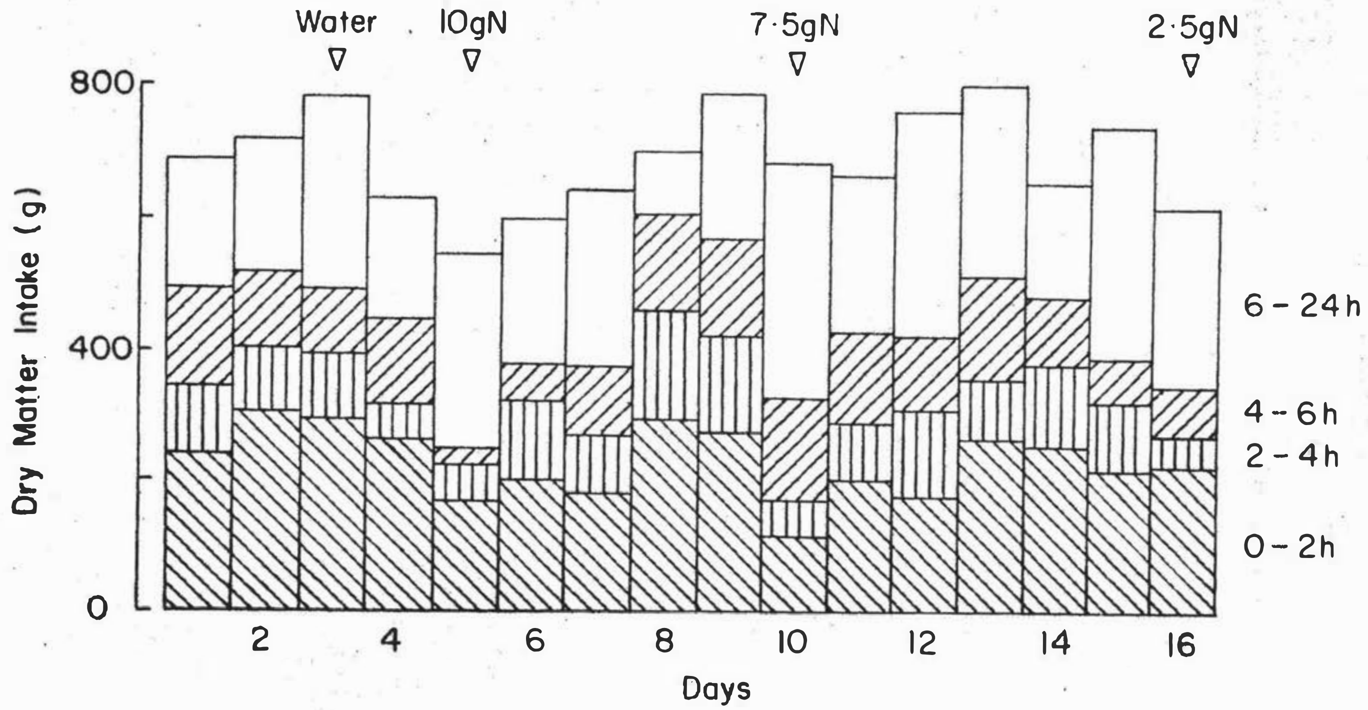

Fig.16. The effect on food intake of infusions of 750ml. water containing urea equivalent to 0, 2.5, 7.5 and 10g nitrogen into the rumen of one sheep (29.5kg). The time of presentation of fresh food is denoted as 0hr; the infusions commenced 1hr before that time and were for 4hr.

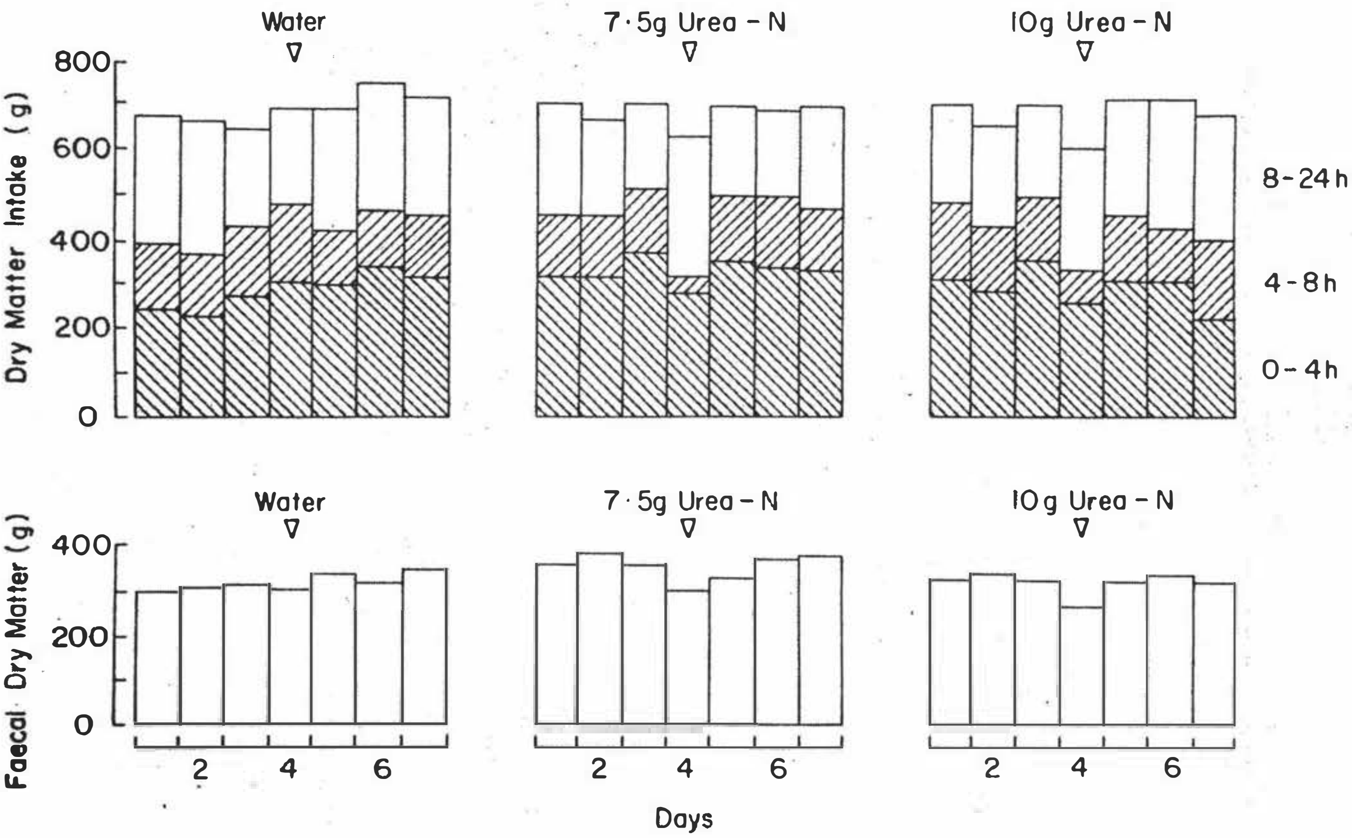

Fig.15. Effect on food intake and faecal dry matter output of intraruminal infusions of 750ml. water containing urea equivalent to 0, 7.5 and 10g nitrogen. Mean of three sheep. The infusions were for 8hr commencing at the time of presentation of fresh food and were applied according to a 3 x 3 Latin Square arrangement with a five day interval between treatment application.

occurred mainly during the 8hr infusion period and was partly compensated for by an increase during the subsequent 16hr. This contrasted with the observation made when casein (70g, equivalent to 10g nitrogen) in 750ml. of water was infused in a similar manner to that of urea: it had no detectable effect on either food intake or faecal dry matter output of the same three sheep.

The effects on food intake and reticulum motility of intraruminal infusions of urea and the relationship between these two parameters were examined in one animal. The food intake of that animal was reduced on the days of urea infusion (Fig.16). The reduction was particularly marked 2-4hr and, in the case of the highest rate of urea infusion, 4-6hr after the time that fresh food was presented. On that occasion the effect on food intake persisted for 2-3 days, for it was not until then that a level of intake similar to that recorded on the days before infusion was observed. The frequency of reticulum contraction was reduced when urea equivalent to 7.5 and 10g nitrogen was infused, reaching a minimum at or shortly after the end of infusion (Fig.17). In the case of the highest nitrogen infusion rate, the frequency was still below normal at the end of the 12hr recording period (Fig.17d). The frequency of contraction recorded on infusion of the lowest nitrogen infusion rate (2.5g, Fig.17b) was generally lower than that recorded on the day that water was infused (Fig.17a).

