THE UNIVERSITY OF ALBERTA

STUDIES IN THE PHYSIOLOGY OF EATING IN SHEEP

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BY

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The undersigned certify that they have read, and recommend
to the Faculty of Graduate Studies and Research for acceptance, a
thesis entitled "Studies in the Physiology of Eating in Sheep"
submitted by Robert John Christopherson in partial fulfilment of the
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ABSTRACT

Experiments were conducted with four Suffolk wethers and five Lincoln ewes in which the relationships between changes in respiration, circulation and body fluids during eating were determined.

Oxygen consumption increased by 60-70% during eating. Brief exposure of the sheep to moderate cold did not alter the magnitude of the change in oxygen consumption of the Suffolk wethers but markedly potentiated the tachycardia associated with eating and decreased the oxygen pulse. Beta-adrenergic blockade with propranolol did not prevent heart rate from increasing during eating in the cold to about 150 beats/minute.

Increases in both arterial and venous P_{CO_2} and plasma bicarbonate concentration and a decrease in blood pH occurred during eating. There was also a significant increase in the venous-arterial difference for P_{CO_2} and plasma bicarbonate.

There was an increase ($p < 0.01$) in hematocrit and blood haemoglobin concentration and a decrease ($p < 0.01$) in mean corpuscular haemoglobin concentration during eating. These values rapidly returned to pre-feeding levels after the end of the meal.

The net effect of the changes in oxygen saturation and haemoglobin concentration was to increase arterial and decrease venous oxygen content during the meal, thus creating a significant ($p < 0.01$) increase in the arterio-venous oxygen difference from 4.4 before to 5.9 ml/100 ml during eating.

Cardiac output determined by the Fick method was increased by about 18% during the early part of the meal but returned to pre-feeding levels before the animals stopped eating. Stroke volume decreased

progressively from the beginning to the end of the meal.

Plasma volume was estimated from the volumes of dilution of the dye T-1824. There was a significant (p < 0.01) decrease in plasma volume during eating in all individual sheep which tended to be related to the amount of feed consumed at a given meal. The mean decrease in plasma volume was 250 ml during eating. Since there was an increase in circulating red blood cell volume, the overall mean decrease in total blood volume was only 140 millilitres.

Thiocyanate ion was unreliable for estimating changes in extra-cellular fluid volume during eating since this ion was concentrated over plasma levels by 10-12 fold in parotid saliva and 3 fold in mixed saliva. This problem was not encountered with thiosulphate ion and the latter was subsequently used to estimate changes in the extracellular fluid volume during eating.

There was a marked day to day variation in the slope of the thiosulphate decay curves both for control trials and for trials in which the animals were fed at 30 minutes following injection of the marker. Thus, standard errors attached to estimates of thiosulphate space were very large, particularly in the post-eating period, but the results suggested that the thiosulphate space decreased progressively throughout the meal by, on average, about 1500 millilitres.

Plasma sodium concentration was increased 10% during eating and remained about 6% above pre-eating levels during the 30 minutes following the meal. Plasma potassium concentration did not change markedly during eating.

The large changes in volume of the extracellular fluids during eating are discussed in relation to the regulation of the circulation and to appetite and satiety in ruminants.

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INTRODUCTION

Eating, one of the more fundamental activities performed by domestic ruminants, is accompanied by marked increases in energy expenditure and cardiac activity which do not generally persist into the immediate post-prandial period. It has been shown that abrupt changes in volume and concentration of the body fluids occur at feeding time, but most studies have described these events during the post-prandial period at which time the processes of digestion and absorption of the feed may influence the changes observed. Thus, it is often difficult to separate the effects on body fluids caused directly by the act of eating and those induced as a consequence of the presence of freshly ingested food in the gut.

The physiological changes associated with the act of eating per se may be relevant to the regulation of energy expenditure and to the determination of satiety in ruminants.

The present study was undertaken to obtain an integrated picture of the changes which occur in respiration, cardiac function and body fluids of sheep during the activity of eating a single meal of hay.

LITERATURE REVIEW

Energy balance in ruminants is dependent upon the relative magnitudes of total energy expenditure and metabolizable energy intake. The voluntary feed intake in ruminants is a major factor determining the efficiency of production (Blaxter, 1969). Studies of the physiological responses and regulatory mechanisms associated with feeding activity in sheep are important because of their relationship to energy expenditure (Graham, 1964; Young, 1966; Webster, 1967) and because of their possible relevance to appetite regulation in ruminants (Ternouth, 1968).

Energy Expenditure

Energy expenditure, in total, is the energy expended in all the functions (e.g. motor, secretory, synthetic, thermoregulatory) of the body. For convenience total energy expenditure may be divided into the following arbitrary groups:

Basal metabolism.

Energy expended in muscular activity.

Energy expended in maintaining homeothermy.

Energy expended as a direct consequence of the ingestion of food.

Basal metabolic rate and fasting metabolism. The "basal metabolic rate" is a calorimetric measurement frequently employed in human physiology to estimate the minimal rate of energy expenditure by the subject. The conditions for measuring the basal metabolic rate are that the subject must be in a post-absorptive state, in a thermoneutral environment and at rest (Brody, 1945). When studying animals it is customary to refer to the minimal energy expenditure as the fasting

metabolism since it is seldom possible to satisfy all of the conditions that have been employed with humans (Blaxter, 1967). Animals, therefore, are studied in a thermoneutral environment under a standard regime of fasting with care being taken to avoid disturbing the animals during the periods of measurement. The animals are allowed freedom to stand and lie while they are within a calorimeter and, thus, the measurements of fasting metabolism include heat produced as a result of limited muscular activity.

Energy expended by sheep during muscular activity. Increased energy expenditure occurs as a consequence of muscular activity. In pen-fed sheep confined to a metabolism stall or calorimeter, body movement is limited to the activities of standing, lying, eating and drinking. At pasture, other activities (in particular walking to graze) become important. The Agricultural Research Council (1965) did not consider that the energy cost of eating per se was sufficiently large to include when calculating the energy requirement of ruminants. This point will be discussed later.

The energy requirements of sheep at pasture have been compared with the requirements of pen-fed animals by estimating the digestible organic matter intake necessary to maintain a constant body weight. Based on this technique, estimates of the increased energy requirements of the animals at pasture have ranged from 24% (Langlands, Corbett, McDonald and Reid, 1963) to 33-275% (Lambourne and Reardon, 1963). The high values in the latter study were estimated during periods of severe weather and are probably not representative of the energy expended primarily for grazing. Blaxter (1967) has predicted that walking to graze would increase the energy expenditure of sheep

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between 10 and 20% of the fasting expenditure. This prediction is based upon estimates of the average daily distance that a grazing sheep walks and estimates of the energy expended by sheep walking on a treadmill inside a calorimeter (Clapperton, 1961). However, the distances walked by sheep may vary according to the density or sparsity of the pasture forage and may be influenced by stocking rate (Young, 1968). Thus the energy expended by grazing sheep might vary considerably depending on the type of pasture available.

Effect of cold environments on the energy expenditure of sheep. Sheep increase their heat production when exposed to environmental temperatures that are below their critical temperature (Joyce and Blaxter, 1964; and Webster, 1966). The critical temperature is the environmental temperature below which an animal must increase its metabolic heat production in order to prevent a decrease in deep body temperature. Above the critical temperature there is a range over which the heat production stays relatively constant (the thermoneutral zone). In this zone, physical thermoregulation, involving vasomotor control, piloerection and evaporative heat loss mechanisms, is effective in maintaining body temperature without increasing metabolic heat production (Blaxter, 1967).

Below the critical temperature, the metabolic rate increases in proportion to decreasing environmental temperature. Webster, Hicks and Hays (1969) showed that exposure of shorn sheep to -30°C elevated their heat production over the basal metabolic rate by four to seven-fold, depending upon the environment in which the animals previously had been maintained. The latter authors, and others (Sykes and Slee, 1968; Webster, Heitman, Hays and Olynyk, 1969) have demonstrated that

cold-acclimation can markedly improve the ability of the animals to maintain homeothermy in a cold environment. Moreover, sheep with a normal winter fleece may have critical temperatures approaching -25 to -30°C, especially following a period of previous cold acclimation (Webster, Hicks and Hays, 1969). Thus, the effects of cold on energy expenditure in sheep will depend upon the resting metabolic rate of the animal and upon factors which influence the magnitude of the insulation provided by the tissues and the fleece.

Effects of feeding on energy expenditure in ruminants. Kellner (1900), in studies with ruminants, showed that the increased heat production associated with an increase in food intake was positively related to the crude fibre content of the ration. He attributed this increased heat production (presumably entirely) to the work of chewing and the heat of fermentation. This became known as the heat increment of feeding (Brody, 1945).

In 1881, Voit (see Lusk, 1931) had suggested a "plethora theory" to account for the increase in metabolic rate that follows feeding. He proposed that "nutrients or fragments of nutrients" arising from the consumed food acted to stimulate cellular metabolism in direct proportion to the amount of nutrients available.

Rubner (cited by Borsook, 1936) showed that the feeding of bones or other non-digestible matter to dogs did not raise their metabolic rate and concluded that the increase in heat production following the ingestion of digestible foods could not be attributed to the visceral work involved in processing the ingesta. Rubner described this phenomenon as the specific dynamic effect (SDE) of food, and later suggested that the stimulation of heat production depended largely on

the amount of nutrients presented and that the different nutrients (protein, carbohydrate and fat) each had specific stimulating effects. Weiss and Rapport (1924) showed that amino acids induced a similar elevation in heat production whether they were injected intravenously or ingested orally. Thus, in non-ruminants there appeared to be no significant heat production associated with the ingestion and digestion of the diet. Rubner proposed that organs such as the liver were the origin of the increased heat production and, in the case of protein, recognized that deamination contributed significantly to the SDE. This idea is somewhat analogous to Voit's "plethora theory".

Rubner, however, did not propose that nutrients acted as general metabolic stimulants as Voit had suggested. Instead he attributed the SDE to the liberation, from intermediary metabolic reactions, of energy which was not available for physiological work but which appeared as heat. It was thus considered to represent a loss (waste) of energy to the animal except at cold environmental temperatures where it could aid in maintaining body temperature (Kleiber, 1961).

The SDE as defined by Rubner and later by Lusk (1931) for monogastric animals (particularly dogs) is generally less than the heat increment of feeding determined in ruminants (Brody, 1945). For ruminants, by 1950, it was still not clear whether the heat increment of feeding represented a very large SDE or the work of ingestion and digestion, or both.

The major end products of the rumen fermentation (the volatile fatty acids) have been administered to sheep singly (Armstrong and Blaxter, 1957) and in various mixtures (Armstrong et al., 1957; Armstrong et al., 1958) and their effects upon heat production were

measured. When acetate alone was injected into the rumen of fasting

sheep, 41-50% of the energy of the acid appeared as heat. That is,

the SDE of the acetate was 41-50%. However, when acetate was adminis-

tered in mixtures containing propionate the SDEs ranged from 12 to

15% of the energy of the acid mixtures. When acetate was given alone

the acid accumulated in the blood and caused a severe acidosis, blood

sugar decreased and there was evidence of an increased protein break-

down. It has been suggested that propionate, because it is glycogenic,

enhances the metabolism of acetate via the tricarboxylic acid pathway

and thereby prevents its accumulation in the body (Hungate, 1966;

Blaxter, 1967). In fasting sheep all mixtures (encompassing a wide

range of acetate: propionate ratios) had similar SDEs, whereas, in

fattening sheep the SDEs increased as the molar proportion of acetic

acid increased (Armstrong et al., 1958). The mechanism by which

acetate causes such a large SDE has not been elucidated.

Also apparent from the above studies is that the SDEs of volatile

fatty acid mixtures given to fattening sheep are higher than when the

same mixtures are given to fasting animals. Therefore, the efficiency

of utilization of the volatile fatty acids for fattening is lower than

for the prevention of body tissue loss in fasting animals. Clearly,

in fattening animals the efficiency of utilization of nutrients is

considerably less than the efficiencies predicted from a consideration

of the $\sim$ P yield per mole of nutrient metabolized (Blaxter, 1967),

while the latter corresponds reasonably well with efficiencies deter-

mined in fasting animals. The biochemically predicted efficiencies

of nutrients for utilization in growth and lactation also tend to be

higher than efficiencies determined calorimetrically (Milligan, 1971).

These discrepancies suggest that in animals fed for production the maintenance energy expenditure and, therefore, requirement is probably higher than in the non-productive state. The latter author has discussed several ancillary factors which could increase the maintenance requirement of an animal in the productive state. Net storage of protein in a constantly turning-over pool would require the protein to be synthesized more than once. Storage of a nutrient prior to utilization as an energy substrate would require the additional expenditure of energy involved in storage of that nutrient. It is also possible that the maintenance of the intracellular electrolyte concentrations could become energetically more expensive in the productive state if the rate of passive leakage of ions were increased as a consequence of hormone changes.

In ruminants, therefore, the end products of digestion do exert a marked specific dynamic effect which represents a significant proportion of the metabolizable energy intake and can probably be attributed to the following losses or expenditures: a portion may arise as heat evolved from exergonic reactions in intermediary metabolism according to Rubner's original concept, a portion may represent energy expended directly for synthesis in the producing animal and a portion may represent an increased expenditure associated with maintenance of the productive state. This concept implies that the ingested nutrients have been absorbed and are being metabolized before the "specific-dynamic effect" becomes apparent.

Similar observations on heat production have been made when ruminants are fed complete rations (Graham, 1964a; Lofgreen et al., 1963). As with the pure nutrients, the increased heat production

associated with ingestion of food is greater when the animals are fed above rather than below maintenance. The increased heat production in this case is, of course the heat increment of ingested feed as originally described by Kellner. These heat increments are always greater than the SDEs resulting from infusions of mixtures of volatile fatty acids, suggesting that they include energy expenditures additional to the SDE.

Other sources of increased heat production associated with the ingestion of feed in ruminants are the energy expended during the activities of eating and ruminating and the heat of fermentation of the ingested feed by rumen microorganisms. Marston (1948b) estimated the heat produced by rumen fermentation to be about 6% of the energy of the carbohydrate fermented. The latter represents a digestive loss which ironically is credited as digestible energy intake by conventional digestion trials. It does contribute to the total heat load of the animal and the energy liberated would thus contribute to the total heat increment of feeding.

Blaxter and Joyce (1963) showed that the metabolic rate of sheep increased very markedly during eating but that this increase did not continue into the post-prandial period. This elevation of metabolic rate may represent the true energy cost of eating and estimates of this expenditure have been reported by Ustjanzew (1911), Graham (1964b), Young (1966), Webster (1967) and Webster and Hays (1968). Table 1 summarizes various estimates reported for energy expenditure directly attributable to eating in sheep:

Table 1. Published estimates of the energy cost of eating in sheep.

Authors	Ration	Energy Cost of Eating (cal/Kg body wt min spent eating)
Ustjanzew (1911)	Timothy hay	14.1
	Timothy chaff	13.1
	Oat grain	15.5
Graham (1964b)	Cut forage	9.0
Young (1966)	Lucerne chaff	5.4-12.4
	Wheaten chaff	8.8
	Concentrate	6.4-7.0
Webster (1967)	Dried grass	22.0
Webster & Hays (1968)	Alfalfa-brome hay	13.8

The increased energy expenditure of sheep during eating has been attributed, in part, to excitement of the animal associated with receiving its feed at regular periods each day (Blaxter and Joyce, 1963). The initial rapid increase in metabolic rate when feed is presented may be associated with excitement and this initial response is probably mediated by the sympathetic nervous system (Young, 1964). Webster and Hays (1968), however, demonstrated that the sustained metabolic and heart rate responses to eating were probably not mediated by the sympathetic system since propranolol (a beta-adrenergic blocking agent) did not abolish either of these responses. Graham (1964b) indicated that the energy cost of eating tended to be greatest when the rate of food intake was greatest. More detailed studies of Young (1966) confirmed this and indicated that the energy expenditure per minute was greatest during the early part of the meal when rate of intake was highest. Towards the end of the feeding period when the rate of eating was slower the rate of energy expenditure was reduced,

but still considerably higher than the resting rate. Sham-feeding of sheep with esophageal fistulae indicated that the metabolic rate increased during eating even though 75% of the food did not reach the rumen. This suggests that the metabolic response during eating is related to the work of eating per se. Concentrate rations are consumed with less effort than roughages, however, the smaller energy cost of eating a given weight of concentrate diet is due primarily to the shorter time spent eating as compared with roughages since the energy expended per minute during eating was similar for all rations (Young, 1966).

Pen-fed sheep generally consume their daily maintenance ration rapidly in one or two short meals and therefore the activity of eating will increase the animals maintenance energy expenditure by only 2-3% (Young, 1966). On the other hand, with animals at pasture, where grazing time may be of the order of 10 hours per day, the maintenance expenditure may be increased by about 20% as a direct consequence of the eating component of grazing (Webster and Hays, 1968). This prediction assumes that there is no compensatory reduction in rate of energy expenditure during prolonged eating periods. Some information is available from the studies of Young (1964) which indicates that the metabolic rate tended to remain elevated throughout 8 hour periods in which feed was continuously available. There was, however, some fluctuation in energy expenditure which could be associated with rate of eating. Feed-lot sheep which are self-fed might also be expected to expend a considerable amount of energy directly associated with eating.