The time spent ruminating was reduced and eating was more evenly spread over the 12hr recording period when urea was infused at the two highest dose rates than on the day the water was infused. These effects are apparent when the data of Fig. 17 are represented in tabular form:

Rate of infusion (g N/4hr)	Total recording time (min)	Time spent at each activity as per cent of total recording time			No.of reticulum contractions/min for:			
					Each activity			12hr recording period
		E *	R	I	E	R	I	
0	720	47.6	33.5	18.9	1.10	1.02	0.77	1.01
2.5	716	39.1	27.0	33.9	0.94	1.04	0.65	0.87
7.5	716	47.5	22.6	29.9	0.85	0.99	0.50	0.78
10.0	708	46.1	9.7	44.2	0.76	0.91	0.52	0.67

*E = eating, R = ruminating and I = idling

These data suggest an inverse relationship between the amount of urea infused and the frequency of reticulum contraction during eating, idling and for the 12hr period as a whole. In contrast, the frequency of reticulum contraction during rumination was affected only slightly.

The infusion of urea equivalent to 10g nitrogen, appeared to have a persistent effect for, on the days following that infusion, the frequency of reticulum contraction progressively increased at a rate similar to that of the increase in food intake (Table 39). A frequency comparable to that recorded on the pre-infusion days was observed on the fourth day after infusion.

TABLE 39. Time spent eating (E), ruminating (R) and idling (I) and the frequency of reticulum contraction during each activity for 8hr commencing 1hr before the start of feeding on the day of infusion of urea equivalent to 10g nitrogen into the rumen (Day 5) and for comparable times on days before and after that of infusion.

Day	Total recording time (min)	Time spent at each activity as per cent of total recording time			No.of reticulum contractions /min for:			
					Each activity			8hr recording period
		E	R	I	E	R	I	
1	424	64.8	27.4	7.8	1.10	1.11	0.76	1.08
2	439	61.0	23.0	16.0	1.13	1.08	0.71	1.05
3	480	55.9	30.4	13.7	1.11	1.03	0.74	1.04
4	444	42.1	35.6	22.3	1.07	1.03	0.79	0.99
5	468	49.0	14.8	36.2	0.76	0.91	0.41	0.66
6	460	71.6	21.7	6.7	0.97	1.00	0.61	0.95
7	467	60.2	11.3	28.5	0.97	0.98	0.73	0.90
9	443	57.0	23.6	19.4	1.10	1.06	0.76	1.03

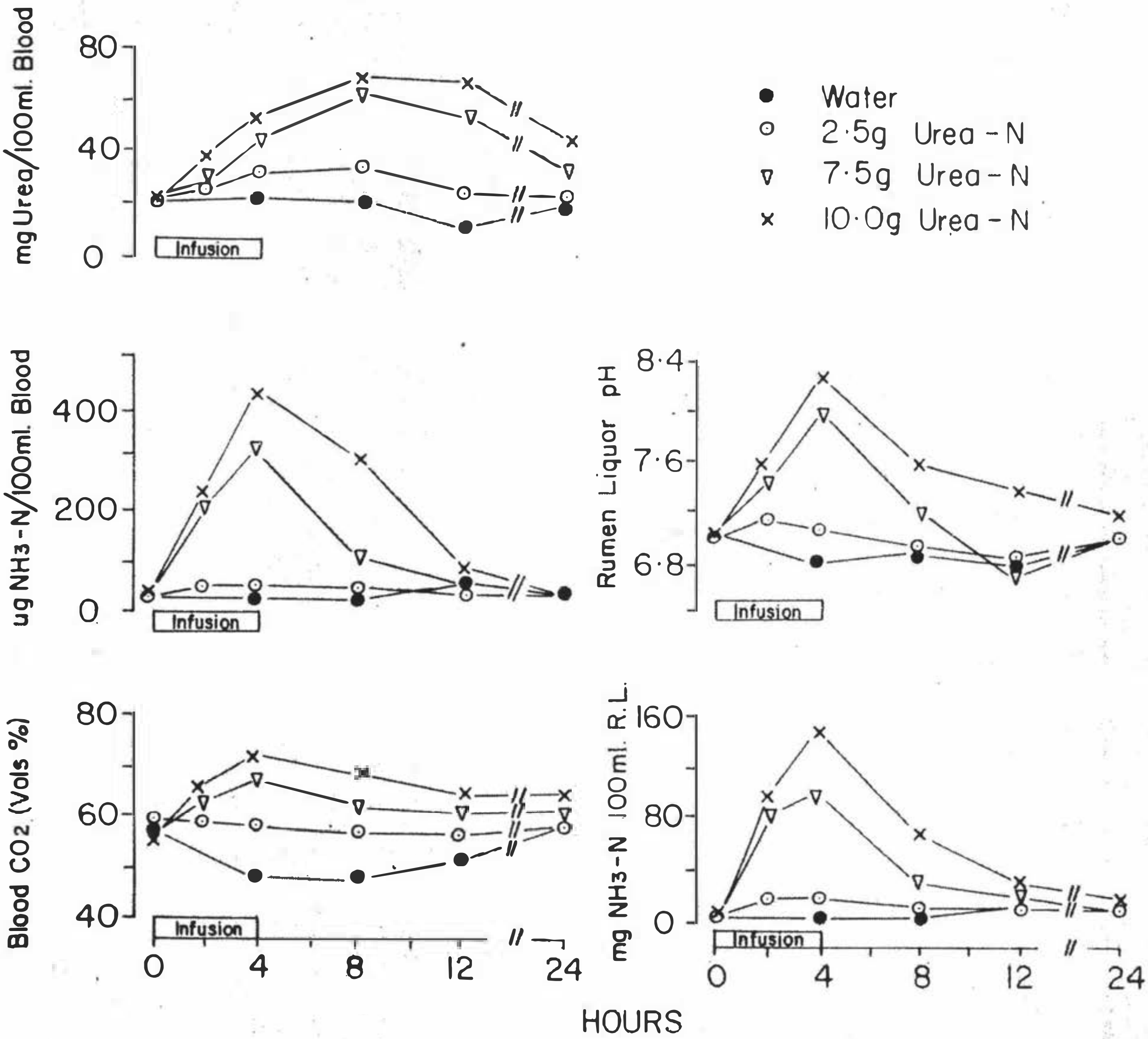

Fig. 18. Changes in ammonia content and pH levels of rumen liquor, urea, CO_2 and ammonia content of venous blood in the experiment shown in Fig. 16 and 17.

The observed maxima of ammonia and pH levels of rumen liquor were at the end of the infusion period (Fig.18). In contrast, the highest levels of urea in the blood occurred 4-8hr after the end of infusion. In each instance, the magnitude of the increase was related to the amount of urea infused.

(b) The effects of intraduodenal infusions of casein on reticulum motility

From the results of Experiment 6 and 8 (Chapter 2) it might be expected that the intraduodenal infusion of a relatively large amount of casein in a small volume of vehicle that was hypertonic relative to duodenal contents would occasion a decrease of food intake. Conversely, it might be expected that the intraduodenal infusion of a relatively small amount of casein in a large volume of hypotonic vehicle would increase food intake. It was reasoned that if the intraduodenal infusion of casein was to affect gut motility, then the effect may be enhanced by one or both procedures. On this basis, two contrasting treatments were compared. They were:

(i) Casein (100g) in 750ml. hypertonic Blaxter's Saline (398 mosm/kg water) and,

(ii) Casein (50g) in 1500ml. hypotonic Blaxter's Saline (106 mosm/kg water).

The control was the 24hr period immediately preceding the start of the infusions. The infusion period was for 8hr (0-8hr) commencing at the time of presentation of fresh food. The sheep (No.87, 29kg B.W. and No.112, 30kg B.W.) were used alternately and the sequence of infusions was selected randomly. Jaw movements and motility of the reticulum were recorded for a 48hr period commencing 24hr before the start of an infusion.

TABLE 40. Food intake, time spent eating (E), ruminating (R) and idling (I) and the frequency of reticulum contraction for each activity for two sheep on the day before (Day 1) and the day of (Day 2) intraduodenal infusions of casein or Blaxter's Saline. Infusions were for an 8hr period starting at the time of presentation of fresh food.

Sheep No.	Day	Food intake (g DM)	Total recording time (min)	Time spent at each activity as per cent of total recording time			No. of reticulum contractions/min for: each activity			24hr period
				E	R	I	E	R	I	
(a) Casein (100g) in 750ml. hypertonic Blaxter's Saline										
87	1	726	1349	16.2	47.1	36.6	1.49	0.99	0.78	0.99
	2	597	1428	16.0	49.2	34.9	1.32	0.88	0.66	0.87
112	1	660	1416	19.2	44.4	36.4	1.10	0.85	0.69	0.84
	2	581	1438	21.3	46.5	32.1	1.02	0.83	0.59	0.79
(b) 750ml. hypertonic Blaxter's Saline										
87	1	613	1374	17.8	51.0	31.2	1.35	0.94	0.78	0.96
	2	543	1434	19.0	46.7	34.2	1.34	0.97	0.82	0.99
112	1	800	1417	22.5	45.0	32.5	1.34	0.97	0.86	1.02
	2	845	1376	26.2	44.7	29.1	1.26	0.97	0.82	1.00
(c) Casein (50g) in 1500ml. hypotonic Blaxter's Saline										
87	1	597	1368	19.5	43.0	37.5	1.29	0.92	0.73	0.92
	2	759	1432	23.0	49.0	28.0	1.24	0.93	0.76	0.96
112	1	746	1426	24.3	43.1	32.7	1.21	0.95	0.76	0.95
	2	937	1434	27.7	46.5	25.8	1.17	1.05	0.82	1.02
(d) 1500ml. Hypotonic Blaxter's Saline										
87	1	723	1436	22.4	43.6	34.0	1.34	0.95	0.81	0.99
	2	783	1436	20.4	47.9	31.7	1.51	1.01	0.86	1.06
112	No data available									

The results are presented in Table 40. When for any one treatment, the results for the day of infusion (Day 2) are compared with those for the day before infusion (no-infusion control, Day 1), the data may be summarised as follows:

(a) The infusion of the preparation containing 100g casein was accompanied by a decrease of both food intake and frequency of reticulum contraction (Table 40a). The effect on motility was observed irrespective of the activity of the sheep.

(b) The infusion of the vehicle alone had no obvious effect on motility even though in one instance it was accompanied by a reduction of food intake (Table 40b).

(c) Neither the infusion of the preparation containing 50g casein nor the infusion of the hypotonic vehicle alone had marked effects on reticulum motility. Both of these treatments were accompanied by an increase of food intake.

(d) An obvious effect on the times spent at each activity was not observed with any of the infusions.

(c) Effect of intraduodenal infusions of an amino acid mixture on reticulum motility, food intake and faecal dry matter output

Two sheep were used. The observations on one (Sheep No.112) were made during Experiment 11 described in Chapter 3 while those on the other (Sheep 87) were made on completion of that experiment. The solutions and their infusion were as described for that experiment.

TABLE 41. Time spent eating (E), ruminating (R) and idling (I) and the frequency of reticulum contraction during each activity for two sheep on the day before (Day 1), the day of (Day 2) and the day after (Day 3) the intraduodenal infusion (0-8hr, Day 2) of an amino acid mixture (1500ml. 0.3g amino acid N/kg B.W.).

Day	Time of day (hr)	Total recording time (min)	Time spent at each activity as per cent of total recording time.			No.of reticulum contractions/min for each activity		
			E	R	I	E	R	I
				Sheep No.112				
1	0- 8	480	53.7	16.7	29.6	1.28	1.04	0.84
	8-16	476	18.3	52.7	29.0	1.34	0.97	0.85
	16-24	461	14.3	47.7	38.0	1.30	0.88	0.80
	0-24	1417	29.0	38.9	32.1	1.30	0.95	0.82
2	0 -8	480	8.1	10.6	81.3	0.85	0.84	0.21
	8-16	480	13.7	0	86.3	0.73	-	0.12
	16-24	462	19.5	41.1	39.4	0.96	0.96	0.59
	0-24	1422	13.6	17.0	69.4	0.86	0.93	0.24
3	0- 8	480	43.4	23.7	32.9	0.92	0.97	0.66
	8-16	464	24.6	45.7	29.7	1.01	0.93	0.65
	16-24	465	1.7	59.6	38.7	1.13	0.82	0.66
	0-24	1409	23.4	42.8	33.8	0.96	0.89	0.66
				Sheep No. 87				
1	0- 8	476	41.8	23.3	34.9	1.33	0.95	0.79
	8-16	480	15.6	52.3	32.1	1.41	0.97	0.85
	16-24	480	10.0	55.0	35.0	1.29	0.94	0.78
	0-24	1436	22.4	43.6	34.0	1.34	0.95	0.81
2	0- 8	480	17.1	0	82.9	1.21	-	0.06
	8-16	480	7.5	16.4	76.1	0.64	1.14	0.16
	16-24	480	11.0	41.3	47.7	0.87	1.01	0.60
	0-24	1440	11.9	19.2	68.9	0.98	1.05	0.22
3	0- 8	465	44.7	12.3	43.0	1.22	1.14	0.72
	8-16	480	15.0	53.6	31.4	1.42	0.99	0.72
	16-24	480	5.2	47.1	47.7	1.00	0.99	0.65
	0-24	1425	21.4	37.9	40.7	1.25	1.01	0.69