Although the influence of various feeding systems and ration

preparation on the energy cost of eating in ruminants has not been investigated, these factors might significantly influence the energy expenditure of the animal. Wainman, Blaxter, Smith and Dewey (1970) found that the heat increment in fattening sheep was about 22% higher for chopped hay than for ground, pelleted hay. A smaller energy expenditure for eating finely ground hay might be anticipated.

Eating involves the obvious muscular activities of chewing and prehension of feed, increased rates of salivary secretion (Denton, 1957; Balch, 1958; Bailey, 1961; McManus, 1961 and Bailey and Balch, 1961) and increased motility of the rumen and reticulum (Schalk and Amadon, 1928; Balch, 1952; Freer and Campling, 1965; Freer et al., 1962 and Christopherson, 1967). These may be considered as primary effector activities requiring the increased expenditure of energy during eating. Rumination (involving chewing, salivation and increased rumen motility) would appear to be an activity comparable to eating, however, estimates of the energy cost of rumination (Ustjanew, 1911; and Graham, 1964b) indicate that this activity is energetically much less demanding than eating. The energy cost of rumination reported by Ustjanzew was 4.0 cal/min/kg body weight compared with energy costs of eating which ranged from 13.1 to 15.5 cal/min/kg. Graham's estimate of the energy cost of rumination was also 4.0 cal/min/kg while his estimate of the energy cost of eating cut forage was 9.0 cal/min/kilogram. Differences in response of the salivary glands (Hungate, 1966) and reticulo-rumen motility (Freer and Campling, 1965) might account for part of the difference in energy expenditure during the activities of eating and ruminating. These authors reported that saliva secretion and reticulo-rumen motility were increased to a greater extent during

eating than during rumination. However, it seems unlikely that these factors alone would account for the 2-3 fold greater energy cost of eating. Further investigation is required to establish the cause of this difference and to evaluate the contribution of the activities of the digestive tract to the total energy expenditure of the animal.

The process of rumination aids in reducing the particle size of coarse feeds and is thought to be initiated reflexly by contact of coarse feed particles with the wall of the reticulo-rumen (Hungate, 1966). Thus, time spent ruminating should be directly related to the quantity of coarse material consumed. Freer et al. (1962) and Freer and Campling (1965) have studied the influence of roughage quality, physical form of the ration and feed intake on the time spent eating and ruminating in cows. The animals spent very little time ruminating when concentrates or finely ground hay were fed. With roughage rations, fast rates of eating were associated with an increased rumination time per kg of feed consumed such that a constant amount of chewing (eating + ruminating) per unit weight of feed was established. The allowance of larger daily amounts of feed resulted in slower rates of eating and less time spent ruminating per kg of feed consumed. With cows fed ad lib relatively more time was spent eating and relatively less time ruminating per kg of food consumed than occurred at restricted intakes. Although the energetics have not been studied one would predict that the energy expenditure due to eating and ruminating per unit weight of feed consumed would be greater in animals fed ad lib than in animals fed restricted amounts of roughage since estimates of the energy expenditure for eating exceeds that for rumination. The cows spent

approximately twice as much time eating and ruminating per unit weight of feed when straw rather than hay was offered ad lib (Freer et al., 1962). When ground pelleted hay rather than long hay was offered the animals spent less time eating and did not ruminate (Freer and Campling, 1965). These factors are likely to influence the maintenance energy expenditure of the animal.

Apart from the activities which occur during periods of eating and ruminating the work of digestion in ruminants must contribute to the heat increment since various gastro-intestinal activities are known to be enhanced in the fed animal. Thus, the rumen motility (both rate and amplitude of contractions) is greater in the fed than in the fasting animal (Atteberry and Johnson, 1969), and is related to the level of feed intake (Freer et al., 1962). Higher resting rates of rumen contraction are found when roughages are fed than when concentrates are fed (Freer and Campling, 1965). The abomasal secretory activity is continuous in nature even during periods when the animal is not feeding or ruminating (Hill, 1955 and Phillipson, 1952) and fasting results in a gradual decline in the rate of acid secretion from this organ. The energetics of these basic digestive functions have not been studied although it is apparent that muscular and secretory work by the digestive tract is continuously being performed.

The heat increment of feeding in ruminants, therefore, includes the separable components of (1) specific dynamic effect of absorbed nutrients (2) the work of digestion (3) the work of eating and rumination and (4) the heat of rumen fermentation. It should be emphasized that all of these components (with the exception of the heat of fermentation) involve the expenditure of energy by the animal for the

performance of work (maintenance and synthesis). The specific
dynamic effect of absorbed nutrients may not be attributable totally
to increased work performance but may reflect variations in the
efficiency with which the energy of $\sim$ P is utilized to perform work.
Since $\sim$ P is used less efficiently for synthesis than for muscular
work or the work of net ion transport (Milligan, 1971), the higher
heat increment of feeding above maintenance may be partly explained
by this reduction in efficiency.

In the foregoing discussion, the terms "specific dynamic effect"
and heat increment have not been used synonymously. The term specific
dynamic effect has been used to describe the increased metabolic rate
associated with metabolism of nutrients once they have been absorbed.
The term heat increment has been used to describe the total increase
in metabolic rate associated with feeding and includes expenditures
for the work of ingestion, cominution and digestion of food plus the
specific dynamic effect and heat of fermentation. Many of the previous
theories on the heat increment of feeding in ruminants have emphasized
the inefficiency aspect primarily in terms of SDE with less attention
being given to the definition of possible essential expenditures of
energy associated with the fed status. It should be advantageous,
therefore, to investigate more thoroughly the increased maintenance
expenditures associated with the ingestion and utilization of feed in
ruminants and to examine the means by which these expenditures are
regulated.

Cardiovascular Changes Associated with Eating in Sheep

Heart rate. During eating, marked increases in the heart rate

have been recorded in sheep (Young, 1966; Webster, 1967; Webster and Hays, 1968; Berzins, 1969) and in cattle (Kelley and Rupel, 1937; Ingram and Whittow, 1962). Since there is a relationship between heart rate and oxygen consumption in man and animals (Henderson and Prince, 1914; Brody, 1945) it has been suggested that heart rate might be used for the prediction of energy expenditure. Predictions of the heat production of men exposed to a variety of conditions have been made using this relationship (Read, 1924; Lundgren, 1946 and Malhotra, Sen Gupta and Rai, 1963). This approach has been used to predict the basal metabolism of ruminants (Blaxter, 1948; Blaxter and Wood, 1951) and gave reasonable results under their conditions. Webster (1967) showed that there was a close relationship between heart rate and energy expenditure during metabolic responses to cold exposure and eating but that the relationship was less exact during eating than during cold stress. Some individual animals exhibited variations in oxygen pulse (ml of oxygen consumed/beat of the heart) either during continuous cold exposure or during the course of each day, which tended to increase the error associated with predictions of energy expenditure from heart rate. In three out of four sheep the errors associated with the prediction were less than 10%. Other workers (Young, 1964; Brockway and McEwan, 1969) considered that heart rate could not be used as an accurate estimate of energy expenditure during eating in sheep because of the large standard error associated with individual regression equations.

The lack of a precise relationship between heart rate and oxygen consumption during eating suggests that factors other than increased energy expenditure may be involved in regulating the heart rate

response during eating. The initial cardioacceleration during feeding was attributed by Young (1964) to the release of adrenaline by the sympatho-adrenal system. The response could be elicited by presenting an empty feed bin to the animal but was transient, lasting only a few minutes. Webster and Hays (1968) demonstrated that the sustained heart rate response during eating was not mediated by the sympathetic nervous system since propranolol did not abolish the response. Treatment with this drug, however, completely prevented the cardioacceleration caused by cold exposure of the sheep.

Increases in heart rate during muscular exercise in dogs (Cronin, 1967) and in man (Epstein et al., 1965 and Cumming and Carr, 1966) also appear to be somewhat independent of the sympathetic nervous system. Donald and Samueloff (1966) showed that the denervated heart of the dog increased in rate during exercise even when blood-borne sympathetic transmitter substances were blocked with propranolol. They postulated that there was an intrinsic mechanism in the heart that brought about cardioacceleration in proportion to the work performed. Although the mechanisms mediating the heart rate increase during eating have not been established, Hays (1968) and Berzins (1969) suggested that relaxation of parasympathetic tone could conceivably account for the increase in heart rate during eating in sheep since atropine infusion during beta-adrenergic blockade with propranolol caused the heart rate to increase to about 120 beats per minute. The heart rates during eating in the study of Webster and Hays (1968) were all below 120 beats per minute and therefore could be accounted for by reduced parasympathetic tone. Further assessment of the involvement of the autonomic nervous system is, therefore, required.

Berzins (1969) found evidence for an enhanced release of ^{131}I from the thyroid gland during eating and suggested that thyroxine secretion was increased during this period. Although experiments with a thyroidectomized animal suggested a possible involvement of thyroxine in the regulation of heart rate during eating, definite conclusions could not be drawn since marked appetite changes associated with thyroidectomy were observed. Also, the metabolic stimulating effects of thyroxine tend to have a long latent period (Guz, Kurland and Freedberg, 1969) and are not characteristic of the rapid responses noted during eating.

The marked increase in heart rate during eating in ruminants implies that an increase in cardiac output occurs at this time. However, since cardiac output is equal to the product of heart rate times stroke volume its magnitude cannot be predicted from a knowledge of heart rate alone.

Blood Flow. There are several reports which relate changes in cardiac output and regional blood flow in ruminants to the consequences of ingesting food. Most of these describe changes in blood flow during the digestion and absorption period. Very few measurements have been made of changes in blood flow during the act of eating.

Sellers et al. (1964) found that blood flow in the right ruminal and omasal arteries of cattle increased by 25 to 30% during eating. The increased flow in these vessels continued beyond the act of feeding for periods up to 90 minutes. Blood flow recorded in the posterior mesenteric and hypogastric arteries, on the other hand, did not increase and the authors suggested that the enhanced ruminal flow was probably not due to an increase in cardiac output. Feeding under

conditions where the rumen fermentation was minimal limited the increase in ruminal blood flow to the period when the animal was eating. Administration of acetate, propionate and butyrate to the rumen also stimulated blood flow. Increased portal blood flow after feeding in sheep has been reported by Bensadoun and Reid (1962). The flow rate was increased within 3-7 hours after feeding and followed similar trends to the changes in rumen VFA concentration. Thus the early post-feeding values were not the highest recorded. Unfortunately, their data does not indicate the extent to which changes in flow may have occurred during the period of eating.

In cattle, Waldern et al. (1963) measured the cardiac output every three hours following feeding. No measurements were made during eating but the maximum cardiac output was recorded at 6 hours post-feeding. The higher cardiac output values tended to be related to concentration of VFA in the rumen and portal blood.

Berzins (1969) found that jugular blood flow in sheep increased by 46-97% during eating. The flow rates were highest during the first five minutes of eating and declined somewhat as the eating period progressed. Immediately following eating the jugular blood flow rates decreased to values below those recorded prior to eating. Fronek (1968) observed rapid increases in heart rate and cardiac output in dogs during eating which he attributed to sympathetic nervous control. Since the animals ate for only 3 minutes the responses may be comparable to the excitement phase in ruminants which occurs when feed is initially presented. It may not be strictly comparable to the responses of sheep during eating for longer periods.

Although it is apparent that the blood supply is increased to

regions of tissue which are more active during eating it is not clear
whether this occurs as a consequence of increasing or redistributing
the cardiac output. It would clearly be desirable to obtain direct
estimates of the cardiac output during eating in ruminants since
changes in the flow in isolated vessels may not be representative of
the overall circulatory response (Sellers et al., 1964 and Milnor,
1968).

Body Fluid Changes in Sheep Associated with Feeding

Evidence of hemoconcentration in sheep following feeding has been
reported (Stacy and Brook, 1964, 1965; Warner and Stacy, 1965; Stacy
and Warner, 1966 and Stacy, 1969) and previous workers (Anderson, 1955
and Schmidt-Nielsen et al., 1958) had noted post-prandial reduction in
urinary flow rates in sheep. These phenomena were attributed to the
abrupt shift of fluid from the blood into the rumen by increased
salivation (Bailey, 1961) during eating and diffusion of water across
the rumen wall (Murray, Reid and Sutherland, 1962). The reduced urine
flows were not associated with changes in renal hemodynamics (Stacy
and Brook, 1964) and were later attributed to the action of anti-
diuretic hormone (ADH) by Stacy and Brook (1965) who found that urine
collected immediately following feeding contained an anti-diuretic
factor. When extracts of post-prandial sheep urine were injected
intravenously into sheep the response resembled the action of ADH. It
is interesting that a similar post-prandial anti-diuresis has been
reported in rats (Kakolowski and Cox, 1968).

The literature relating to volume changes in the blood and
extracellular fluid (ECF) during, rather than following eating, is

scanty. The hemoconcentration which follows feeding in sheep has been
shown to include increased plasma protein concentration (Stacy and
Brook, 1964; Berzins, 1969) and gradually increasing plasma osmotic
pressure and Na^+ levels (Stacy and Brook, 1965; Warner and Stacy, 1965
and Ternouth, 1967). The changes in plasma protein concentration
tended to be transient, returning to normal within about 2 hours
following feeding. On the other hand, the plasma osmotic pressure and
Na^+ concentration tended to increase more gradually and remained
elevated for several hours. The increased plasma protein concentrat-
ions have been interpreted as a decrease in plasma volume. Blair-West
and Brook (1969) reported that the plasma volume decreased by 150 to
250 ml. in 30 minutes after the animal started to eat and that most of
this decrease had occurred by 5 minutes after eating began.

Marked changes in rumen electrolyte levels occur after feeding.
Warner and Stacy (1965) found that rumen contents tended to be
hypotonic to blood when feed was witheld from the sheep. When the
sheep were fed the rumen fluid rapidly became hypertonic to plasma
and the increased osmotic pressure was attributed mainly to K^+ released
from the feed plus increased VFA concentration. The Na^+ concentration
of rumen fluid decreased following feeding at a time when plasma Na^+
concentration was rising. Stacy and Warner (1966) estimated the
balances of water and sodium in the rumen following eating and con-
cluded that Na^+ absorption from the rumen was enhanced at this time.
The water entering the rumen could be accounted for by saliva
secretion alone and they found no evidence for a significant net
inflow of water across the rumen epithelium. Later they reported that
net transmural movement of water from the blood to the gut was less

than 100 ml. per hour after feeding whereas before feeding there was a net absorption of water from gut to blood of 100-300 ml/hour (Stacy and Warner, 1968). The net movement of water in either direction across the rumen wall, thus, appeared to be a slow process compared with total entry and exit of water from the foregut. Willis (1964) did not observe hyperosmolarity of the rumen contents of sheep following feeding. Instead the plasma osmotic pressure remained above that of rumen fluid at all times. The difference between the above studies might have been due to ration differences. Warner and Stacy included 10 grams of NaCl with each meal whereas Willis fed hay alone.

Ternouth (1967) also observed hyperosmolarity of rumen fluid after feeding. The rumen fluid became hypotonic again within 3 hours if the sheep had access to water but required over 10 hours to become hypotonic when water was witheld. The animals drank the majority of their daily water intake during the first two hours following feeding. The author found that net movement of water from the blood across the rumen epithelium was quite large during the first two hours following feeding and again between the fourth and eighth hours. This is in contrast to the observations of Stacy and Warner (1968) and Willis (1964). The latter author observed a similar net water absorption from rumen to blood at all times following feeding even though the water flow in both directions was highest immediately after feeding. Net water movement tended to follow osmotic gradients across the rumen wall.

In so far as it is possible to generalise from the studies cited above and others (Parthasarathy and Phillipson, 1953; and Hyden, 1961)

there would seem to be agreement that net movement of water across the rumen wall tends to follow osmotic pressure gradients, but that differences of opinion exist as to the magnitude of net water movements associated with a given osmotic pressure gradient.

Water movement between the gut and the blood may be influenced by the transport of individual solutes (Curran and Solomon, 1957). These authors observed that water movement across the ileum of the rat varied directly with sodium movement and that, in the absence of sodium, water absorption was greatly reduced. This relationship between sodium and water absorption has been confirmed by other investigators (Curran, 1960; Clarkson and Rothstein, 1960; Annegers, 1961 and Rossi and Copraro, 1961). Curran (1960) postulated that net water absorption was dependent upon net solute flow and Engelhardt (1963) suggested that absorption and secretion of solutes across the rumen epithelium may be involved in the regulation of water movement. These observations indicate that water transport may be intimately related to metabolic activity. The enhanced post-prandial absorption of Na^+ from the rumen which was observed by Stacy and Warner (1966) was associated with a high osmotic pressure of the rumen contents. However, since the rumen Na^+ concentration was always lower than plasma sodium concentration the absorption of this ion must have occurred by means of an active transport mechanism involving energy expenditure. The mechanism by which active Na^+ absorption was stimulated under these conditions is not clear. The authors suggested that hypertonicity of the rumen contents appeared to be an adequate stimulus, but how this stimulus could eventually lead to enhancement of the active transport of Na^+ has not been explained. The authors ruled out ADH as a possible

mediator of the enhanced Na^+ absorption since sham feeding sheep with esophageal fistulae did not result in increased Na^+ absorption, although, systemic renal and plasma effects were observed. It is possible that aldosterone secretion is increased during the post-feeding period since Blair-West and Brook (1969) observed increases in plasma renin levels in sheep after feeding. The renin-angiotensin system is known to be intimately involved in the regulation of aldosterone secretion (Ganong et al., 1966). Since aldosterone has been shown to cause increased Na^+ absorption from the small intestine (Edelman et al., 1968), the possible involvement of aldosterone in regulation of the electrolyte balance in sheep following feeding merits further study.

Although the ionic composition of saliva may vary considerably, this secretion tends to remain approximately isotonic with plasma (Kay, 1960 and Bailey, 1961). Thus, enhanced saliva secretion during eating would be expected to reduce the volume of the extracellular fluid, but not necessarily alter its osmotic pressure. There has been no detailed study made of electrolyte concentrations in the plasma during the initial period of eating, except for a few observations reported by Ternouth (1968). While this author concentrated mainly on changes occurring over several hours following feeding it seems likely that the samples which were drawn during the first 30 minutes represent the actual eating period. The results suggested that plasma Na^+ and osmolality do not increase until one or two hours after feeding. This confirms the observations of Stacy and co-workers. Serum K^+ levels were highest at feeding time, dropped abruptly by 30 minutes and remained unchanged thereafter. The serum levels of Cl^-

and phosphate appeared to change in relation to Na^+ and osmolar changes although the increased anion levels appeared to preceed the changes in Na^+ and osmolar concentrations. The nature of these serum electrolyte changes suggests that during the post-feeding period water is removed from the extracellular phase by extra-salivary pathways, thus supporting the results of Ternouth (1967) which indicated a net movement of water across the rumen wall at various times after feeding.

Since changes in concentration of serum electrolytes do not distinguish between changes in water volume of that phase and changes in quantity of solute in that phase it would be desirable to have estimates of actual volume changes. Blair-West and Brook (1969) found that plasma volume drops very rapidly during eating in sheep. Since the plasma volume represents only about 20% of the total extracellular fluid volume (ECF), the changes in the latter should more closely reflect the magnitude of the fluid shifts that occur during eating. Ternouth (1968) reported a 10% reduction of the thiosulfate space in sheep one hour after feeding and then a subsequent return to normal by the 3rd hour, post-feeding. It was suggested however that water movement from the intracellular phase to the ECF would result in an underestimation of the overall movement of ECF into the digestive tract.

The extent to which the fluid shifts during feeding involve the intracellular compartment are not known. Water movement into and out of cells tends to be related to solute movements and in general the intracellular and extracellular phases are maintained in osmotic equilibrium (Cizek, 1968). Changes in the osmotic pressure gradient between the ECF and the rumen during and after feeding will give rise

to osmotic pressure changes not only in the ECF but inevitably in the intracellular space as well. The increased release of ADH in sheep following feeding (Stacy and Brook, 1965) is known to be associated with the osmotic withdrawal of water from the intracellular compartment, specifically the osmoreceptor areas of the hypothalamus (Verney, 1947).

Again, it is not possible to say precisely whether the stimulus to ADH release is due to the fluid changes taking place during eating, which may be isotonic, or to the rearrangement during the post-prandial period of water and osmotically active particles such as sodium.

Methods for estimating the volume of various body fluid compartments in ruminants have been discussed by Hix _et al_. (1959). The methods involve calculation of the volume of distribution of easily determined labels which have been added to the fluid compartments. Total body water has been estimated from the volume of distribution of tritiated water and antipyrine since these are thought to become distributed throughout all fluid compartments. Plasma volume is commonly estimated from the volume of distribution of Evans Blue which binds to plasma proteins and thus remains intravascular for a considerable period of time. Hix (1959) used thiocyanate ion to estimate the extracellular fluid volume of ruminants. The size of the thiocyanate space in sheep, cattle and goats was approximately 30% of the body weight and, therefore, measures a larger space than thiosulphate (Dalton, 1964; English, 1966; Holmes and English, 1969). In humans and dogs thiosulphate space tends to be similar but slightly larger than the inulin space (English, 1966). Hix (1959) considered that the thiocyanate space was a better estimate of ECF volume since it entered

most transcellular fluids and would therefore include a measure of gastro-intestinal secretion. Inulin on the other hand, was considered to be restricted mainly to the plasma and interstitial fluid. Thiosulphate ion probably is distributed in a similar manner to inulin. Although Holmes and English (1969) reported that thiosulphate appeared to enter the rumen in very small amounts, Ternouth (1968) found no evidence that thiosulphate entered the gastro intestinal tract.

MacFarlane et al. (1958) reported thiocyanate spaces for sheep which ranged from about 25 to 37% of body weight. Differences due to changes in nutritional status were observed. Thiosulphate space of sheep ranges from 15 to 26% of body weight (English, 1966; Ternouth, 1968 and Holmes and English, 1969). Thus a 50 kg sheep would be expected to have a thiosulphate space of about 10 l and a thiocyanate space of roughly 15 l. Salivary secretion rates in sheep deprived of feed and water overnight have been estimated by Stacy and Warner (1966) to range from 4.5 - 6.4 ml/minute. During eating the saliva secreted/gm of dry feed was found to be about 2.7 millilitres. If a sheep consumes 300 gm of dry feed during 30 minutes, its extra rate of salivary secretion due to eating would be about 645 millilitres. This is equivalent to about 6.5% of the thiosulphate space of the animal. While this is not a large percentage of the ECF, the rate of removal is quite impressive. In this regard, the homeostatic responses of ADH secretion (Stacy and Brook, 1965) and renin secretion (Blair-West and Brook, 1969) following feeding are very appropriate in that they will help to counteract the possible osmotic and volume stresses induced by eating. Another response which undoubtedly helps to maintain circulatory integrity is the marked increase in hematocrit that occurs during

eating (Berzins, 1969). This author suggested that splenic contraction and mobilization of stored red blood cells was partly responsible for the increased hematocrit associated with feeding. However, the extent to which red blood cell mobilization can compensate for the reduced plasma volume during eating has not been established.

Post-prandial changes in the blood acid-base balance and augmented renal excretion of calcium and magnesium in sheep have been reported by Stacy (1969). There was a post-prandial increase in plasma calcium and magnesium concentration and fall in blood pH and plasma bicarbonate concentration. The increased urinary excretion of Ca^{++} and Mg^{++} following feeding was associated with a marked fall in urine pH. This confirmed earlier reports on changes in urine pH of ruminants following feeding (Kinne et al., 1961; Stacy and Brook, 1964). Stacy (1969) attributed the drop in blood pH following eating to the removal of HCO_3^- from the ECF by the saliva which, in ruminants, contains relatively high levels of this anion (Kay, 1963). The lowered buffering capacity due to salivary removal of HCO_3^- would coincide with the increased rate of metabolism associated with eating in ruminants and, therefore, accentuate the tendency towards acidosis.

In general, there is a considerable amount of literature in existence which describes changes in body water and electrolytes at and around the time of feeding. It is, however, seldom possible to distinguish between the effects of eating per se and the consequences of digestion and absorption of the recently ingested food. Therefore, further analysis of the body fluid and electrolyte changes during the act of eating in ruminants is clearly required.

Regulation of Feed Intake

The regulation of feed intake is part of the overall system that regulates energy exchange (Conrad, 1966). This system is very complex and may involve chemosensitive (Kennedy, 1953; Mayer, 1955), thermosensitive (Strominger and Brobeck, 1953) and other reflex mechanisms that provide feedback signals to the central nervous system. The latter integrates the afferent signals and may respond by evoking either facilitation or inhibition of feeding behaviour.

An inherent stimulus to feeding behaviour is the total energy demand upon the animal (McClymont, 1967). In both ruminants and monogastrics, cold temperature, lactation, low degree of body fatness and hyperthyroidism all lead to increased energy intake over the long term. Muscular work in sheep has been shown to increase feed intake (Blaxter, 1969).

The existence of a long term regulation of feed intake is obvious since man and adult animals tend to maintain a relatively constant body weight for a long period of time and this equilibrium state may be achieved with great precision at different levels of intake. For example, Mayer (1967) showed that rats with lesions in the ventromedial hypothalamus over-ate and became obese, but once they reached a certain degree of obesity their body weight stabilized at a higher level and their feed intake actually returned towards normal levels.

Increased feed intake occurs in rats in response to prolonged cold exposure (Cottle and Carlson, 1954). These authors demonstrated that the adjustments in feed intake do not occur immediately but require a period of several days before a new intake level is reached. This implies that some delayed metabolic adjustment is necessary.

Sudden exposure to cold often reduces feed intake initially until the animals become adjusted (Mayer, 1967). Similar adjustment lags have been noted in other experiments involving changes in caloric density of the diet (Strominger, Brobeck and Cort, 1953) or intragastric feeding of a portion of the diet (Janowitz and Hollander, 1955).

Thus, it is clear that a mechanism for long-term regulation of feed intake does exist. Moreover Edholm et al. (1970) have shown that in man there appears to be no short-term correlation between energy expenditure and energy intake. This suggests that the long term regulation of appetite is of paramount importance. However, it is difficult to visualize how the long-term energy status of the body might be transduced into specific stimuli regulating appetite and satiety on particular occasions. Harvey (1969) has postulated a lipostatic theory of long-term regulation of feed intake but the means by which the body can directly or indirectly sense changes in fat content have not been established and are difficult to visualize.

The other important aspect of appetite control is the short-term regulation of feed intake (Mayer, 1967). This involves a number of factors which operate to turn feeding behaviour "on" and "off" and ultimately determine the size and frequency of individual meals. Although a distinction is made between long-term and short-term regulation of feed intake, long-term regulation must ultimately operate in conjunction with short-term mechanisms.

Since the unit of energy input is the meal (Baile, 1968), it is important to determine the mechanisms regulating hunger and satiety with respect to individual meals.

Oropharyngeal factors are not thought to be very important in

limiting the appetite of ruminants (Balch, 1958; Campling and Balch, 1961). When swallowed boluses of hay were removed by hand from the rumen of cows during eating the animals prolonged their feeding time and consumed 177% of their normal voluntary intake. It was suggested that neither exhaustion of salivary secretion nor fatigue of jaw or rumen muscles are important in determining the time at which a cow stops eating roughage, although, the cows did slow down in their rate of eating during the later stages of the meal. With dogs, Janowtiz and Grossman (1949) found evidence for a small degree of satiety arising from the oropharyngeal region. Epstein (1967) demonstrated that rats could regulate intake adequately even when they received their entire diet intragastrically by operating a bar-pressing device. This illustrates that oropharyngeal factors are not essential to the regulation of energy intake but does not rule out their involvement in the feeding behaviour of animals under more normal circumstances. Palatability, the property of feed for eliciting olfactory, gustatory and tactile stimuli, (McClymont, 1967), has been shown to influence the selection by ruminants of pasture forage (Bland and Dent, 1964; Fontenot and Blaser, 1965 and Heady, 1964). The selectivity involved a preference for plants with a high soluble carbohydrate and protein content and low structural carbohydrate and lignin content. Palatability factors also influence the voluntary intake of ruminants offered a single choice of feed. Thomas et al. (1961) attributed poor consumption of high moisture silage to some constituent of the juice in the silage and Roe and Motterhead (1962) found that some strains of pasture grass contain a water-soluble, volatile substance which reduced the voluntary intake of sheep grazing pure stands of this forage.

In ruminants there is a positive relationship between voluntary intake and the digestibility of roughages (Crampton, 1957; Crampton et al., 1960). The latter authors suggested that recurring hunger in ruminants is primarily determined by a reduction in rumen load and that appetite for forage is correlated with the rate of cellulose fermentation by rumen microbes. Further studies have illustrated that the voluntary intake of roughages is controlled by capacity of the digestive tract and interrelated factors such as digestibility and rate of passage of digesta through the tract (Blaxter, Wainman and Wilson, 1961; Campling et al., 1961, 1962, 1963; Freer et al., 1962 and Freer and Campling, 1965). The work of Campling's group indicated a relationship between voluntary intake of roughages (but not of concentrates) and the rate of disappearance of digesta from the rumen such that there was a relatively constant weight of dry matter in the rumen just before the next meal. The rate of onward passage of residues out of the reticulo-rumen in cows depends on the time taken to reduce roughage particles to a size small enough to pass through the reticulo-omasal orifice and is, therefore, a function of cellulose digestion and the extent of cominution by chewing. Hungate et al. (1959) demonstrated that the rate of fermentation within the rumen of small ruminants is faster than in large ruminants. The high energy intake per unit of body size in the suni, a small African antelope, is associated with a rapid rate of rumen fermentation and passage of feed through the digestive tract.

The positive relationship between voluntary intake of dry matter and digestibility is apparently established only when digestibility is below 67% (Conrad, 1966). The latter author postulated that for highly

digestible feeds, other factors are more important than gut-fill in determining intake and it would seem that, under these conditions, ruminants may respond to changes in caloric density of the diet in a manner similar to that exhibited by monogastrics (Kleiber, 1961). That is, dry matter intake would be expected to decrease when the digestibility of the diet is very high.

Chemostatic satiety signals may become important in ruminants receiving high concentrate rations (Baile, 1968). With monogastrics, the glucostatic theory of Mayer (1955) for short-term appetite regulation has received considerable support despite several deficiencies (Brobeck, 1968). Hunger and satiety in monogastrics have been shown to be related to the rate of glucose utilization. Injections of insulin that lower blood glucose levels tend to result in increased feed intake but there is lack of conclusive evidence that high blood glucose levels reduce appetite. There is some question as to the location of glucoreceptors. Mayer (1955) proposed that receptors sensitive to glucose were located in the ventro-medial nucleus of the hypothalamus, however, Epstein and Teitelbaum (1967) report that rats with lesions in the ventro-medial nuclei respond to insulin hypoglycemia by increasing feed intake. This does not necessarily deny Mayer's hypothesis since insulin could exert an appetite stimulating effect unrelated to its hypoglycemic action. They found evidence that the lateral hypothalamic regions may be the sites sensitive to hypoglycemic-induced feeding behaviour. When lesions were placed in the lateral hypothalamic regions the rats did not increase feed intake in response to hypoglycemia, whereas they still increased their intake in response to caloric dilution of the diet and exposure to low ambient

temperatures. These observations suggest that glucostatic mechanisms may not be of importance in the regulation of intake in response to changes in diet caloric density or ambient temperature. It has been demonstrated that monogastrics can regulate caloric intake when carbohydrate-free diets are fed (Brobeck, 1968). This casts doubts on the role of dietary carbohydrate in the control of hunger and satiety even in monogastric animals.

In ruminants the very nature of the rumen fermentation process precludes the absorption of significant amounts of glucose from the digestive tract (Weller and Gray, 1954) and thus, glucose is not expected to be important in determining satiety in ruminants. Neither blood glucose nor insulin levels change appreciably with feeding (Manns and Boda, 1967 and Simkins et al., 1965a). Intravenous (Dowden and Jacobson, 1960 and Vallance and McClymont, 1959), intraperitoneal (Simkins et al., 1965b) and intracerebral-ventricular (Baile and Mahoney, 1967) injections of glucose cause little or no depression of feed intake. Severe hypoglycemia caused by insulin injections in goats does not cause eating (Baile and Mayer, 1968a).

Attention has been focused on the possibility that volatile fatty acids (VFA's) might cause satiety in ruminants (Dowden and Jacobson, 1960). Intravenous injections of acetic acid, propionic acid and sodium acetate reduced feed intake of cows when given at the level of 12.5% of the caloric value of the maintenance requirement. Rook et al. (1960) showed that intraruminal infusion of acetate at a rate of 3500 kcal/day reduced the feed intake of cows significantly. The experiments of Dowden and Jacobson (1960) have been criticized on the basis that unphysiological blood levels of the infused volatile fatty acids

were produced (Balch and Campling, 1962). More recent work (Baile and Mayer, 1967) has indicated that acetate injected intraruminally during eating in goats reduced meal size but did not change meal frequency. Injection of 60 or 90 mMole/meal of acetate reduced oral intake of a liquid diet by 40 and 60 percent respectively. Other studies have indicated a similar effect in sheep (Baile and Phander, 1966; Ulyatt, 1965) and cattle (Montgomery et al., 1963; Rook et al., 1960 and Simkins et al., 1965a). Intravenous injections of similar dose rates of acetate in goats had little or no effect on feed intake (Baile, 1968). Therefore, it was suggested that the acetate sensitive receptors are located on the luminal side of the rumen wall. Administration of acetate into the abomasum had no effect on feed intake. The importance of acetate receptors in the rumen as a mediator of satiety will depend on the time course of the increase in rumen acetate concentration during feeding. Various authors (Phillipson and McAnally, 1942; Masson and Phillipson, 1951; Annison and Lewis, 1959; and Bensadoun and Reid, 1962) have reported that peak values for rumen VFA concentrations occurred at 3-6 hours following feeding and Waldern et al. (1963) reported maximum rumen VFA concentration between 3 and 9 hours after feeding. In more recent studies with sheep maximum rumen VFA concentrations occurred at 4 hours (Warner and Stacy, 1965) and 1 to 3 hours (Ternouth, 1967) after feeding. In the two latter studies, marked increases in the rumen VFA concentration were evident within one hour of feeding. While Warner and Stacy (1965) allowed the animals access to feed for either 2 or 4 hours they did not indicate whether the animals ate continuously during the time that feed was available. They did indicate, however, that the amount of feed

consumed was greater during the first hour than during the subsequent
1 1/2 hour period. In these studies the animals were generally offer-
ed feed only once daily and, thus, the post-prandial changes in rumen
VFA concentration may have been greater than the changes expected when
animals are fed at more frequent intervals during the day (Satter and
Baumgardt, 1962). Although rumen acetate levels may have contributed
to satiety following feeding in the studies cited above, it is apparent
that acetate would be likely to exert its greatest inhibitory influence
several hours rather than immediately following feeding. Ash (1959)
had previously suggested that VFA receptors might exist in the rumen
wall. This suggestion was based on the observation that VFA solutions
or vapors when introduced to the rumen caused inhibition of rumen
motility independently of any changes in rumen or blood pH. Since
Baile and Pfander (1966) found that acetate (intraruminally) caused
inhibition of rumen motility there is some support for the proposal
of Baile (1968) that acetate may be involved in the determination of
satiety in ruminants.