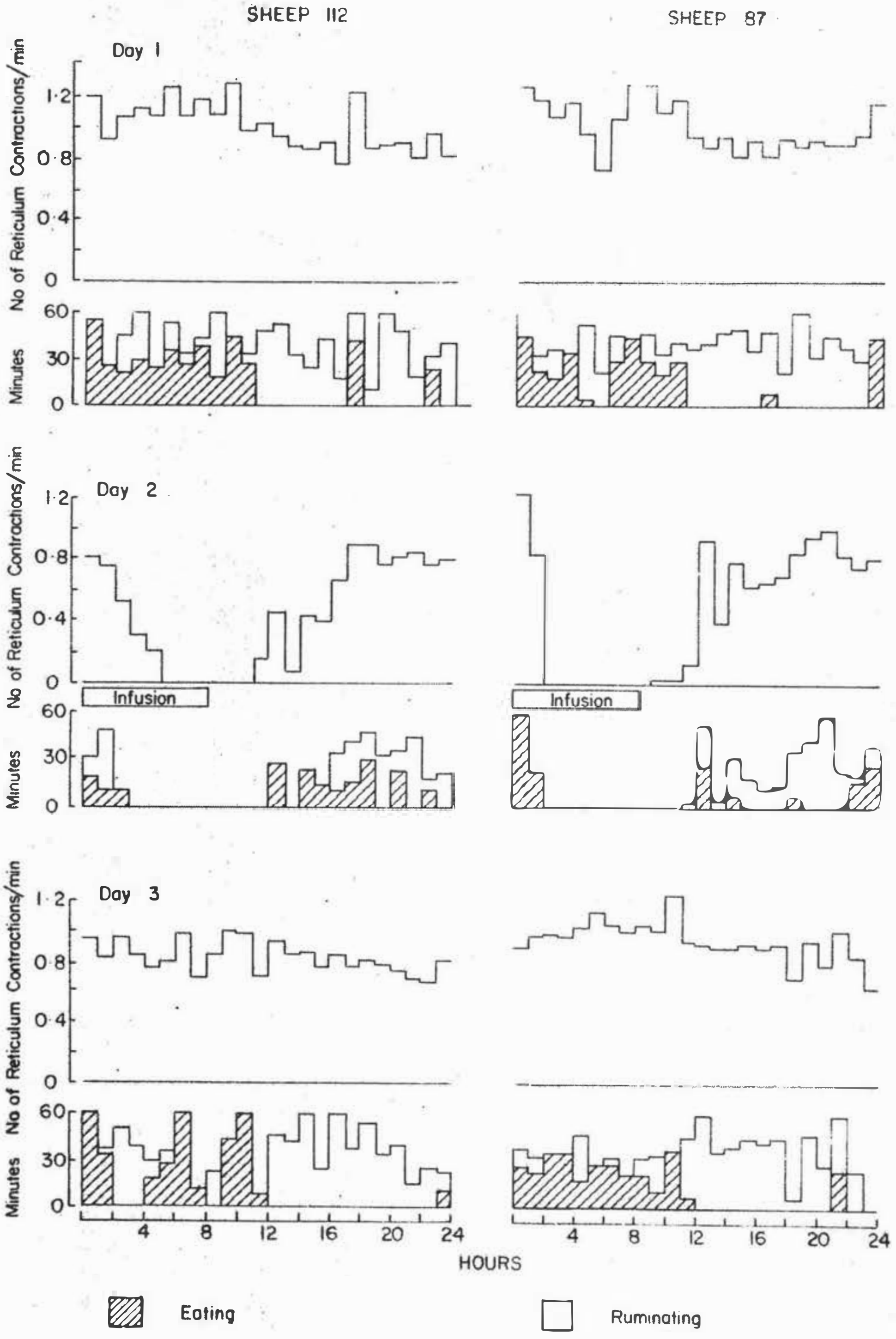

Fig.19. The frequency of reticulum contraction and the circadian pattern of eating and ruminating on the day before (Day 1), the day of (Day 2) and the day after (Day 3) the intraduodenal infusion of an amino acid mixture to two sheep. The infusions (1500ml., 17mg nitrogen/min) were for 8hr commencing at the time of presentation of fresh food.

The marked reduction in food intake and faecal dry matter output on the day of infusion of the amino acid mixture is illustrated by the following data:

Day	Food intake (g DM/day) for: Sheep 87	Food intake (g DM/day) for: Sheep 112	Faecal output (g DM/day) for: Sheep 87	Faecal output (g DM/day) for: Sheep 112
1.Day before infusion	628	789	368	455
2.Infusion day	259	290	177	243
3.Day after infusion	460	590	283	324

Less obvious effects were apparent on the day after infusion.

The infusion of the amino acid mixture had a marked effect on the reticulum motility of both sheep (Fig.19); contractions of the reticulum being absent for 6-7hr on the day of infusion. The latency of response was markedly less in one sheep than in the other. Contractions reappeared 1-3hr after the end of infusion at an irregular and increasing frequency. Neither eating nor rumination occurred during the time that contractions of the reticulum were absent; both activities recommenced after the reappearance of reticulum contractions. As judged by frequency of reticulum contraction, the effects of the infusion persisted until the day after infusion (compare Days 1 and 3, Fig.19).

The effects of the infusion on frequency of reticulum contraction were most marked while the sheep were eating or when they were idle; this was in contrast to the lack of effect when the sheep were ruminating (Table 41). These effects were most marked on the day of infusion (Day 2, Table 41). Similar but less marked effects were observed on the day after infusion (Day 3).

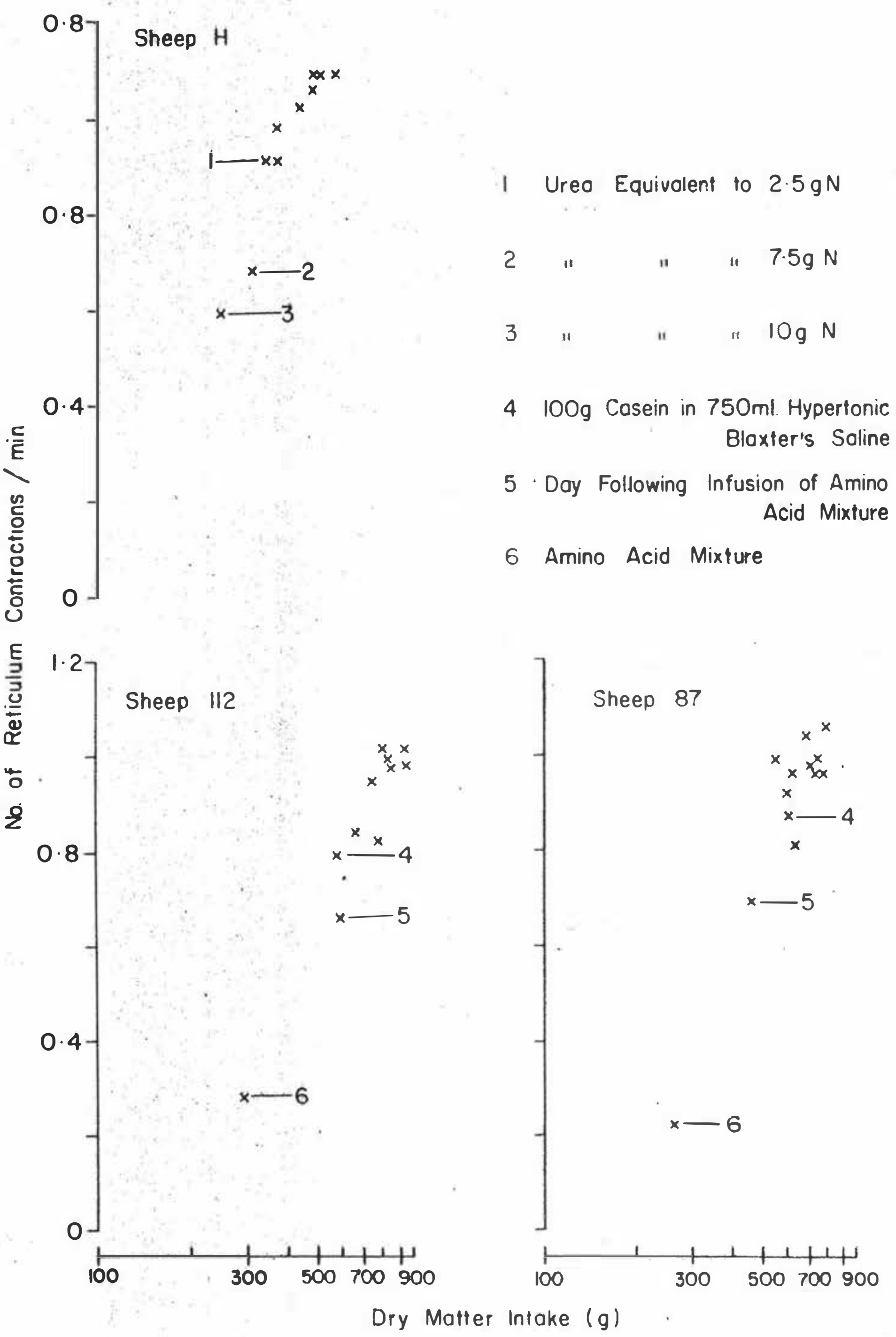

Fig.20. Relationship between the mean frequency of reticulum contractions and food intake for each of three sheep. The data for Sheep H are recalculated from the data presented in Table 38, Fig.16 and text and are for the 6hr commencing at the time of presentation of fresh food. The data for Sheep 112 and 87 are from the data presented in Tables 40, 41 and text and are for the 24hr period commencing at the time of presentation of fresh food. Note that a similar linear relationship was apparent for each sheep.

The infusion of the amino acid vehicle, Salt Solution, was not accompanied by any marked change in the frequency of reticulum contraction or activity of the sheep (Table 42). This was so even though it was accompanied by an increase in faecal dry matter output and, in one sheep, an increase of food intake.

TABLE 42. Time spent eating (E), ruminating (R) and idling (I) and the frequency of reticulum contraction on the day before (Day 1) and the day of (Day 2) and intraduodenal infusion (0-8hr, Day 2) of the amino acid vehicle (Salt Solution, 1500ml.)