One criticism of the studies carried out by Baile and coworkers
that merits consideration involves a possible confounding of changes
in water and electrolyte balance with various treatments. For
example, intraruminal infusion in goats of about 1500 mEq acetate per
day caused a reduction in grain intake (Baile and Mayer, 1968) but the
infused acetate was accompanied by an equivalent amount of sodium ion.
This is roughly equal to the amount of Na^+ that normally enters the
rumen of a 40 kg sheep per day (Dobson, 1961). The extent to which
this might influence appetite is not known, however, very marked
changes in rumen osmolality can occur after feeding in sheep (Warner

and Stacy, 1965 and Ternouth, 1967). Stacy and Warner have shown
that administration of NaCl or sodium salts of VFA's to the rumen
results in an increased Na^+ concentration of rumen fluid that is
sustained for several hours. Ternouth (1968) has suggested that tissue
dehydration resulting from a rapid shift of the extracellular fluid
into the gut of ruminants at feeding time may be a factor that deter-
mines satiety when highly digestible feeds are consumed. Baile and
Phander (1966) found that intraruminal injection of 250 mMoles of sa-
line twice daily before feeding caused a 25% reduction in feed intake
by goats as compared with a 60% reduction when acetate was injected.

Although the hypothalamic mechanisms regulating food intake and
water intake are distinct from each other, the regulation of food
intake appears to be correlated with water exchange (Anand, 1968).
During and after feeding there is a movement of fluid out of the rest
of the body into the digestive tract and it has been proposed (Adolph,
1947; Lepkovsky et al., 1957) that this may be a factor causing
satiety. Brobeck (1960) suggested that water may provide a common
denominator necessary for interrelationships in control of feeding,
drinking, body temperature, activity, body size and energy intake.
There is evidence for the presence of osmoreceptors in the stomach
and duodenum (Janowitz, 1967). Hyperosmotic solutions placed in the
stomach of monogastrics tend to retard gastric emptying. The duodenal
osmoreceptor mechanism has been shown to cause inhibition of gastric
acid secretion even from autotransplanted pouches (Sharma, 1967),
suggesting that the response might be mediated by a humoral agent.
Verney (1947) obtained evidence for the existence of osmoreceptors
in the central nervous system which are involved in the release of

anti-diuretic hormone. Stevenson (1967) has pointed out that, "although non-isotonic solutions can cause non-specific excitation of neurons, there is sufficient evidence for a specific osmoreceptor response of some cells of the nervous system as compared with others exposed to the same stimulus".

In the rat, hypertonicity of the extracellular fluid has been found to diminish food intake (Schwartzbaum and Ward, 1958; Smith, 1966 and Gutman and Krausz, 1969). The latter study, however, indicated that the "drive to eat" could overcome the inhibitory effect of increased plasma osmotic pressure. Hypovolemia was shown to suppress feed intake and the authors concluded that "thirst" tends to reduce feed intake since it was also found that isotonic saline loading, intraperitoneally, suppressed drinking but increased feed intake. Thirst has been observed in man following a heavy meal (Gregersen, 1941) and was attributed to reduced salivary flow.

In cattle, restriction of water intake to 50% of the ad lib amount caused a slight decrease in feed intake and an associated increase in dry matter digestibility (Phillips, 1961). High saline content of drinking water (Pierce, 1966) or high sodium chloride content of the feed (Wilson, 1966) can reduce the feed intake of sheep.

Although the evidence at present is variable and inconclusive for the involvement of body water and electrolyte changes in appetite regulation of ruminants there are reasons to suggest that they may have an influence. In view of the systemic alterations in water and electrolyte balance that have been demonstrated following feeding in sheep the relationship between feed intake and water and electrolyte status deserves further study.

The control of food intake has been suggested to occur as a part

of the overall body temperature regulating system (Brobeck, 1948, 1960). There is a well documented negative relationship between environmental temperature and food intake in all homeotherms that have been studied (Hamilton, 1967). Brobeck's original proposal was that "animals eat to keep warm and stop eating to prevent hyperthermia" and it was suggested that the "specific dynamic effect" of the food would increase body temperature and cause satiety (Strominger and Brobeck, 1953). Andersson and Larsson (1961) found that heating the preoptic and anterior hypothalamus of goats inhibited feeding, whereas cooling these areas caused little or no change in feed intake. During the cooling treatments the rectal temperature rose to 41°C, suggesting that cold-defense mechanisms had been activated. Feeding behaviour in these preparations was not inhibited by the high rectal temperatures although goats normally refuse to eat when the rectal temperature is above 40°C (Appleman and Delauch, 1958). Although these experiments suggest a relationship between appetite regulation and body temperature regulation, they have been criticized on the grounds that the temperature changes induced were very unphysiological (Grossman, 1968).

Increased hypothalamic temperatures during periods of feeding in rats have been reported (Abrams and Hammel, 1964). This observation together with experiments of Hamilton and Brobeck (1964) suggested that feeding is related to body temperature regulation. The latter authors found that rats with lesions in the anterior hypothalamic area eat more than controls in the heat but eat less than controls in the cold. However, Grossman (1968) concluded that the changes in hypothalamic temperature of the rat do not show any consistent relationship to the development of satiety during eating.

In the goat, hypothalamic temperatures have been found to remain constant during eating or decrease during forced feeding via rumen fistulae (Baile and Mayer, 1968b). Neither initiation of eating nor cessation of eating could be attributed to changes in hypothalamic temperature, nor could any correlation between feeding behaviour and rumen or horn temperatures be discerned. There are, however, reports that the onset of feeding induces an immediate temperature elevation in the carotid artery and jugular vein of the ox (Ingram and Whittow, 1962) and sheep (Mendel and Raghavan, 1963). The increase in jugular temperature was greater than that of the carotid blood, thus decreasing carotid-jugular temperature difference. A similar response was observed during rumination. When the animal stopped eating there was an immediate decrease in jugular blood temperature. Attebury and Johnson (1969) found that high environmental temperatures depressed the amplitude of rumen contractions in cattle. The effect of environmental temperature on rumen motility was thought to be direct since feed intake was controlled at the same level at all air temperatures. With data collected from the same experiment, Kelley et al. (1967) reported that the levels of total volatile fatty acids in the rumen were depressed when the cattle were exposed to 38°C. This would suggest that VFA's are probably of less importance in determining satiety at high environmental temperatures.

While the exact role of hypothalamic or other deep-body temperature receptors in the regulation of feed intake has not been clearly established, there are instances where increased deep body temperature could conceivably be related to inhibition of feeding. Very drastic decreases in feed intake have been reported for a number of species

at high environmental temperatures (Heitman and Hughes, 1949; Kleiber, 1961; Brody, 1945; Johnson et al., 1963; Davis and Merilan, 1960; Johnson and Yeck, 1967). In view of the marked increase in metabolic rate during eating in ruminants (Young, 1966; Webster, 1967) one would expect a high environmental temperature to impose a significant stress on the animals during eating since thermal polypnea is markedly reduced during this activity (Young, 1966). Bhattachyra and Warner (1968) found that the infusion of cold (5°C) water into the rumen of dairy cows during eating increased feed intake 24% whereas warm (49°C) water reduced intake by 9%. These treatments were associated respectively with a decrease and increase in tympanic membrane temperature.

In a cold environment, the "specific dynamic effect" of food is useful for helping to maintain homeothermy (Kleiber, 1961) and under these conditions a rise in deep body temperature probably would be less important in determining satiety than in a thermoneutral or warm environment. The extent to which the increased heat production during eating in ruminants can substitute for cold induced thermogenesis has not been established. In any event, feed intake of ruminants has been shown to increase during cold exposure (Webster et al., 1969; Webster, Chlumecky and Young, 1970; Wodzicka-Tomaszewska, 1964). The study of Webster et al. (1969) indicated that sheep exposed to a constant cold stress in a cold room increased their feed intake in relation to the degree of cold stress, whereas with sheep exposed to the fluctuating outdoor environment, the feed intake was less closely related to the thermal demand of the environment but tended to increase gradually throughout the winter. The resting metabolic rate of the outdoor animals also increased throughout the winter in a manner not directly

related to the thermal demand of the environment. The reasons for
the different appetite responses of cold room acclimated and winter
acclimatized sheep have not been elucidated but are probably related
to the characteristic metabolic adjustments observed in the respective
groups (Webster et al., 1969).

It has been shown in rats that the involvement of thyroid hormones
(Hamilton, 1967) and glucostatic mechanisms (Epstein and Tietelbaum,
1968) are not essential for increasing feed intake during cold exposure.
The extent to which appetite responses to cold might be mediated by
thyroid activity in ruminants is not definitely known. However, thyro-
xine treatment has been shown to increase feed intake in ruminants
(Lambourne, 1964) and thyroid activity is increased during cold expo-
sure. As the regulatory mechanisms by which ruminants increase their
energy expenditure in a cold environment have not been clearly estab-
lished (Webster et al., 1969), one cannot identify with certainty any
specific factor that may be responsible for increasing feed intake in
the cold. A generalization that has been made is that feed intake is
increased in response to the increased energy expenditure by the animal
in the cold. However, if energy intake were precisely adjusted to
energy expenditure one could not account for the fact that animals can
store energy in the form of body fat.

Kennedy (1961) has proposed a "lipostatic" theory of appetite
regulation which states that feed intake and activity are adjusted in
response to changes in body content of fat, and that, in the long run,
animals attempt to maintain a constant fat content in the body. This
theory is supported by the work of Cohn and Joseph (1962) with rats
but according to Blaxter (1967) "can hardly apply to ruminants" since

mature dairy and beef animals vary so markedly in body fat content.
One could say as much of humans. Baile and Mayer (1966), however,
have demonstrated that, by placing lesions in the ventro-medial hypo-
thalamus, hyperphagia can be induced in goats. The animals overeat
and gain considerably in body weight for a period of time but then
maintain a constant weight again at a higher level. This suggests that
removal of some of the satiety mechanisms will allow the animal to eat
more food, but that eventually some other factor assumes a role in
limiting intake. Whether this observation in goats is an example of
a lipostatic regulation remains to be established.

Many different factors may be involved in determining the volun-
tary feed intake of animals and no single theory of appetite regulation
seems to account adequately for all the variations observed. There is
probably no single mechanism that determines satiety under all circum-
stances, but rather, as circumstances (diet composition, environment,
feeding system) change, different satiety mechanisms or combinations
of mechanisms probably assume roles of greater or lesser importance.
McClymont (1967) has suggested that inhibitory stimuli all have cer-
tain thresholds and that all above-threshold stimuli can summate to
produce an additive inhibition of feed intake. A similar proposal has
also been made for facilitatory stimuli.

Since appetite is probably the single most important determinant
of production efficiency in ruminants (Blaxter, 1969) a better under-
standing of the mechanisms regulating energy intake under various en-
vironmental, feeding and management situations would be of direct value
to the animal industry. Studies of the physiological changes that

occur during eating in ruminants may help our understanding of this
problem.

EXPERIMENTS CONDUCTED AT THE UNIVERSITY OF ALBERTA

An investigation was made of the extent and time course of changes in the respiration, circulation and body fluids of sheep during the activity of eating a single meal of hay.

EXPERIMENTAL METHODS

A. Animals and Management

All trials were performed on mature sheep. The breed, sex and body weights of the animals are listed in table 13 (Appendix page 131). The particular sheep used in each phase of the study are outlined below.

The animals were housed at the Environmental Laboratory, The University of Alberta, in individual wooden stalls within a large holding room, the temperature of which was maintained between 15 and 18°C. During any extended periods when the animals were not used for experiments they were housed in small group pens in the environmental laboratory.

On non-experimental days the sheep were fed twice daily 400-600 grams of chopped alfalfa-bromegrass hay. Cobalt iodized salt blocks and water were available at all times. On days when the sheep were used for experiment, the morning feed was witheld and the trials were conducted between 12:00 noon and 4:00 p.m. Since the sheep are normally fed at 4:00 p.m. the trials were, therefore, conducted 20 to 24 hours following the previous meal. Prior to their use in experiments the sheep were trained and therefore were accustomed to the experimental procedures employed.

The basic procedure employed for each eating trial is described briefly below. A more detailed description of the different surgical

and experimental techniques follows thereafter.

1. Animal preparation was carried out either on the afternoon preceding or the morning of the experimental day. Preparation included clipping the wool on the ventral surface of the neck for catheterization; clipping areas on the chest girth for placement of ECG surface electrodes and the insertion of jugular vein and carotid artery indwelling catheters.

2. At least one hour before starting each trial, ECG surface electrodes were fixed to the skin and the animal was placed in a metabolism crate. Heart rate was recorded and used as an indication of the energy expenditure of the animal immediately before each trial. After the heart rate had declined and remained at a steady level for 30 minutes, it was assumed that the animal had recovered from any excitement associated with handling. Experimental measurements and sampling were then started.

3. Each trial consisted of three periods: before, during and immediately after eating. Unless otherwise indicated the animals were offered feed for a period of 30 minutes. The pre- and post-eating periods were usually of at least 30 minutes duration.

B. <u>Surgical Procedures</u>

1. Carotid artery exteriorization: Five Lincoln ewes were prepared with carotid arterial loops by the surgical procedure outlined below.

Food and water had been witheld from the sheep for 24 hours. Wool was clipped from the neck, and surgical anaesthesia induced by slow intravenous injection of sodium pentobarbital[1]. The dose required

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[1] Abbott Laboratories Ltd., Montreal, Canada.

to induce anaesthesia was about 15-25 mg/kg. An endotracheal cannula
with inflatable cuff was inserted immediately via the larynx. Anaes-
thesia was subsequently maintained by periodic injections of sodium
pentobarbital. The skin on the neck was washed and treated with two
applications of iodine solution and sterile drapes were applied leav-
ing only the ventral surface of the neck exposed for surgery.

A fifteen cm. paramedial skin incision was made on the neck
starting about 4 cm. below the mandible and about 4 cm. to the right
of the midline. Blunt dissection was used to separate the sterno-
thyroid and omohyoid muscles and to expose the carotid artery. A
suitable length of artery (about 10 cm) was dissected free from its
connective tissue sheath with care being taken to avoid damage to the
vagus nerve which was left intact. The free loop of artery was
elevated by gentle traction and held in a superficial position by an
assistant while the fascia of the sternothyroid and omohyoid muscles
were sutured together beneath the loop with continuous chromic gut
sutures. A second skin incision was made 4 cm. lateral and parallel
to the initial incision, thus, creating a skin flap which was subse-
quently sutured into a loop enclosing the length of artery. The free
margins of the two skin incisions were sutured together beneath the
exteriorized loop with interrupted silk sutures. The skin incision
lines were treated with antibacterial powder and a gauze bandage was
wrapped around the loop to prevent adhesions between the loop and neck
suture lines. The animals were treated with daily intramuscular
injections of antibiotic for four days following surgery. The bandage
was removed from the loop on the second day following surgery and the
animals were inspected daily until the skin sutures were removed on

the tenth day. Experiments were not conducted with the sheep until at least six weeks after the date of surgery.

2. Cannulation of the Parotid Salivary Gland Duct:

Parotid salivary duct cannulae were established in two sheep during acute experiments. Anaesthesia and surgical preparation was carried out as described above.

A four cm. skin incision was made on the lateral aspect of the lower jaw. The parotid salivary duct was dissected from the surrounding tissue for a length of 2 cm. Ligatures were placed (but not tied) around the duct and a small transverse incision was made through the duct wall. An 18-gauge polyethylene catheter was inserted through the incision and directed proximally for a distance of about 5 centimetres. The ligatures were tied to secure the catheter. The experiments involving saliva collection were conducted while the animal was under anaesthesia. These particular animals were maintained under anaesthesia for other experimental purposes after which they were sacrificed.

C. Blood Sampling Procedure

Mixed venous blood samples were obtained through a polyethylene catheter[2] previously inserted via a jugular vein. The catheter was advanced down the jugular vein until a distinct pulse could be observed by movement of a small air bubble introduced into the saline-filled catheter. After this pulse was first observed, the catheter was advanced two inches further. If the pulse was still present the catheter tip was judged to be in the right ventricle of the heart. Samples drawn from this catheter were considered to be representative

[2] Clay Adams, Division of Becton, Dickinson and Co., Parsippany, N.J., U.S.A.

of mixed venous blood.

Arterial blood was obtained via a catheter inserted into the exteriorized carotid artery.

The distal end of each catheter was fitted with a three way tap. The intravenous and intra-arterial catheters were left in the animals for only a few hours and in no case were they left for a period longer than 48 hours. Between samples the catheters were flushed with sterile saline containing 20 IU/ml Heparin.

D. Physiological Measurements

1. Oxygen Consumption:

In sixteen trials with 4 Suffolk wethers, oxygen consumption ($\dot{V}_{O_2}$, ml/min or litres/hr) was determined using the open-circuit respiration apparatus described by Webster and Hicks (1968). The animals were trained to stand with their head in a ventilated hood (Webster and Hays, 1968) in which feed could be presented to the animal by opening a sliding door to the feed compartment (Berzins, 1969). The ventilation rate of the hood was about 60 litres per minute. Eight of the sixteen trials with wethers were conducted at an air temperature of 15°C (thermoneutral) and eight were conducted at an air temperature of -15°C (cold). The fleece depths of the wethers during this experiment ranged from 45-50 millimetres.