Day	Total recording time (min)	Time spent at each activity as per cent of recording time			No.of reticulum contractions/min for each activity			Food intake (gDM/day)	Faecal output (gDM/day)
		E	R	I	E	R	I		
				Sheep No.87					
1	1436	22.4	43.6	34.0	1.34	0.95	0.81	716	330
2	1412	24.8	42.8	32.4	1.38	0.99	0.84	680	344
				Sheep No.112					
1	1440	28.4	47.0	24.6	1.30	0.96	0.73	857	479
2	1434	25.8	47.1	27.1	1.28	0.95	0.76	955	483

DISCUSSION

A striking result of the series of experiments described in this chapter was the demonstration that those infusions which elicited a reduction of food intake also reduced reticulorumen motility. This was so irrespective of the nature of the material administered, the route of infusion and the duration of the effect on food intake. A common relationship existed between food intake and reticulum motility regardless of whether or not an infusion was made and whether or not an infusion effected a change of food intake (Fig.20). Alternative interpretations that may be placed on these data include:

(a) That a reduction of reticulorumen motility was the primary cause of the reduced food intake. It is generally accepted but largely unproven that the motility of the reticulum and rumen assist in the transfer of digesta to the omasum and to more caudal regions of the gut (Reid, 1963). Balch & Campling (1962), Blaxter (1962) and Campling (1966) have emphasised the possible importance of flow of digesta from the reticulorumen in determining food intake.

(b) That a reduction of motility was a consequence of reduced food intake. The control of the motility of the rumen and reticulum is largely reflex in nature and the importance of afferent stimuli arising from the gut itself has been emphasised (Comline & Titchen, 1960; Titchen, 1960; Titchen & Reid, 1965). The nature of some of the receptors concerned (e.g. those sensitive to stretch and tactile stimulation of the walls of the reticulum and rumen) suggest that the efficacy of the afferent input to the medullary centres may be dependent in part on the level of food intake.

(c) That the relationship was due to indirect causes. Changes in either the level of excitability of the nervous system or in the influence of the higher centres may have had an effect that was common to both food intake and reticulorumen motility.

Evidence enabling a distinction to be made between these possibilities was not obtained in the experiments reported here. It is clear that the interrelationships between nitrogen infusions, gut function and food intake are complex.

A marked reduction of faecal dry matter output accompanied the intraduodenal infusion of the amino acid mixture. It was also observed when urea was infused into the rumen of the feeding animal. Such a reduced output could be due to a reduction in the rate of propulsion of digesta along the gut however occasioned. If this were so, then it is not obvious why a reduction of faecal dry matter output was observed in Chapter 3 when an intraduodenal infusion of casein or casein hydrolysate elicited an increase of food intake. Striking though these reductions were, a decrease of even greater proportions was recorded by Egan & Moir (1965). These workers found that the intraduodenal infusion of either urea or casein increased the consumption of a low quality roughage by sheep. Although no comment on it was made in their published paper, the data of their Table 5 reveal that the increase of food intake was accompanied by an almost equivalent reduction of faecal dry matter output.

The time course of the effects on food intake of intraruminal infusions of urea was similar to that previously observed when casein was administered by the intraduodenal route or when casein hydrolysate was given intravenously (Chapter 3). In each case, the effects occurred mainly during the period of infusion, irrespective of whether an increase or a decrease of food intake was observed. These observations emphasize that any proposed mechanism must be able to account for the rapid onset of the effects of the infusions on food intake as well as the existence of both short and long term effects. More persistent effects were observed when urea equivalent to 10g nitrogen was infused into the rumen over a 4hr period commencing 1hr prior to the presentation of fresh food. Again, effects comparable to this were observed when amino acid containing materials were administered; in this case the intraduodenal infusion of the amino acid mixture.

In the case of urea, the persistent effects may have been due either to changes in the ecological balance of the various micro-organisms in the rumen microbiota or to deleterious effects on microbial activity. On the other hand, the similarity of effects between urea and the amino acid mixture may indicate the animal itself to be involved, including perhaps, damage to the rumen epithelium or to receptors located in the walls of the reticulorumen and elsewhere in the body.

On occasions the administration of urea by either dosing or infusion was without obvious effect on the reticulorumen motility of the fasted animal. This was so even though substantial changes in blood and rumen liquor composition were detected. This may indicate that there is a threshold to the effects of urea although no evidence of this was apparent from the changes in the concentration of those blood and rumen liquor parameters that were measured.

The various effects observed during the time that a reduction of reticulorumen motility was apparent may be of some moment when considering the site and mechanisms of action of the nitrogenous infusions. The observation that abnormal forms of reticulorumen motility occurred at that time may indicate that:

(a) The effects on motility were not a consequence of a reduction of food intake.

(b) Contractions of the rumen are more susceptible to inhibition than are those of the reticulum; this is true for at least some forms of stimuli (Titchen, 1960; Reid & Titchen, 1965).

(c) The infusion was acting to inhibit some forms of motility or the contractions of some areas of the reticulorumen in preference to others. Some support for this suggestion is found in the observation that the frequency of contraction of the reticulum during rumination was relatively resistant to change. This was so during the infusion of both urea and amino acid containing material. It is emphasized however, that the incidence of rumination was markedly reduced, almost as if the infusions acted to reduce the initiation of rumination but were with little effect on the mechanisms responsible for its continuation. In order to establish these suggestions on a more convincing basis it is clear that a study of reticulorumen motility more detailed than that undertaken here is required; in particular one that enables the relative occurrence of 'A' and 'B' sequences of contraction to be assessed. Alternatively, the reduced incidence of rumination when urea was infused into the rumen of the feeding animal may be explicable in terms of taste stimulation. Urea is known to have an unpleasant taste in man. It is possible that the recycling of urea in the saliva and the intermittent regurgitation of digesta during rumination may have accorded a strong and disagreeable stimulus to receptors located in the mouth, pharynx and even the oesophagus; a stimulus which resulted in a reluctance of the sheep to ruminate or the early termination of an episode of rumination. Such a suggestion may seem unlikely in view of the report that "sheep readily consumed large amounts (up to 100g) of urea per day provided the concentration of urea in the ration did not exceed 6%" (Coombe et al., 1960). It is also to be remembered that a reduced incidence of rumination accompanied the infusion of both the amino acid mixture and the casein hydrolysate.

The magnitude of the reduction of motility was dependent in part on the dose rate of urea, the activity of the sheep and the sheep itself. The less frequent occurrence of reticulum contractions in the lying or prone animal as compared with that when the animal was in the standing position has been noted by other workers (Freer et al., 1962; Dziuk & McGauley, 1965). It is also known that the frequency of contraction of the reticulum varies according to whether the animal is eating, ruminating or idle (Balch, 1952; Reid, 1963). The tendency for the most marked effects on motility to occur during the time that the frequency of contraction was normally the least suggests that the infusions acted to accentuate effects that are characteristic of the normal animal. Even more striking was the appearance of contractions at an enhanced frequency when fresh food was offered to the fasted animal. It was as though the stimuli afforded by the presentation of food overcame a reduced level of sensitivity or increased inhibition consequent on the prior administration of urea. When eating ceased after an initial period of vigorous eating, the return to a sub-normal frequency suggests that a reduction of excitatory stimuli, associated perhaps with the partial satisfaction of "hunger", allowed the inhibitory effects of the infusion to become dominant once more. Changes in the balance of excitatory and inhibitory stimuli may explain why a higher rate of urea infusion to the feeding animal resulted in a less marked reduction of motility even though considerable quantities of ammonia were detected in the peripheral blood. By inference, the inhibitory effects of the amino acid mixture were of sufficient magnitude to bring about a complete cessation of the contractions at a time that feeding would be normally taking place.

The intraduodenal infusion of urea to the fasted animal was without obvious effect on the motility of the reticulorumen. This is in accord with the commonly held belief that urea owes its toxicity to the excessive production of ammonia consequent on its hydrolysis by the rumen microorganisms. Acid-sensitive receptors, which when stimulated, inhibit contractions of the reticulum and rumen have been postulated to occur in the reticulorumen walls (Ash, 1959). Although Iggo (1957) concluded that there are receptors in the cat stomach which respond specifically to alkaline solutions, Ash found that introduction into the rumen of buffered solutions in the pH range 7.9 - 10.1 was without marked effect on reticulorumen motility. It was suggested that the epithelium may have been impermeable to the solutions used. Ammonia readily crosses cellular barriers when in an unionised form, the extent of ionisation being less at high than at low pH (Warren, 1962). This is reflected in the observation by Hogan (1961) that the rate of passage of ammonia from rumen contents through the rumen wall was directly related to pH and ammonia content of the rumen liquor. In the present work, a reduction of motility was not related to a combination of high pH and ammonia levels of rumen liquor (Table 38, Fig.11 & 13). This might suggest that alkali-sensitive receptors, if present, were not involved in the inhibition of motility.

The enzyme urease occurs in the stomach of man and many other mammals (Kornberg & Davies, 1955). Since the enzyme is rapidly inactivated by acid and pepsin, it is thought to be situated in the stomach wall at a site protected from the action of these agents (Kornberg & Davies, loc.cit). "Urea available for hydrolysis comes in contact with the enzyme as water containing it flows from the plasma through the mucosa during gastric secretion " (Davenport, 1966, p.113). The result is that some of the acid secreted is neutralised by the ammonia formed during the hydrolysis. The extent of neutralisation is dependent on the urea levels in the blood; it may amount to half the acid secreted when large amounts of urea (10-25g) are ingested (Fleshler & Gabuzda, 1965; Davenport, loc.cit.). Titchen (1958) has shown that the introduction of acid into the abomasum of the decerebrate sheep serves as a strong stimulus to contraction of the reticulum. On this basis it might be argued that in the ruminant, elevation of blood urea levels may result in the withdrawal of this stimulus with a consequent reduction in the frequency of reticulum contraction. The absence of any such reduction when urea was infused into the duodenum of the fasted sheep suggests that under those conditions, urea was not acting in this manner.

In contrast to the absence of effects when urea was infused into the duodenum, the same route of administration of the amino acid mixture led to a profound inhibition of reticulorumen motility. The effects were similar to those induced by the intraduodenal infusion of fat or fatty acids (Titchen et al. 1966)in that there was in both instances a marked effect on food intake and reticulorumen motility. McLeay (1967) has shown that included in the effects of fats and fatty acids, whether administered by the intraduodenal or intravenous route, is a reduction in the volume and concentration of acid secreted by a form of separated (Heidenhain) pouch of the abomasum. The mechanisms whereby these effects were induced has not been established. In animals with a simpler form of stomach, the intraduodenal instillation of proteins or the products of protein hydrolysis as well as fats and fatty acids inhibits gastric motility and secretion (Thomas & Crider, 1939; Thomas, 1957) and also delays the rate of stomach empting (Peraino et al., 1959). Thomas (1957) has suggested that a reflex mechanism is involved but the intraduodenal instillation of peptones to dogs has been found to inhibit the motility of fundic pouches deprived of sympathetic and vagal innervation (Johnson & Magee, 1965) and of transplanted gastric pouches (Brown et al., 1967). Sircus (1958) has concluded that an osmoreceptor mechanism, sensitive to changes in the osmolality of duodenal contents consequent on the duodenal instillation of hypertonic solutions of saline, proteins or fats, may cause the release of a humoral agent. Menguy (1960) has discussed the possibility that some of the inhibitory effects of fats might be due to osmotic stimulation. The possibility that the hormone, enterogastrone, may play an important part in regulating the motility and secretion of the ruminant gut has been discussed by Titchen et al. (1966) and in greater detail by McLeay (1967). McLeay concluded (p.126) "It appears unlikely.....that a single mechanism operates in mediating the effects on gastric secretion and motility: rather a number of mechanisms acting seperately, additively or synergistically should be envisaged whether they be blood levels of fatty acids, glucose or amino acids, inhibitory hormones or an inhibition of stimulatory hormones, peripherally or centrally sited osmoreceptors or even neural reflexes". The relevance of these remarks to the present work is suggested by:

(a) The striking difference between the effects of intraduodenal infusions of the amino acid mixture and those of casein. The amino acid mixture appeared to act at sites or on mechanisms that were not accessible to the casein. In addition, the effects of the amino acid mixture were persistent suggesting that on those occasions changes of a profound nature may have taken place.

(b) The intraduodenal infusion of casein had at the most only a slight effect on motility even though it was administered at a dose rate that was higher in terms of nitrogen than that of the amino acid mixture. What effect was observed appeared to depend on the vehicle as much as the casein. It is possible that the effects of the casein were mediated by receptors that were located in the walls of the intestines or the portal vascular field and that stimulation of those receptors led to a reduction of abomasal outflow with a consequent distension of that organ. Such a distension would lead to a reduction of reticulorumen motility (Phillipson, 1939; Titchen, 1960). It is suggested that there is in addition some mechanism sensitive to circulating levels of amino acids, one which may account for the more marked effects of the amino acid mixture. It is also possible that the amino acid mixture stimulated receptors in the intestinal walls more effectively than did casein.

(c) It seems probable that the intravenous infusion of casein hydrolysate (Chapter 3) effectively inhibited reticulorumen motility. There was a striking similarity between its effects on the activity of the sheep (as judged from the records of jaw movements) and the effects of the amino acid mixture.

Some emphasis has already been placed on the similarity of effects induced by urea to those induced by amino acid containing materials. The most marked similarity perhaps, was seen in the effects of the infusions on the demeanour of the sheep. Irrespective of whether the treatment imposed was the administration of urea into the rumen, the intraduodenal infusion of the amino acid mixture or the intravenous infusion of casein hydrolysate, a condition of somnolence with an associated depression of sensitivity prevailed during the later stages of the infusion. It seems reasonable to propose that not only was there a similarity in the effects of the infusions but also in the mechanisms mediating those effects. There is a substantial body of evidence to indicate that such was the case, and that the afferent stimuli were in part dependent on, or closely associated with, changes in electrolyte and/or water metabolism. In previous chapters of this thesis it was suggested that some of the effects induced by the administration of amino acid containing materials were dependent on changes of this nature. This led to the demonstration that the intraduodenal infusion of casein was accompanied by a potassium retention of considerable magnitude. There is strong but less direct evidence that in the case of urea too, its administration into the rumen may affect electrolyte distribution. Clark & Lombard (1951) in their studies on the causes of urea toxicity found that a complete cessation of rumen contractions immediately followed the intravenous injection of either 100ml. of 1% NaOH or 150ml.

of a 1% solution of ammonia. They considered this to constitute "convincing proof of the central origins of the ruminal paralysis" and that the paralysis was not directly due to alkaline conditions in the rumen but to "an upset in the acid-base balance of the blood". Weiss (1953) found that after dosing Na_2CO_3 into the rumen, there was an inverse relationship between the CO_2 combining power of the blood and the ability of the sheep to eructate air that was insufflated into the rumen. The impairment of reticulorumen motility persisted for as long as the CO_2 combining power of the blood was enhanced - up to 72hr after the dosing of the Na_2CO_3. In the present work, evidence to suggest that the intraruminal administration of urea was accompanied by changes in the acid-base balance of the blood is seen in the effect of this treatment on the CO_2 content of venous blood (Fig.11, 13 and 18). It is emphasised that in the experiments with fasted sheep there appeared to be little relationship between this and the reduction of motility. Additional evidence was obtained from the inhibition of reticulorumen motility produced by the intravenous infusion of $NaHCO_3$. This observation is regarded as being of some moment even though a response was obtained on only one occasion. It contrasts with those situations where NaOH or ammonia solutions have been employed (Clark & Lombard, 1951); procedures which may be regarded as being grossly unphysiological and likely to induce intense irritant effects either locally or at more distant sites. It is clear that a study of other agents, both gaseous and liquid, that are known to induce a state of alkalosis or acidosis would be of interest.

The manner in which urea may effect changes in acid-base balance remains one of speculation. It was apparently not dependent on the appearance of ammonia in the peripheral circulation. A clue to its elucidation is suggested by the work of Engelhardt (1963b). He found that the intraruminal injection of 2.5 - 5.0g urea was accompanied by a 5 - 10 fold increase in the concentration of sodium in the rumen liquor. He suggested that the active transport of sodium from the rumen was inhibited and that a diffusion in the reverse direction occurred. Whatever the mechanism, it is clear that a major redistribution of electrolytes, and in all probability, water had occurred; it is also clear that the retention of sodium and potassium observed on the intraduodenal infusion of casein (Chapter 4) may have in fact, reflected their transfer from the tissue to the rumen. The investigation of the role of the reticulorumen and possibly other regions of the gut in the determination of electrolyte and water distribution within the ruminant would be of obvious interest.