A further series of thirteen trials were conducted with four Lincoln ewes at an air temperature of 15 to 18°C.

Oxygen consumption was calculated by multipling the ventilation rate (litres/hr STPD) by the decrement in oxygen concentration between the air entering and leaving the ventilated hood.

2. Heart Rate:

Heart rate was recorded throughout most of the trials conducted during this study. Surface electrodes were taped to the skin at three sites around the circumference of the chest just caudal to the thoracic limbs. One electrode was placed just behind each elbow and the third was placed dorsally just behind the left shoulder. Electrical contact between the skin and electrode was achieved by a layer of electrode paste. The ECG signal was recorded using a high gain pre-amplifier (type 350-2700)[3] and Sanborn recorder (type 7700)[3]. In some trials the ECG signal was displayed on an oscilloscope screen and visual counts were made at 5 minute intervals instead of using the recorder.

Heart rate changes during eating were recorded in nearly all trials. Two series of trials with three wethers were conducted in cooperation with F.L. Hays specifically to investigate heart rate changes during eating at different degrees of cold stress.

In the (a) series each animal had a fleece depth of about 50 mm. and were fed after two hours exposure to an air temperature of -15°C. One control and one treatment trial was conducted on each animal.

In the (b) series the same design and air temperature was used except that the animals were shorn and two control and two treatment trials were conducted with each animal.

In both series (a) and (b), the treatment consisted of an intravenous injection of propranolol[4] (0.5 mg/kg) one hour prior to cold

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[3] Hewlet Packard, Canada, Ltd., Point Claire, Quebec.

[4] Ayerst Laboratories, Saint Laurent, Quebec

exposure followed by a second injection of the drug after the animals had been in the cold for one hour.

Series (a) and (b) have been designated "cool" and "cold" respectively.

3. Blood gas and Blood Acid-Base Analysis

In seventeen trials with four Lincoln ewes, blood pH, P_{O_2} and P_{CO_2} determinations were made on samples of mixed venous and arterial blood drawn simultaneously at regular intervals before, during and after eating.

The blood samples were drawn into plastic syringes, the barrels of which had been lightly greased to prevent entry of air into the syringe. The syringes were immediately capped and placed in an ice bath until the samples were analyzed.

Analysis of blood for pH, P_{O_2} and P_{CO_2} was carried out using the Radiometer blood micro system (BMS3)[5] which incorporated the model G298A pH, model E5047 P_{O_2} and model E5036 P_{CO_2} electrodes in a thermostatic bath maintained at 39°C. The above determinations were done in duplicate for each sample.

The electrodes of the "blood micro system" were calibrated immediately before analysing each set of samples and frequent calibration checks were carried out during the analysis of each set. The high and low calibration values for P_{O_2} encompassed a range from 0 to 140 mm. Hg and for P_{CO_2} the range was from about 18 to 80 mm. Hg, the absolute values depending upon concentration of the standard gas and the barometric pressure at the time of analysis.

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[5]
Radiometer, Copenhagen, Denmark.

Plasma bicarbonate concentration was calculated from a knowledge of blood P_{CO_2} and pH according to the "Henderson-Hasselbalch equation" employing a pK value of 6.10 and a solubility coefficient of 0.03.

Oxygen saturation of the hemoglobin was calculated using the Radiometer blood-gas calculator, type BGC1[5] which incorporated the oxygen dissociation curves shown in Figure 1.

4. Cardiac Output Measurements

(a) In thirteen trials with four Lincoln ewes, the cardiac output was determined before, during and after eating according to the "direct Fick" procedure. These determinations of cardiac output were based upon a knowledge of oxygen consumption and arterio-venous blood oxygen differences measured simultaneously. The measurements of oxygen consumption and blood oxygen saturation have already been described.

In order to determine the oxygen content of the blood, hemoglobin (Hb) determinations were made using the "Sahli-Adams method" (Schalm, 1965).

Oxygen content of the blood (C_{O_2}, ml/100 ml) was calculated assuming that hemoglobin carried 1.34 ml O_2/gm at 100% saturation with O_2.

Thus C_{O_2} = [1.34 x gm Hb/100 ml] X [% O_2 saturation/100]
Cardiac output (Q, litres/min) was calculated from rate of oxygen consumption ($\dot{V}_{O_2}$, ml/min) and arterio-venous C_{O_2} difference by:

$$Q = \dot{V}_{O_2} / (Ca_{O_2} - Cv_{O_2})$$

Stroke volume (svml blood/heart beat) was calculated by:

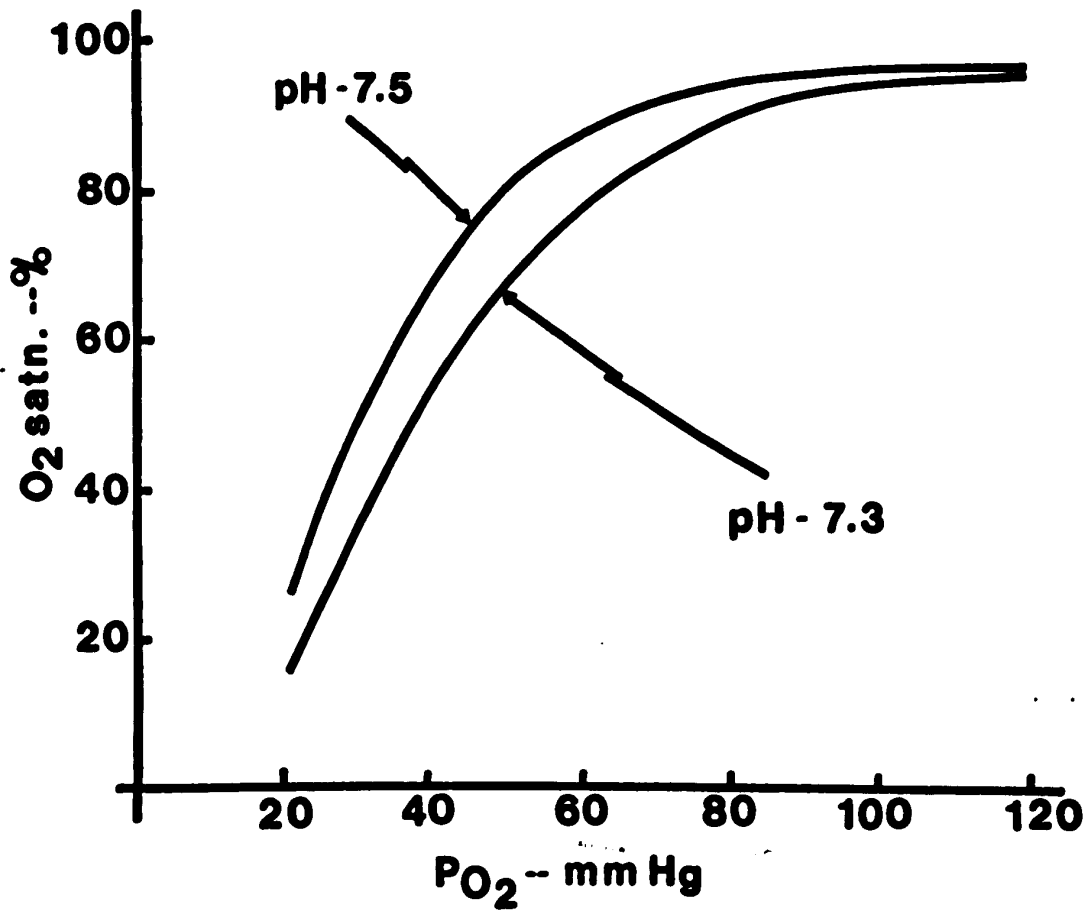$$\text{Stroke volume} = Q/\text{heart rate}$$

Figure 1. Examples of the oxygen dissociation curves used for estimating the oxygen saturation of hemoglobin.

Hematocrit (Hct, %) values were determined on arterial blood samples according to the microhematocrit procedure. Blood was drawn (after thorough mixing) into heparinized capillary tubes. The capillary tubes were sealed at one end and were centrifuged for 15 minutes at 12,500 rpm in a microhematocrit centrifuge[6].

(b) A series of trials with three Lincoln ewes was conducted to evaluate a dye-dilution technique for estimating cardiac output.

It was considered that the continuous infusion of dye into the venous blood returning to the right ventricle might lead to the establishment of an asymptotic concentration of dye in the arterial outflow from the left ventricle before dye recycling occurred. If a true plateau does occur and if the time during which the plateau remains stable can be predicted, a single arterial blood sample drawn at a suitable time during the plateau period could be used to estimate the asymptotic dye concentration in the blood leaving the heart. From a knowledge of the asymptotic dye concentration (C_m, mg/l in the plasma) and the infusion rate of the dye (I, mg/min) the cardiac output of plasma (Q_p, litres/min) could be calculated according to the following formula: $Q_p = I/C_m$.

In order to calculate total blood flow, the hematocrit must be determined and the formula for true cardiac output (Q) becomes:

$$Q = Q_p/(1 - Hct)$$

This approach for determining cardiac output, if valid, would eliminate the need to measure oxygen consumption and would have the advantage of requiring only a single blood sample drawn at a suitable

[6] International Equipment Co., Needham Heights, Mass., U.S.A.

time during dye infusion. This would allow measurements of cardiac output to be repeated at frequent intervals.

To assess the validity of the technique, the time course of dye appearance in the arterial blood was determined as follows:

The blue dye, "bromsulphthalein" (BSP)[7] was chosen since it binds to plasma proteins and (at least initially) remains intravascular, and because it is cleared relatively rapidly and primarily by the liver (Cornelius, 1963). Thus, repeated infusions should not result in large accumulations of the dye in the circulation.

Bromsulphthalein was infused continuously for 30 seconds via a jugular catheter, at a rate of 50 mg/min. The infusion was made using a model 600-950V, Harvard syringe-pump[8] and a 20 ml glass syringe. The same calibrated syringe was used in all trials.

At the same instant that the infusion was started a peristaltic pump (model 1215, Harvard)[8] was switched on. This pump drew a continuous sample of arterial blood via a carotid arterial catheter. The distal tip of the sampling catheter was shifted from one collection tube to the next at 4-second intervals for a period of 60 seconds. Thus, blood samples were collected at 4-second intervals during the 30-second dye infusion and for a further 30 seconds following the cessation of infusion.

The blood samples were centrifuged for 20 minutes at 3000 R.P.M. and the plasma was separated for determination of the dye concentration.

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[7] Hynson, Westcott and Dunning, Inc., Baltimore, Maryland, U.S.A.

[8] Harvard Apparatus Co. Ltd., Dover, Mass., U.S.A.

Plasma samples were diluted with distilled water and NaOH (0.1N) was added to develop the dye color. The optical densities of the diluted samples were read in a Spectronic-20 colorimeter[9] at 580 mμ. The instrument was set to zero absorbance with diluted pre-infusion plasma and a calibration curve was established using a series of BSP standard solutions appropriately diluted with reagent and pre-injection plasma.

5. Blood and Plasma Volume Measurements

Twenty-five trials with four Lincoln ewes were conducted to determine the plasma and total blood volumes before, during and after eating. Eight control trials in which the animals were not fed were also conducted.

(a) Plasma volume was estimated from the volume of distribution of T-1824[10] following intravenous injection of this dye. Although, more correctly called the T-1824 space, for convenience, this compartment will be referred to as the "plasma volume".

At zero time, a single intravenous injection of T-1824 (25 mg) was made. Mixed venous blood samples were drawn at 10-minute intervals. After an interval of 60 min (17 trials), 120 min (5 trials) or 30 min (3 trials) the animals were offered feed for 30 min during which time blood samples were drawn at 5 minute intervals. Following removal of the feed blood sampling was continued for at least another 30 minutes.

The blood samples were centrifuged for 20 min at 3000 R.P.M. to separate the plasma. The plasma samples were diluted and the T-1824

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[9] Bausch and Lomb, Canada, Scarborough, Ont.
[10] General Diagnostics Div., Warner-Chilcott, Morris Plains, N.J.,U.S.A.

dye concentration was estimated from the optical density determined
at 620 mμ in a Spectronic-20 colorimeter.

In eight control experiments, the animals were not fed, and
T-1824 concentrations in the plasma were determined at intervals
that corresponded with those in the eating trials.

For analysis, the plasma optical density was plotted versus time
after injection on a semi-log graph. Absolute plasma volume was
estimated by extrapolation of the decay curve to give the zero time
dye concentration (C_o, mg/ml). The following formula was used to
calculate plasma volume (PV, ml) where D = injected dose of dye (mg):
PV = D/C_o.

The changes in the T-1824 decay curve associated with eating were
used to estimate changes in plasma volume.

(b) Hematocrit was determined by the microhematocrit procedure.

(c) Total blood volume (BV) was estimated from a knowledge of
the PV and hematocrit (Hct) according to the following equation:

$$BV = PV/[1.0 - (0.96 \times Hct/100)]$$

The factor (0.96) is a correction for the quantity of plasma
trapped in the column of packed red cells.

6. Estimation of the Extracellular Fluid Volume (Thiocyanate
and Thiosulphate spaces)

The volumes of distribution of thiocyanate ion and thiosulphate
ion were determined using the single-injection technique that was
used for studying the T-1824 space.

The decay curves obtained from eating and control trials were
used to estimate changes in the thiocyanate and thiosulphate spaces
associated with eating.

(a) Thiocyanate space was estimated during twelve eating and eight control trials with four Lincoln ewes. Approximately 15 mg/kg of sodium thiocyanate[11] (in a solution containing 50 mg/ml) was injected in a single dose at zero time. Plasma thiocyanate concentration was determined on samples drawn at various times following injection.

Plasma thiocyanate determinations were made according to the procedure of Meier (1963).

During several trials, samples of mixed saliva were obtained by holding a sponge in the animal's mouth for a few minutes. The saliva was pressed from the sponge and collected in test tubes. Saliva samples collected before and after injection were analysed for thiocyanate concentration.

During an acute experiment, one parotid salivary duct was cannulated and samples of saliva collected and analysed before and after injection of 15 mg/kg of sodium thiocyanate.

(b) Thiosulphate space was estimated during 13 eating trials and 8 control trials with four Lincoln ewes.

Sodium thiosulphate[11] was made up in isotonic solution, i.e. a 5% solution of the hydrated salt. Each sheep was given an injection over 4 min, of 200 ml of solution which contained 6.37 gm of pure Na thiosulphate. The midpoint of the injection period was used as zero time for purposes of data analysis.

Blood samples were collected at five minute intervals for the first hour and at 10-20 minute intervals during the second hour following injection.

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[11] Fisher Scientific Co., Ltd., Montreal, Quebec.

During all eating trials, the animals were allowed to eat between the 30th and 60th minute following injection of thiosulphate.

The thiosulphate concentration in plasma samples was determined according to the procedure of Varley (1966).

During three eating trials and three control trials urine was collected quantitatively via bladder catheters and analysed for thio-sulphate content. The cumulative urinary excretion of thiosulphate was determined for each trial.

Samples of parotid saliva were collected from an anaesthetized sheep before and after injection of 200 ml of 5% Na thiosulphate. Saliva samples were analysed for thiosulphate concentration.

For analysis, plasma thiosulphate concentration vs time was plotted on a semi-log graph.

Thiosulphate space (T, litres) prior to the onset of the meal was calculated in each trial from the extrapolated zero-time plasma concentration (G, mg/litres) and the injected dose (D, mg) by:

$$T = D/G$$

Changes in the decay curve occurring during and after eating were compared with the control experiments in which the sheep were not fed.

7. Plasma Concentrations of Na^+ and K^+

An experiment was conducted with three ewes and one wether to determine the concentrations of Na^+ and K^+ in mixed venous plasma samples collected during 30 minute periods before, during and after eating.

The Na^+ and K^+ contents of each sample were analysed in duplicate using an atomic absorption spectrophotometer (Techtron, type AA4)[12].

[12] Techtron PTY. Ltd., Melbourne, Australia.

8. Statistical analysis of the data were performed according to Steel and Tory (1958). The time course of changes in the various measurements before, during and after eating are described by the means and standard errors of the means for each time interval. Tests of significance were performed by use of the unpaired t-test in which the means of all values during eating or after eating were compared with the mean of all pre-eating values.

RESULTS

Feed Consumption

The mean intakes of hay and times spent eating by the sheep in various trials are summarized in Table 2. In general the Suffolk wethers consumed much more hay at a single meal than did the Lincoln ewes. This may have been because the Lincoln ewes were catheterized and somewhat disturbed by the taking of blood samples during the meal, although every effort was made to minimize this disturbance.

Oxygen Consumption and Heart Rate

Changes in $\dot{V}_{O_2}$ and heart rate recorded from the Suffolk wethers offered feed for 60 minutes at air temperatures of +15°C and -15°C are shown in Figure 2. The increase in $\dot{V}_{O_2}$ during eating was virtually the same at the two temperatures, an increase of 60-70% above pre-eating values. On the other hand, the increase in heart rate during eating was very much greater at -15°C. This mild cold stimulus, which did not elevate $\dot{V}_{O_2}$ very much, markedly exacerbated the tachycardia during eating. Oxygen pulse, therefore, was considerably lower before eating at -15°C and decreased even further during the meal. At +15°C there was no apparent change in O_2 pulse during the meal but the values increased somewhat after eating.

The effects of beta-adrenergic blockade with propranolol on heart rate during eating in cool (-15°C, fleece) and cold (-15°C, shorn) environments are shown in Table 3. These trials, which were conducted with F.L. Hays, confirmed the intense tachycardia that was observed during eating in the cold in the previous experiment. Also, the heart rate during eating, even after propranolol treatment in the cold,

Table 2. Intake of hay and time spent eating (mean ± SE) by sheep during various experimental measurements

Animals and Experimental Conditions	Measurements	Number of Trials	Hay Consumed (gm)	Time spent eating (min)
Suffolk wethers				
Fleece +15°C	$\dot{V}O_2$ and HR	8	709 ± 26	58 ± 1.3
Fleece -15°C	$\dot{V}O_2$ and HR	8	659 ± 18	57 ± 2.1
Fleece -15°C	HR	3	617 ± 48	60[1]
Fleece -15°C + propranolol	HR	3	485 ± 189	60[1]
Shorn -15°C	HR	6	558 ± 74	60[1]
Shorn -15°C + propranolol	HR	6	651 ± 53	60[1]
Lincoln ewes				
15 to 18°C	$\dot{V}O_2$ + Q	13	312 ± 15	30 ± 1.1
" " "	PV and BV	25	317 ± 24	30 ± 1.3
" " "	Thiosulphate space	13	248 ± 25	22 ± 1.6

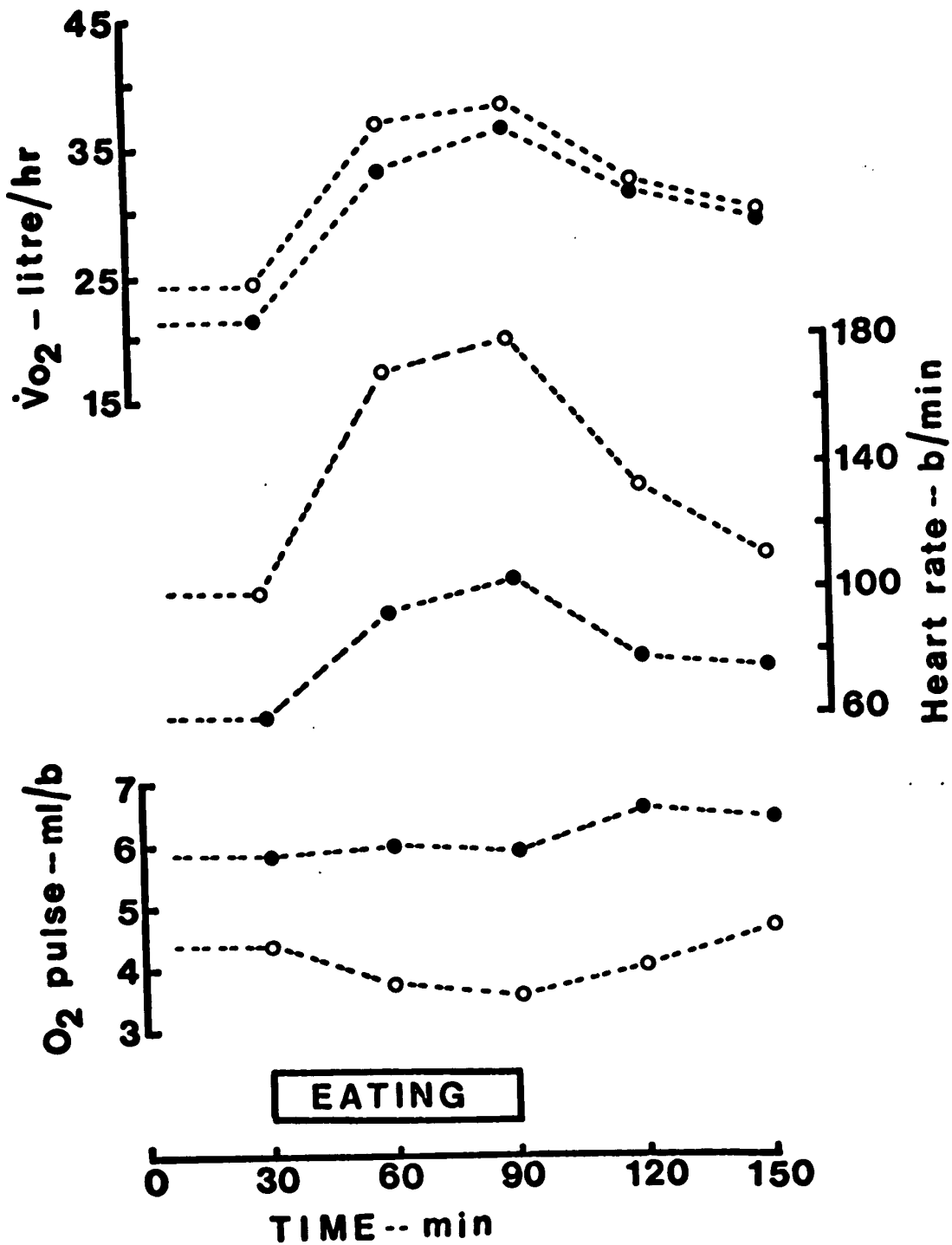1 Animals ate for 60 minutes at which time the feed was removed.

Figure 2. Changes in $\dot{V}_{O_2}$, heart rate and oxygen pulse in Suffolk wethers during eating in thermoneutral (solid circles) and cold (open circles) environments. Each point is the mean of 8 trials.

Table 3. Mean values (±SE) for heart rate of Suffolk wethers before, during and after eating in cool, and cold environments with and without propranolol treatment

Environment	Cool		Cold	
Treatment	Control	Propranolol[2]	Control	Propranolol[2]
No. of Observations	3	3	6	6
Periods[1]				
1	80 ± 3	75 ± 2	80 ± 3	77 ± 2
2	73 ± 2	72 ± 3	91 ± 8	73 ± 5
3	85 ± 8	81 ± 4	180 ± 25	118 ± 8
4	85 ± 3	72 ± 3	178 ± 18	103 ± 11
5	165 ± 19	100 ± 6	230 ± 17	135 ± 10
6	190 ± 18	128 ± 12	233 ± 14	147 ± 8
7	154 ± 26	127 ± 15	210 ± 16	129 ± 10
8	138 ± 18	128 ± 16	200 ± 17	133 ± 12

[1] The sheep were transferred from a thermoneutral (+18°C) room into a cold room at the end of period 2 and then were allowed food during periods 5 and 6. All periods were of 30 minutes duration.

[2] Two injections of propranolol (0.5 mg/kg) were given, one at the end of period 1 and the second at the end of period 3.

increased to an average of about 150 beats/minute. In individual trials, the heart rate during eating after propranolol treatment was on occasions as high as 170 beats/minute.

The $\dot{V}_{0_2}$ and heart rate changes of the four Lincoln ewes offered feed for 30 minutes at +15°C presented a picture essentially similar to that of the Suffolk wethers. The values for $\dot{V}_{0_2}$ increased by about 60% during eating from about 250 ml/min to about 400 ml/min and heart rate increased from about 80 to about 120 beats per minute. These measurements, which were taken simultaneously with measurements of blood gas concentrations and used to assess cardiac output and stroke volume, will be considered in more detail below.

Blood Gas Analysis

Mean values for blood P_{CO_2}, pH and plasma bicarbonate before, during and after eating are presented in Figure 3. Comparisons among the overall means are given in Table 4. Both arterial and venous P_{CO_2} rose sharply during eating and remained elevated during the 30 minutes following the meal. Mean values during and after eating were both significantly elevated ($p < 0.01$). Values for Pv_{CO_2} decreased slightly following the meal but Pa_{CO_2} remained at about the level attained during eating. The changes resulted in a significant increase in $[Pv_{CO_2} - Pa_{CO_2}]$ during eating ($p < 0.01$).

Both arterial and venous blood pH decreased abruptly during eating to values which were, on the average, about 0.06 pH units below pre-feeding values ($p < 0.01$). Blood pH recovered somewhat, but not totally after eating. During the 30 minutes following the meal the mean values were still significantly lower than pre-feeding values ($p < 0.01$).
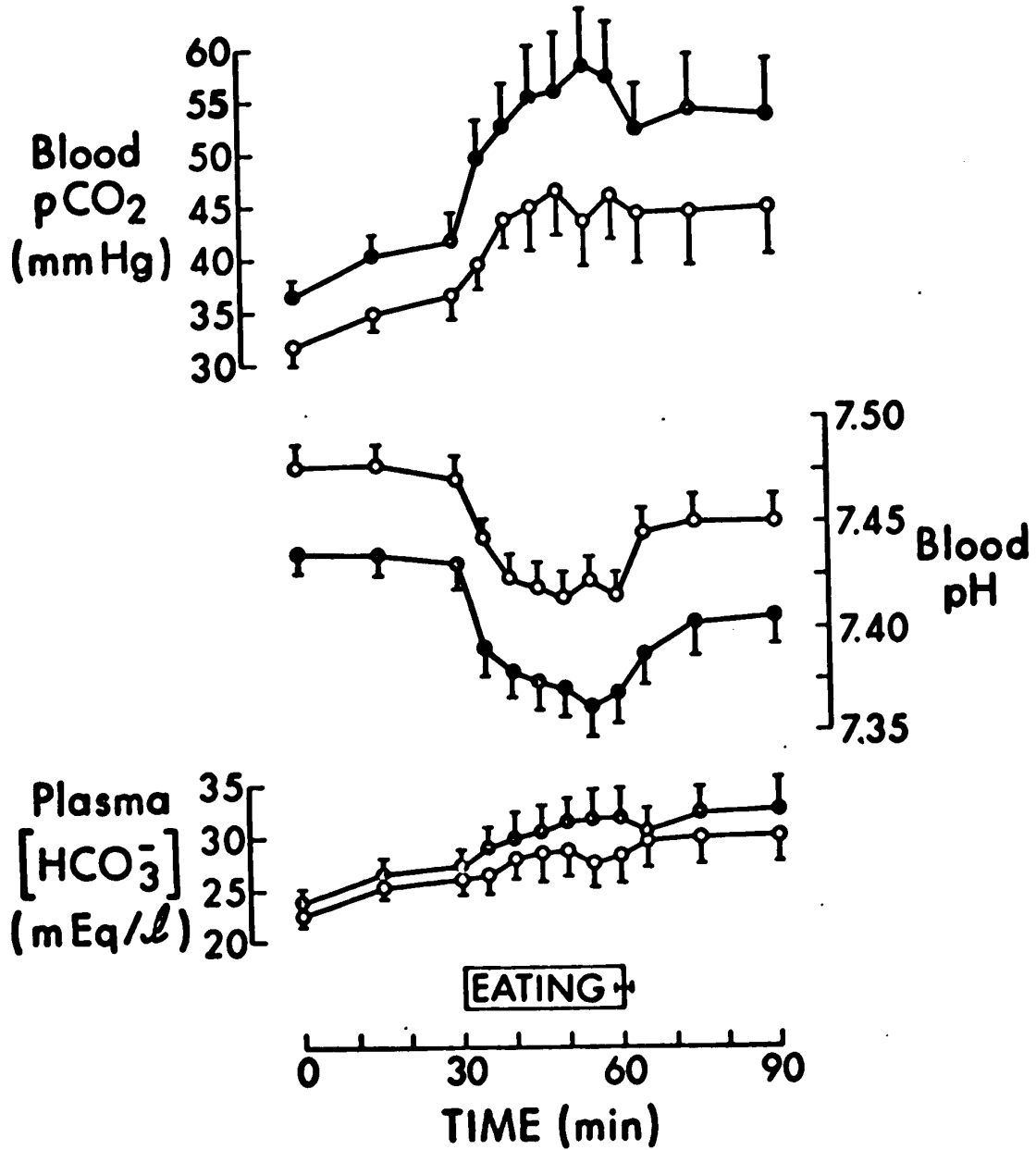
Figure 3. Values (mean±SE, n = 13) for blood P_{CO_2}, pH and plasma bicarbonate in Lincoln ewes before, during and after eating. (Solid circles – venous blood; open circles = arterial blood). Measurements were made while the sheep were in the ventilated hood.

Venous plasma bicarbonate concentrations showed a progressive increase to values that were significantly higher ($p < 0.01$) during and after eating than before the meal. The change in arterial plasma, although significant ($p < 0.05$), was smaller than for venous plasma. The venous-arterial difference in plasma bicarbonate concentration, therefore, was increased significantly ($p < 0.01$) during the meal.

Table 4. Comparison of mean values for blood pH, P_{CO_2} and plasma HCO_3^- determined before, during and after feeding

	Before Eating	During Eating	After Eating
pH_a	7.474	7.422**	7.448**
pH_v	7.433	7.374**	7.398**
Pa_{CO_2} (mmHg)	34.4	44.0**	44.7**
Pv_{CO_2} (mmHg)	39.5	54.8**	53.6**
$Pv_{CO_2} - Pa_{CO_2}$ (mmHg)	5.08	10.81**	8.94**
Plasma $[HCO_3^-]$ (mEq/l)			
a	24.6	27.7*	29.7**
v	25.5	30.7**	31.7**
v - a	0.98	3.08**	1.98

Mean values marked with asterisks differ significantly from the pre-eating mean. * ($p < 0.05$) ** ($p < 0.01$).

Values for blood P_{CO_2} and pH determined in 4 trials during which the animals were not in the hood, showed trends similar to those obtained when the sheep were in the hood (Figure 4).

Figure 5 shows the mean values for hematocrit, hemoglobin and MCHC, and comparisons of the overall means are given in Table 5.
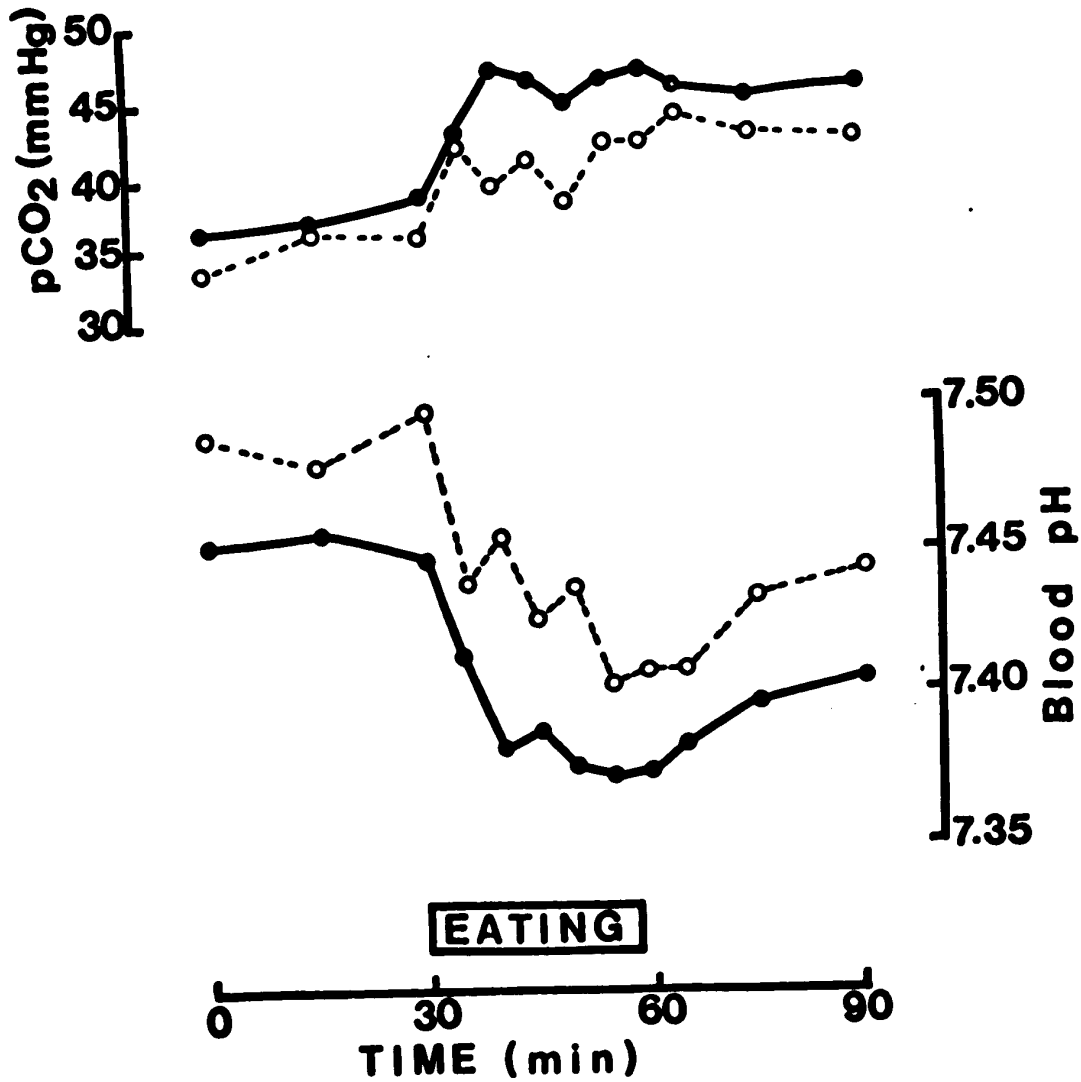
Figure 4. Blood P_{CO_2} and pH determined when the sheep were not in the ventilated hood. (Solid circles = venous blood; open circles = arterial blood). Each value is the mean of 4 trials.
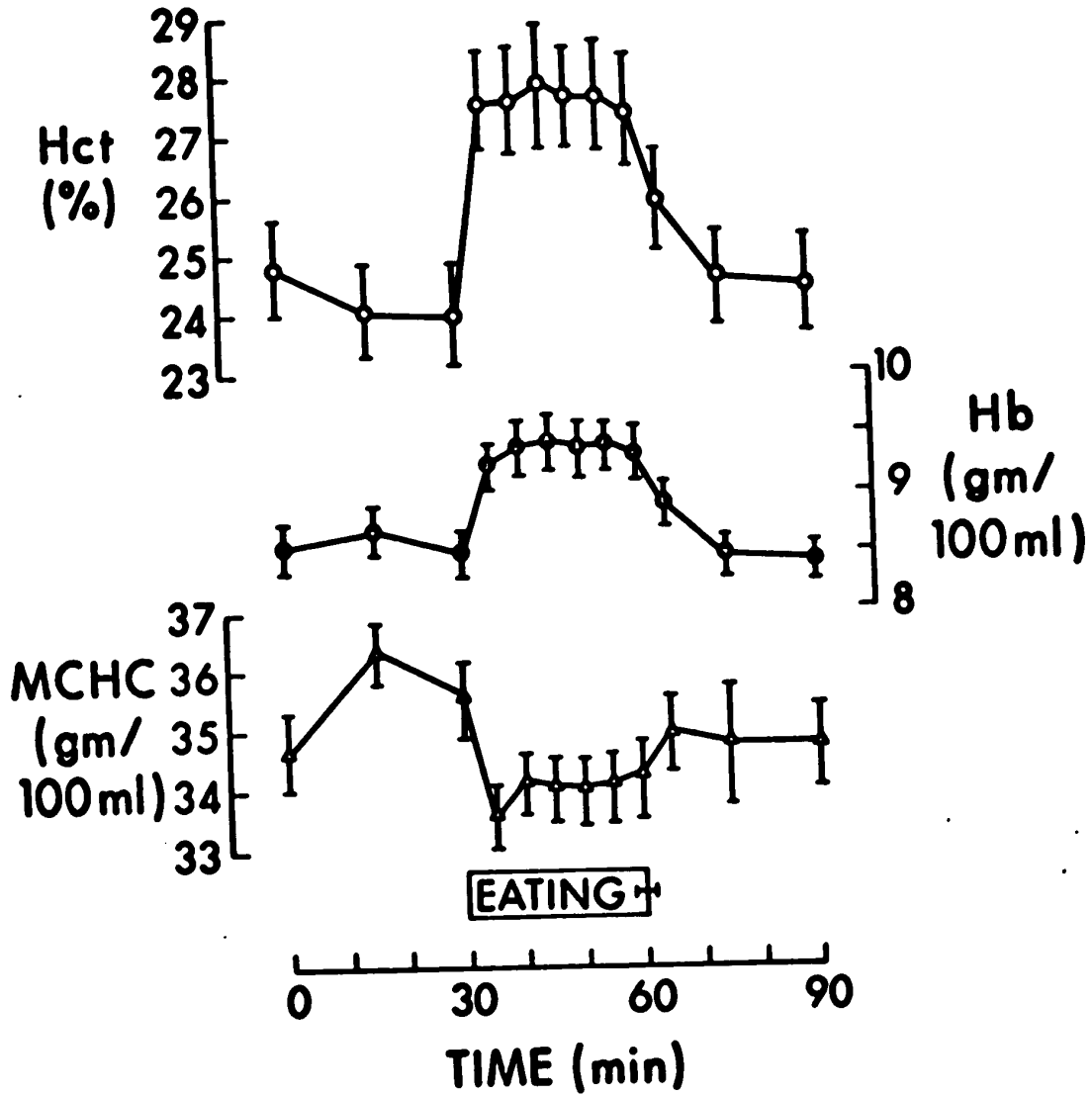
Figure 5. Hematocrit (Hct), hemoglobin (Hb) and mean corpuscular hemoglobin concentration (MCHC) in Lincoln ewes before, during and after eating. (mean±SE, n = 13).