CHAPTER 6

GENERAL DISCUSSION

This thesis has been concerned with the question as to how intraduodenal infusions of protein modify the food intake of sheep. Emphasis has been directed towards a definition of the effects induced and of the mechanisms involved. The outstanding conclusion that has emerged has been that the situation is one of considerable complexity. The main points leading to this conclusion are summarised below.

1. The infusion of amino acid containing materials induced a multiplicity of effects. A change of food intake was just one of those effects. Others included changes of water intake and retention, electrolyte and faecal dry matter excretion, blood composition and reticulorumen motility.

2. The effects of the infusions were modified by several factors.

(a) Nature of the amino acid material administered: an unequivocal increase of food intake could be demonstrated only when casein was the material administered. The effects of other materials tried were either equivocal or a clear inhibition of food intake.

(b) Route of infusion: an increase of food intake was observed only when infusions were made into the duodenum.

(c) Dose rate: on increasing the dose rate of casein, the excitatory effect on food intake was either reduced or changed to one of inhibition.

(d) Vehicle: the response to an intraduodenal infusion of casein was determined in part by the composition of the vehicle. It was also modified by a preliminary infusion of water. On occasions, infusion of the vehicle alone exerted a marked effect on food intake.

(e) Animal variation: on occasions marked variation occurred between and within animals. No obvious differences were observed to account for this variation.

3. The effects of the infusions on food intake were variable. This was so when intraduodenal infusions of casein were administered each day for several days and when they were restricted to a single 8hr infusion. In the latter case, the same procedure might induce either an increase or a decrease of food intake.

4. The effects of the infusions on food intake were rapid in onset and of short duration. They generally occurred during the period of infusion irrespective of whether a decrease or an increase of food intake was observed.

It would seem unlikely that a single mechanism could account for all the features listed above. More probably a number of mechanisms were operative, mechanisms involving receptors located at various sites in the body and responding to a range of stimuli. Depending on the various factors modifying the effects of the infusions, one or another mechanism or group of mechanisms predominated. Thus, the net effect on some occasions was an excitation of food intake, on others, no obvious change, and on others again, an inhibition.

The identity of the mechanism(s) involved remains uncertain. Although some clues as to possible mechanisms were obtained in this study, precise definition was not possible. Rather, the observations made here have served to indicate the wide range of problems which must be resolved before such a definition can be attempted. Those recognised so far include:

(i) The relationships between changes in food intake and the other effects induced by proteinaceous infusions: the effect on food intake may be dependent on one or more of the co-existing effects on blood composition, gastric motility, etc. Neither the evidence provided by the experiments described here, nor that found in the literature, allows any more than speculation as to the nature of these relationships. This should be borne in mind when considering the suggestion, discussed below, relating to the importance of water and electrolyte metabolism.

(ii) The properties of casein, casein hydrolysate and amino acid solutions determining their contrasting effects when infused into the duodenum: it remains uncertain as to whether the predominating factors are chemical, e.g. the extent of hydrolysis of protein, the species and proportions of amino acids present, or physical, e.g. the osmolality of the solutions.

(iii) The primary site of action: any suggestion that the stimuli leading to an increase of food intake were dependent on the presence of the infused material in the gut, or events associated with its digestion or absorption must be regarded as speculative.

(iv) The role of the vehicle: that the role of the vhicle may not be passive was indicated by the demonstration that an increase in food intake could accompany the infusion of the Salt Solution (Experiment 11) or, following a preliminary infusion of water, Balxter's Saline (Experiment 7). Such effects might be expected as a consequence of interaction of the vehicle and the digestion of nitrogen in the gut; i.e., the infusion of the vehicle affected the metabolism and/or the absorption of nitrogenous compounds. It remains a possibility, of course, that infusions of the vehicle and of the vehicle plus casein increased food intake by different mechanisms.

(v) The time relationships between infusion and response: the short latency and short persistence of response make it unlikely that the mechanisms responsible involved changes in the activity of rumen micro-organisms or correction of a protein or amino acid deficiency. One mechanism that exhibits a short latency between stimulus and response is that of gastric distension. The importance of this mechanism has been recently re-emphasised by Anand & Pillai (1967) who believe that during immediate post-prandial period, afferent stimuli arising from the gut are important in determining satiation and that "this state of satiation is later on maintained as a result of changes produced in the internal environment".

The elucidation of the problems outlined above will require a more extensive investigation than was possible here.

A marked difference is apparent between the results of Egan (1964) and those reported here. That two apparently similar investigations should produce such divergent results again emphasises the complexity of the effects induced by nitrogen supplementation and the importance of the experimental conditions in determining those effects. It seems likely that the following factors may have contributed to the differences.

(a) Breed of sheep: the breed used by Egan was Merino. Some strains of that breed are adapted to harsh conditions and their ability to withstand the effects of heat stress and dehydration has been established (MacFarlane, Morris & Howard, 1958; MacFarlane, Morris, Howard, McDonald & Budtz-Olsen, 1961). In contrast, the Romney breed used here is generally considered unsuitable for survival under severe conditions. That the conditions imposed in the present experiments were severe is suggested by the abnormal physiological condition of the ryegrass fed sheep used in the experiments on electrolyte excretion (Chapter 4).

(b) Electrolyte status: In view of the possible relationship between electrolyte status and the effects of casein infusion, the addition by Egan of a mineral mixture to the diet (3% of the dry weight) and his use of a 1% sodium phosphate solution as the casein vehicle may have been important. Such a procedure might lead to enhanced kidney function and water intake (Potter, 1961; Wilson, 1966) and the maintenance of a satisfactory sodium status, all of which may influence the response to proteinaceous infusions.

(c) Diet: The diet used by Egan was chaffed oaten straw. Although comparable in nitrogen content and dry matter digestibility to the ryegrass straw used here, it is possible that the two diets resulted in important differences in digesta composition.

(d) Housing: The sheep used by Egan were kept in individual pens, presumably allowing a greater degree of exercise than that allowed by the sling and crate used here. The importance of the conditions under which the animals were housed is suggested by the reports that food intake, water intake and efficiency of protein utilisation are modified by environmental temperature and exercise (Leitch & Thomson, 1944; Tredwell, Flick & Vahouny, 1957; Meyer & Hargus, 1959; Payne & Jacob, 1965; Schmidt & Widdowson, 1967).

(e) Casein: The contrasting results may have arisen in part from differences in the physical properties and chemical composition of the casein preparation used. Apart from nitrogen content which was similar to that of the casein used here, Egan did not report the nature of the casein he used.

In view of the differences between the results of Egan and those reported here together with the suggestions that have been made to account for them, it is of interest to consider the theory proposed by Egan to account for his observations. It was based on the assumption that sheep fed a low quality roughage may experience a deficiency of protein and that an intraduodenal infusion of casein served to correct that deficiency. He proposed (Egan, 1964, p.270)

> "The sheep limits feed intake to a level where all energy absorbed can be efficiently utilised, and resists the continuous imposition of energy loads which taxes the animal's physiological ability to utilise or dispose of energy. When sheep are fed a diet of low protein roughage, ability to cope with energy load is increased by giving supplementary protein. Greater dry matter intake is permitted even though this may result in greater 'fill' of the alimentary tract."

The work of Meyer & Hargus (1959) was quoted in support of the proposal. These workers suggested that the intake by rats of a low protein diet was limited by their ability to utilise energy. More recently, Clawson (1967) has found evidence to suggest that the intake by pigs of a low protein diet is decreased when the energy content of the diet is increased. Egan made no suggestion as to how the protein supplements increased the ability of the sheep to utilise energy although evidence was obtained to indicate that protein supplements increased the rate of disappearance of injected acetate or propionate (i.e. an energy load) from the peripheral circulation. It is to be remembered however, that Weston (1966b) has suggested that such an observation may be explicable in terms of increased digestible energy intake rather than the protein supplement *per se*.