There was a rapid increase in hematocrit from 24% before to about 27.5% during the meal. The hematocrit values were significantly elevated during eating ($p < 0.01$) but returned to pre-feeding values following the meal. Hemoglobin values increased significantly during eating ($p < 0.01$) and appeared to reflect the changes in hematocrit.

MCHC values were variable before feeding and showed a small, but significant decrease during the meal ($p < 0.01$).

Mean values for blood P_{O_2} are shown in Figure 6. Comparison of mean values before, during and after eating are given in Table 5.

Table 5. Comparison of mean values for hematocrit, hemoglobin, MCHC and blood oxygen determined before, during and after eating

	Before Eating	During Eating	After Eating
Hct(%)	24.3	27.6**	25.0
Hb(gm/100 ml)	8.59	9.38**	8.62
MCHC(gm/100 ml)	35.54	34.06**	34.86
Pa_{O_2}(mmHg)	69.5	67.9	69.8
Pv_{O_2}(mmHg)	34.0	30.6**	31.7*
aO_2 satn.(%)	90.2	87.4**	88.9
vO_2 satn.(%)	52.6	41.5**	44.8**
Ca_{O_2}(ml/100 ml)	10.40	11.27**	10.53
Cv_{O_2}(ml/100 ml)	6.02	5.31**	5.34**
$[Ca_{O_2} - Cv_{O_2}]$(ml/100 ml)	4.38	5.96**	5.21**

Mean values marked with asterisks differ significantly from the pre-eating mean. * ($p < 0.05$, ** ($p < 0.01$).

Values for Pa_{O_2} did not change during eating although Pv_{O_2} underwent a small, but significant decrease ($p < 0.01$). In some individual

trials, a decrease in Pa_{O_2} was recorded in occasional samples during eating but no consistent trend was established.

Values for percent oxygen saturation of hemoglobin, which are dependent upon both P_{O_2} and pH, are also summarized in Figure 6 and Table 5. There was a slight, but significant ($p < 0.01$) decrease in arterial oxygen saturation during eating. Venous oxygen saturation decreased markedly during eating and recovered slightly following the meal. Mean values for venous oxygen saturation both during and after eating were significantly lower than pre-feeding values ($p < 0.01$).

Ca_{O_2} increased markedly during eating ($p < 0.01$) and reflected the increase in hemoglobin concentration since arterial oxygen saturation did not change very much. Values for Cv_{O_2} did not show an abrupt shift during eating although they tended to decrease progressively from the beginning to the end of the meal and the mean values during and after eating were significantly ($p < 0.01$) lower than pre-eating values. In this case the decrease in oxygen saturation tended to mask the increase in hemoglobin concentration during eating, such that Cv_{O_2} did not change rapidly (Figure 6).

The overall effect of the above changes in the arterial and venous blood was to create a significant increase in $[Ca_{O_2} - Cv_{O_2}]$ from 4.4 ml/100 ml before to 5.9 ml/100 ml during eating ($p < 0.01$). mean values for $[Ca_{O_2} - Cv_{O_2}]$ decreased somewhat after the meal but remained significantly higher than pre-feeding values ($p < 0.01$).

Cardiorespiratory Function During Eating

The data given above for $\dot{V}_{O_2}$, heart rate and $[Ca_{O_2} - Cv_{O_2}]$ were used to determine the changes in cardiac output and stroke volume of
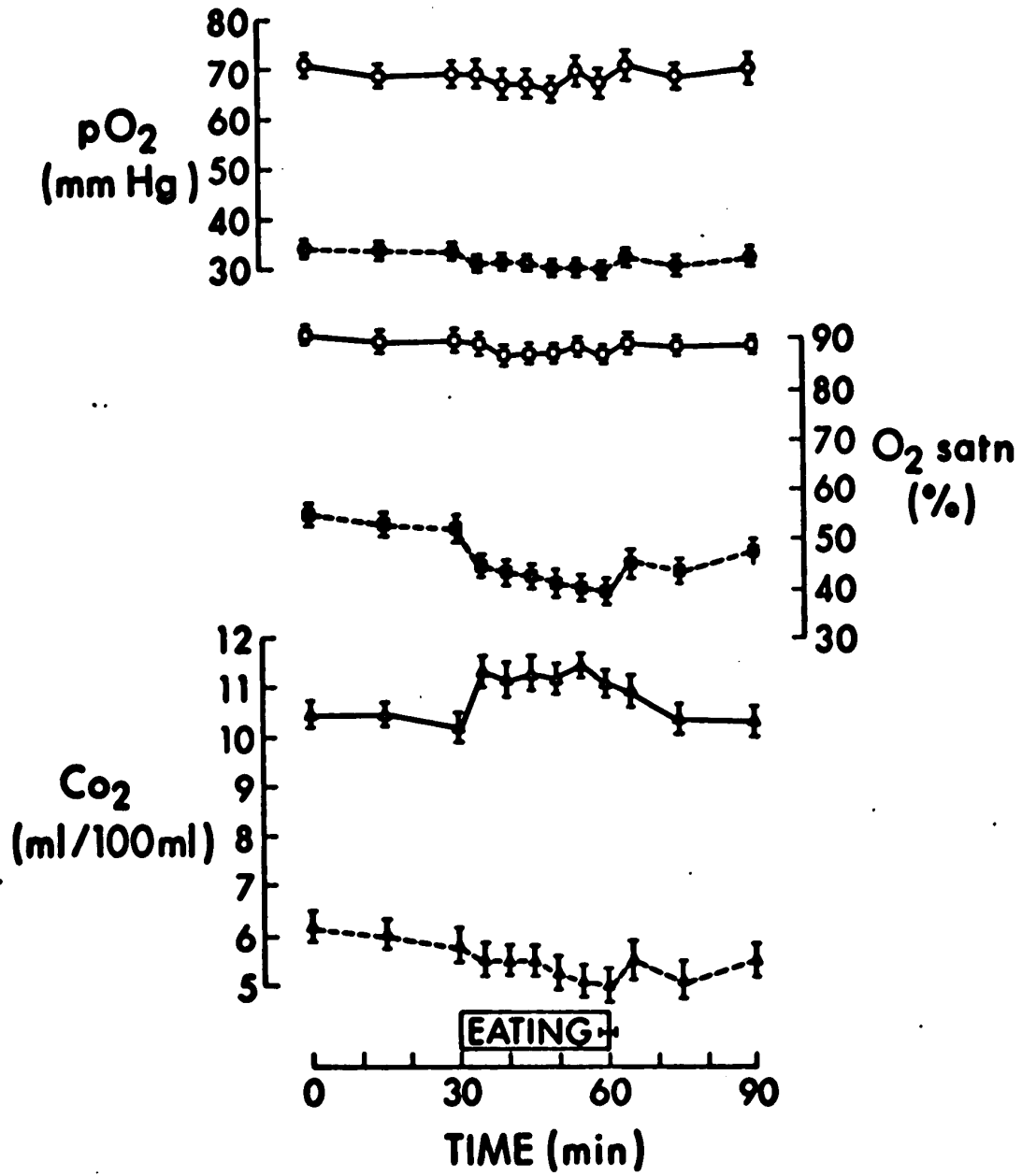
Figure 6. Blood P_{O_2}, oxygen saturation and C_{O_2} in Lincoln ewes before, during and after eating. (mean$\pm$SE, n = 13). Solid symbols = venous blood; open symbols = arterial blood.

the sheep during eating. All these data are combined in Figure 7 which brings together, in an integrated form, the different changes in cardiorespiratory function during eating. Table 6 gives the comparison of mean values before, during and after eating.

The figure shows that the increases in $\dot{V}_{O_2}$ and heart rate is not accompanied by a comparable increase in cardiac output despite the observed relationship between $\dot{V}_{O_2}$ and heart rate. Cardiac output increased, at the most, by 15-18%, compared with a 50-60% increases in heart rate and $\dot{V}_{O_2}$ during eating. This increase took place in the first 10 minutes of the meal and then declined even while the animals continued to eat. Stroke volume decreased progressively during eating and recovered somewhat following the meal.

Table 6. Comparison of mean values for oxygen consumption, heart rate, cardiac output (Fick) and stroke volume determined before, during and after eating

	Before Eating	During Eating	After Eating
$\dot{V}_{O_2}$ (ml/min)	254.9	382.8**	300.0**
Heart rate(beat/min)	80.1	114.5**	97.1**
Cardiac Output(l/min)	6.07	6.75**	5.97
Stroke volume(ml/beat)	76.3	59.6**	62.9**

Mean values marked with asterisks differ significantly from the pre-eating mean. ** (p<0.01).

Cardiac Function Determined by BSP - Dilution

Examples of individual dye-dilution curves are presented in Figure 8 which shows the time-course of changes in plasma dye concentration during and following a 30-second continuous infusion of

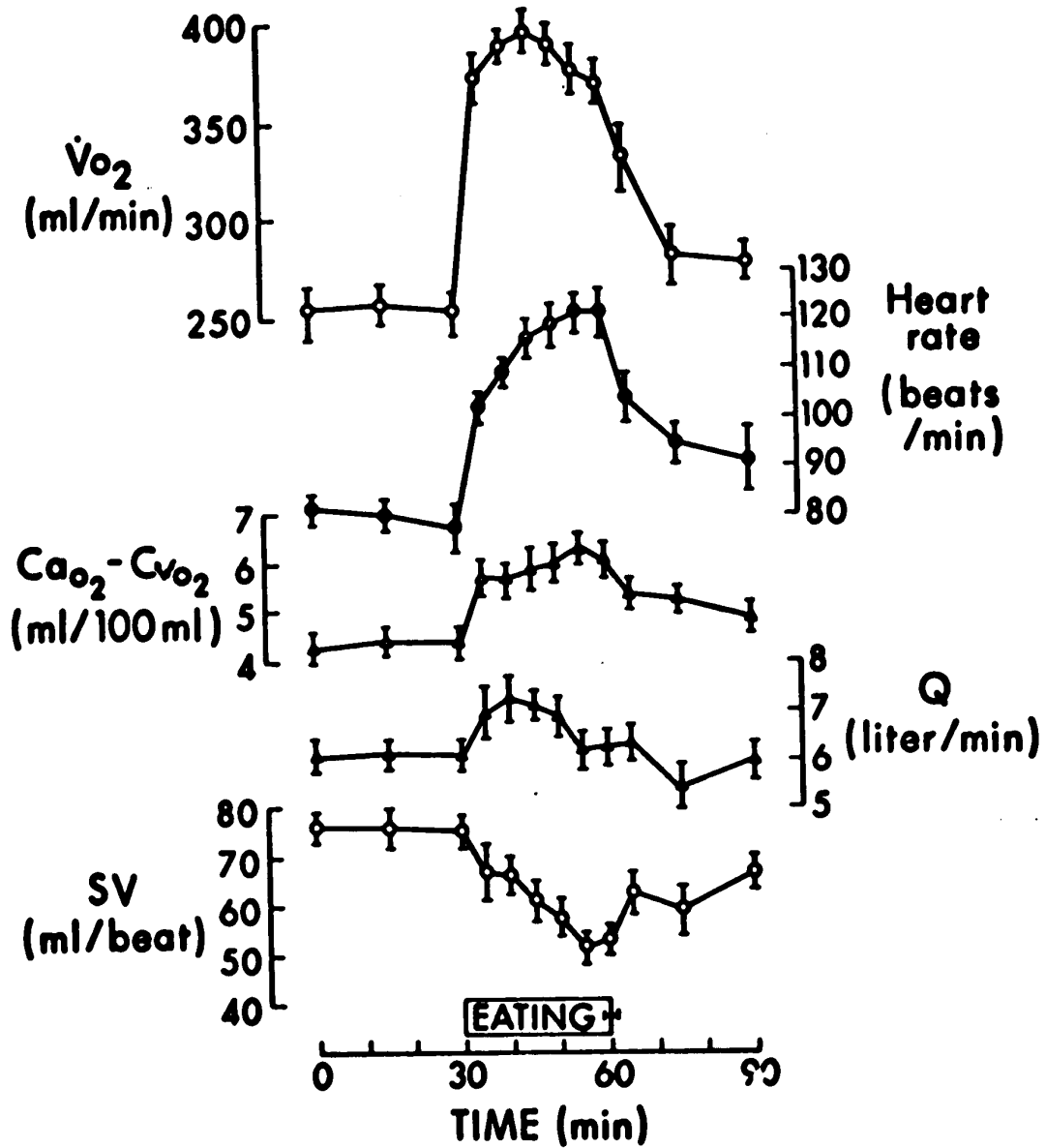**Figure 7.** Summary of changes in cardiorespiratory function in Lincoln ewes during eating. Values for $\dot{V}_{O_2}$, heart rate, arterio-venous CO_2 difference, cardiac output (Q) and stroke volume (SV) are presented as the mean±SE of 13 trials.

bromsulphthalein via the jugular vein. When sampling continuously

from the carotid artery, there was a delay of 12 to 16 seconds before

the initial appearance of dye in the sample. Approximately 6 seconds

of the delay can be accounted for by the dead space in the sampling

catheter. The latter estimate is based upon the average appearance

time of the dye when sampling from a jugular vein site just downstream

from the tip of the infusion catheter. The remaining 6-10 second

delay is the minimum time taken for labelled blood to traverse the

heart chambers and pulmonary circulation. In five out of six compari-

sons the initial appearance of dye in the arterial blood occurred

earlier while the sheep were eating than while they were resting.

Following the initial delay, the arterial dye-dilution curves

showed a rapid rise in plasma dye concentration which was usually

completed within about 8 seconds. This rapid rise was followed by a

period of slowly increasing dye concentration. A true plateau was not

established on the dye-dilution curves. After reaching a maximum the

dye concentration decreased rapidly over about 8 seconds and then

levelled off for the final 8-12 seconds of the sampling period.