It is true that there is some evidence to suggest that the amount of protein absorbed by the ruminant may be less than optimal. It is largely indirect in nature, for the symbiotic association between the rumen microorganisms and the host animal leads to practical difficulties when techniques of protein evaluation that have been successfully applied to animals with a simpler form of stomach are applied to the ruminant (Annison & Lewis, 1959). An increased nitrogen retention has been observed when the nature of the protein component of the diet is such that the net loss of nitrogen in the rumen is restricted (see reviews by Chalmers & Synge, 1954; Chalmers, 1954 and also Jasiorowski, 1960;

Whitelaw & Preston, 1963; Chalmers, Jayasinghe & Marshall, 1964; Tagari, Krol & Bondi, 1965) or when protein supplements are added to the duodenum (Chalmers, 1954) or abomasum (Little & Mitchell, 1967). The improved weight gain and utilisation of feed when sheep (Gordon & Tribe, 1952; Moir & Somers, 1957; Rakes, Lister & Reid, 1961) and calves (Putnam, Gutierrez & Davis, 1961) are given their food at frequent intervals throughout the day rather than as one or two feeds may also be attributed to an improvement in the protein nutrition of the animal (Moir & Somers, 1957; Mochrie, 1964; Hungate, 1966, p.416).

That fermentation in the rumen results in an excess of energy relative to protein is suggested by the clear demonstration (Meyer & Nelson, 1963) that sheep and cattle convert a smaller percentage of digested food into protein and a larger percentage into fat than do the rat and chicken. It has been established that the feeding of diets low in protein or deficient in some amino acids results in a relatively greater fat content of blood, liver and other tissues than when a normal diet is fed. This applies to the chicken (Thomas & Combe, 1967), mouse (Leveille & Sauberlich, 1964), rat (Harrill & Gifford, 1965; Morris, Arata & Cederquist, 1965) young pig (Filer & Churella, 1963), milk fed lamb (Walker & Cook, 1967), humans (Viteri <u>et al</u>., 1964) and probably other species. As pointed out by Hungate (1966, p.328) "the rumen anaerobiosis imposes a thermodynamic limit on the extent of host protein synthesis".

The casein supplement may have corrected a deficiency of some essential amino acid rather than a quantitative lack of protein. Methionine supplements have been shown to improve the utilisation of dietary nitrogen on some occasions (Loosli & Harris, 1945; Lofgreen, Loosli & Maynard, 1947; Barth, McLaren, Anderson, Welch & Smith, 1959) but not on others (Gallup, Pope & Whitehair, 1952). Reed, Moir & Underwood (1949) concluded that rumen bacterial protein was mildly deficient in methionine and suggested that the benefits obtained by adding methionine to some diets was explicable in terms of an improvement in the quality of the protein utilised by the host animal. Additional evidence that sulphur containing amino acids may be limiting on some occasions is suggested by the increased wool growth (Reis & Schinckel, 1963; Reis, 1967) and efficiency of feed utilisation (I.D. Sibbald, pers.com.) obtained when the acids are administered in a manner which reduces their metabolism by rumen microorganisms. A deficiency of lysine has been suggested as a factor limiting weight gain of calves (Whitelaw & Preston, 1963) and milk production by cows (Bigwood, 1964). The nature of the diet appears to be important in determining the quality of the protein absorbed by the host animal (Reed et al., 1949; Abdo, King & Engel, 1964; Conrad, Hibbs & Pratt, 1967). Clarke, Ellinger & Phillipson (1966) have described the influence of the diet on the amino acid composition of the digesta passing to the duodenum of sheep. The diet had a marked effect on the amount of each amino acid reaching that organ. Large disparities between the amino acid composition of the diets were reduced but not eliminated by the time that the digesta reached the duodenum. The manner whereby the diet influences the quality of the protein reaching the intestines may be determined in part by the effect of the diet on the qualitative composition of the rumen microbial population. There is some evidence to indicate that the quality of bacterial protein depends on the species and strain of the bacteria providing that protein (Kaufman, Nelson, Brown & Forbes, 1957; Bergen, Purser, Cline, 1967).

Impressive though this evidence appears, the experimental results described here make it unlikely that the effects of protein or amino acid supplementation on food intake or any other aspect of ruminant production can be solely explained in terms of a unitary hypothesis which claims that the effects are due to a correction of a protein or an amino acid deficiency. That not only food intake but also gut function, water intake, electrolyte metabolism, wool growth, and feed conversion efficiency are involved clearly indicates that a number of mechanisms are likely to be implicated.

The effect on wool growth of supplements of protein or amino acids has recently assumed importance. Increases in its rate of growth of up to 200% have been achieved by the abomasal infusion of casein, methionine, or cysteine (Reis & Schinckel, 1963; 1964; Reis, 1967). Lesser increases (of the order of 60%) have been obtained by the oral administration of these compounds subsequent to their treatment by a process which reduces their susceptibility to microbial attack (Fraser, 1967). It does not appear to have been established what effects are induced by infusions directly into the blood stream. Observations that must be accounted for by any mechanism that is proposed include:

(a) Casein is at least three times more effective than any of the proteins so far tried (including gelatin, blood meal, fish meal and soya bean meal). This is so even though methionine is the most limiting amino acid in casein for growth of rats (Harper, 1959).

(b) The abomasal infusion of small amounts (0.5 - 2g/day) of methionine or cysteine increases the growth and sulphur content of wool and the body weight of sheep. At higher dose rates (6 - 10g/day) wool growth is depressed but not its sulphur content. In contrast to the effects on wool growth, the rate of bodyweight gain increased with increasing dose rate of amino acid (Reis, 1967).

(c) An increase in the rate of wool growth of approximately 240%

has been achieved by adding 20% of sodium chloride to a constant daily allowance of a diet containing 180g linseed meal (Fraser, 1967).

These observations suggest that the explanation of the increased wool growth like that of increased food intake is complex. The marked increase of wool growth induced by the addition of salt to the diet poses the question of whether at least part of the effects are associated with, or dependent on, changes in electrolyte and water metabolism of the sheep. The nature of the mechanisms that would account for this relationship is obscure.

In the case of food intake it is more certain that there is a close relationship with water intake and that changes in one induce changes in the other. A large number of observations to indicate that this is so have been presented in Chapter 1. An osmoreceptor mechanism is thought to be involved. It is reasonable to argue that the increased water intake that accompanies the ingestion of food arises from the need for additional water to excrete the solutes that are ingested or are formed during the digestion of food (Radford, 1959). It may also be accounted for in part by the increased osmolality and decreased volume of extracellular fluid consequent on the transfer, during feeding, of water and solutes from the extracellular fluid to the gut lumen. Yet to be established however, is the manner in which water intake influences food intake. This applies both to the depression of food intake that follows water deprivation (Adolph, 1947, Strominger, 1947; English, 1966) or the administration of hypertonic solutions (Schwartzbaum & Ward, 1958; Smith, 1966) and the increase of food intake that on occasions has been observed when hypotonic solutions are administered or when water is added to the diet (Archdeacon & Allen, 1948; Strominger <u>et al</u>. 1953; Schwartzbaum & Ward, 1958; Smith, 1966). Mechanisms which might be involved in such interrelationships are implicit in Oatley's model for the regulation of water drinking (Oatley, 1967). Oatley suggested that signals arising from receptors which are sensitive to changes in the osmolality and volume of the extracellular fluid are involved not only in the regulation of water intake but may also form part of the afferent input to the hypothalamic centres controlling food intake. As well as explaining the relationships between food and water intake, changes in these signals might also explain the effects of experimental procedures involving the administration of various solutions to various sites in the body. Alternatively, the experimental procedures may result in cellular changes (pH, membrane permeability, chemical or electrical gradients) which affect the sensitivity of receptors involved in the regulation of a wide range of processes, of which wool growth, gut function and food and water intake are but easily recognised examples. Mechanisms based on ideas such as these have the attractions that their initiation may not depend on any one metabolite, the latency between cause and effect may be short or long, and they provide an explanation of how several effects may be induced by one experimental procedure.

The simple experiments attempted here have not provided an answer to the questions of how intraduodenal infusion of protein increase the food intake of sheep. More questions have been posed than solved; questions which should provide a basis for the reasoned approach to future work. In particular, the results strongly suggest that osmometric mechanisms may be involved in the regulation of food intake and in determining the effects of infusions of nitrogenous materials. It is also clear that an understanding of the mechanisms whereby the productive processes of the ruminant are influenced by proteinaceous infusions will require much further work, including a detailed examination of the effects of clearly defined solutions administered at different dose rates to different sites in the body. Attempts to prove or disprove these assertions should not only facilitate an understanding of the effects on food intake of casein infusions but should also make a significant contribution both to physiology and animal production.

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