During two trials, blood samples were drawn continuously from the

inferior vena cava to assess the rate of recycling of the dye. Dye-

dilution curves from these trials are also presented in Figure 8 for

comparison with the results of arterial sampling. It is apparent that

recycling of the dye from the systemic circulation was occurring very

rapidly and, thus, preventing the appearance of a true plateau on the

arterial dye-dilution curve. The rate of accumulation of the dye in

the inferior vena cava samples was very similar to the slowly rising

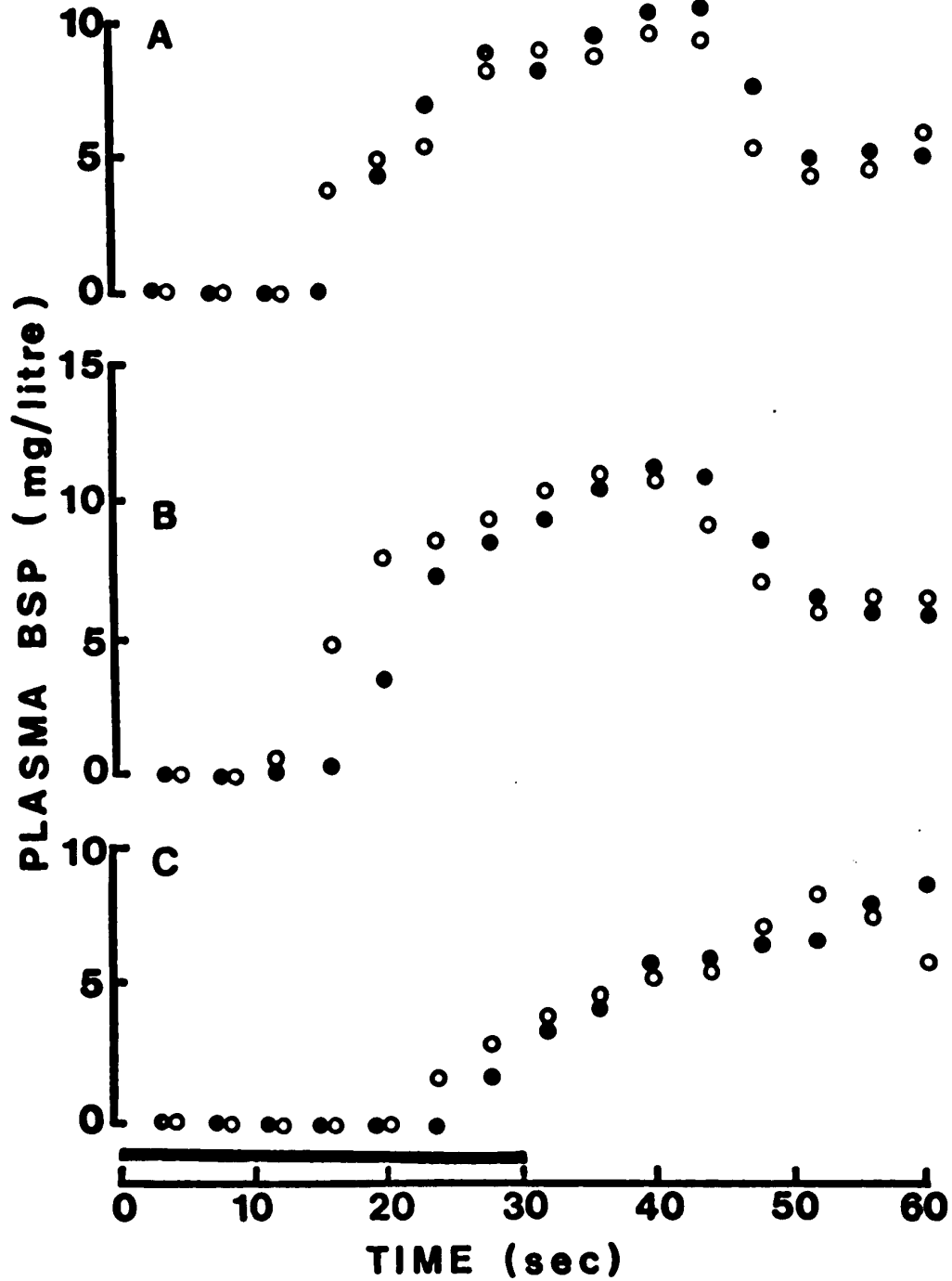phase of the arterial curve, suggesting that the latter could be used

Figure 8. Individual dye dilution curves determined during continuous infusion (solid bar) of bromsulphthelein via the jugular vein in sheep. In A and B, blood samples were drawn from the carotid artery and in C from the inferior vena cava. Measurements were taken while the sheep were resting (solid circles) and while they were eating (open circles).

to estimate the rate of recycling.

The contribution of recycling to the plasma BSP concentration at the 25th second of infusion was 1.9 ± .19 mg/litre for the sheep while resting and 2.5 ± .17 mg/litre while eating. Each of these values represents the mean (±SE) of six experiments. These correction factors were respectively 24% and 30% of the mean total BSP concentration determined simultaneously.

Volume of the Circulation

Decay curves for T-1824 (on a semi-logarithmic graph) during control and eating trials performed on the same sheep are shown in Figure 9. In control trials, when the sheep were not fed, the T-1824 decay curves were linear and repeatable for individual animals. Eating resulted in a departure from the linear decay such that plasma dye concentrations increased during the meal, thus indicating a reduction in plasma volume.

Estimates of the mean plasma volume for individual sheep before and during eating, together with values for hematocrit and total blood volume are presented in Table 7.

There was a significant (p<0.01) decrease in the plasma volume of all individuals during eating. Based on the overall means the decrease in plasma volume during eating was about 250 millilitres.

There was a sufficient increase in hematocrit during eating such that the total blood volume did not decrease to the same extent as the plasma volume. In two individual sheep there was no significant change in total blood volume, whereas, the other two individuals showed a significant decrease (p<0.01) during eating.
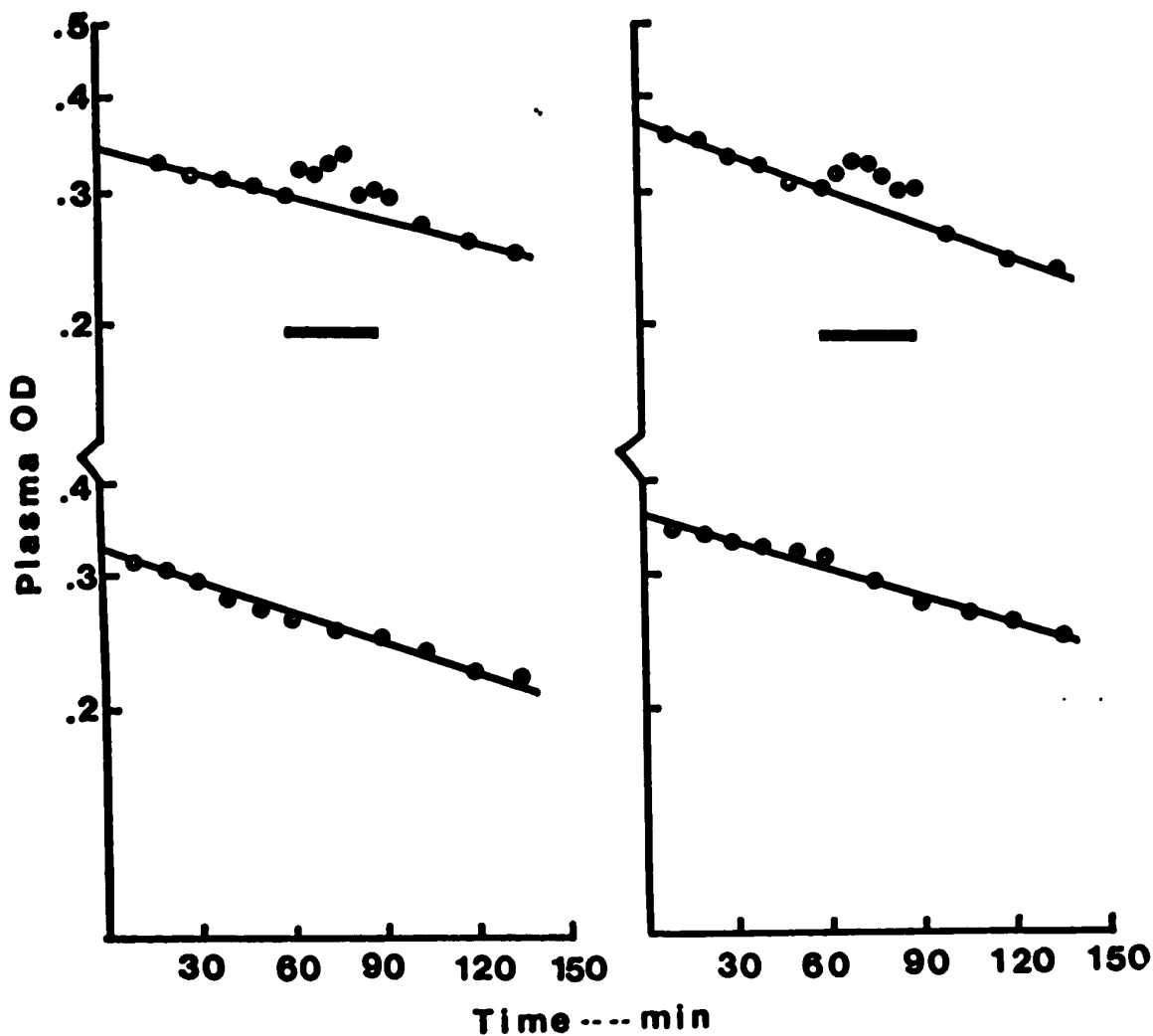
Figure 9. Plasma T-1824 concentration (O.D.) v.s. time after inject-
ion (plotted on semilogarithmic graphs) from four individual
trials with one Lincoln ewe. The upper graphs were obtain-
ed during trials in which the sheep was allowed to eat
(open bars) between 60 and 90 minutes. The lower graphs
were obtained during two control trials in which the sheep
was not fed.

Table 7. Comparison of mean values for plasma volume, hematocrit and whole blood volume determined during eating with values determined before eating

Sheep	Before Eating			During Eating		
	PV (ml)	Hct (%)	BV (ml)	PV (ml)	Hct (%)	BV (ml)
312	3110	27.8	4245	2750**	32.0**	3944**
329	3065	26.1	4087	2869**	29.9**	4028
324	3032	27.7	4141	2916*	31.4**	4177
466	3210	23.8	4155	2819**	27.8**	3847**
All Trials	3086	26.8	4162	2827**	30.8**	4020**

Means with asterisks differ significantly from the pre-eating mean.
* ($p < 0.05$), ** ($p < 0.01$).

The time course of the changes in plasma volume, hematocrit and total blood volume is shown in Figure 13 and will be discussed later.

Extracellular Fluid Volume

Thiocyanate space: Figure 10 shows the decay curves on a semi-logarithmic scale for plasma thiocyanate determined during control trials and during trials in which the animals were fed at 30, 60 or 120 minutes following a single injection of thiocyanate.

The thiocyanate decay curves during control trials exhibited more than one exponential decay phase. Also, there were very irregular changes in the thiocyanate decay curves during eating, which could not be interpreted in terms of changes in fluid volume. Only in the trials during which the sheep were fed at 60 minutes post-injection was there an apparent increase in the plasma thiocyanate concentration.
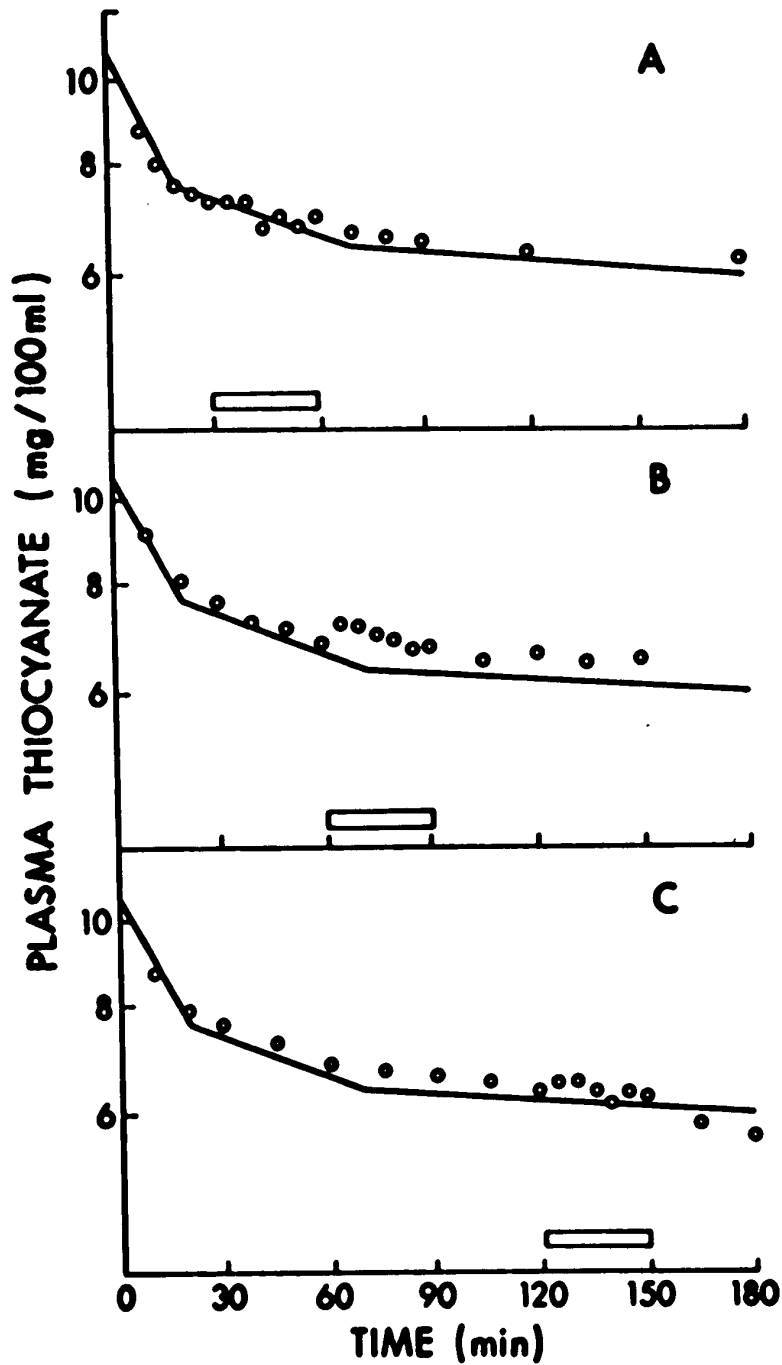
Figure 10. Semilogarithmic graphs showing the plasma thiocyanate decay patterns determined during control trials (solid curve) and during trials in which the animals were allowed to eat (open circles). Feed was offered at 30 minutes after injection in (A), at 60 minutes in (B) and at 120 minutes in (C).

Saliva tests: Thiocyanate concentrations in mixed and parotid saliva together with plasma values determined simultaneously are shown in Table 8. Thiocyanate ion was concentrated over plasma levels about 12-fold and 5-fold in samples of parotid and mixed saliva respectively.

The use of thiocyanate as a means for estimating changes in extracellular fluid volume was subsequently abandoned because of the high concentrations which were secreted in the saliva and because of the lack of a single, exponential decay pattern.

Table 8. Concentration of thiocyanate (mg/100 ml) in plasma and saliva determined at various times following injection of sodium thiocyanate into a conscious sheep and an anaesthetized sheep

Time(min)	Conscious Sheep		Anaesthetized Sheep	
	Plasma	Mixed Saliva	Plasma	Parotid Saliva
10	8.5	25.6	8.7	72.4
20	7.3	20.8	7.6	85.0
30	6.9	14.3	7.6	86.6
40	---	----	7.2	85.0
50	---	----	7.2	85.5
60	6.6	22.8	7.2	88.2
75	---	----	7.0	86.6
90	6.4	18.9	6.8	87.0

Thiosulphate Space

Saliva test: Table 9 shows the values for thiosulphate concentration in parotid saliva and plasma during 60 minutes following injection of 6.37 gm of sodium thiosulphate into an anaesthetized sheep. Only trace

amounts of thiosulphate appeared in the saliva during the first 40 minutes following injection, and none thereafter. It was concluded that thiosulphate would probably not be selectively removed from the extracellular fluid by saliva secretion during eating.

Table 9. Concentration of thiosulphate (mg/100 ml) in plasma and parotid saliva of an anaesthetized sheep at various times following intravenous injection of sodium thiosulphate

Time(min)	Plasma	Parotid Saliva
5	99.4	0
10	60.8	2.24
15	52.3	1.02
30	40.7	1.02
40	33.9	1.02
50	26.9	0
60	22.0	0

Thiosulphate decay: In the control trials there was considerable variation in the rate of disappearance of thiosulphate from the plasma. This variation existed both between different sheep and between successive trials on the same sheep. Figure 11 shows thiosulphate decay curves on a semi-logarithmic graph for an individual sheep during two control and two trials in which the animal was fed at 30 minutes post-injection. The control decay curves were not always linear on a semi-logarithmic plot (Figure 11-c). There was some indication that irregularities of the decay curves in control trials might have been associated with the sheep becoming restless (as indicated by an
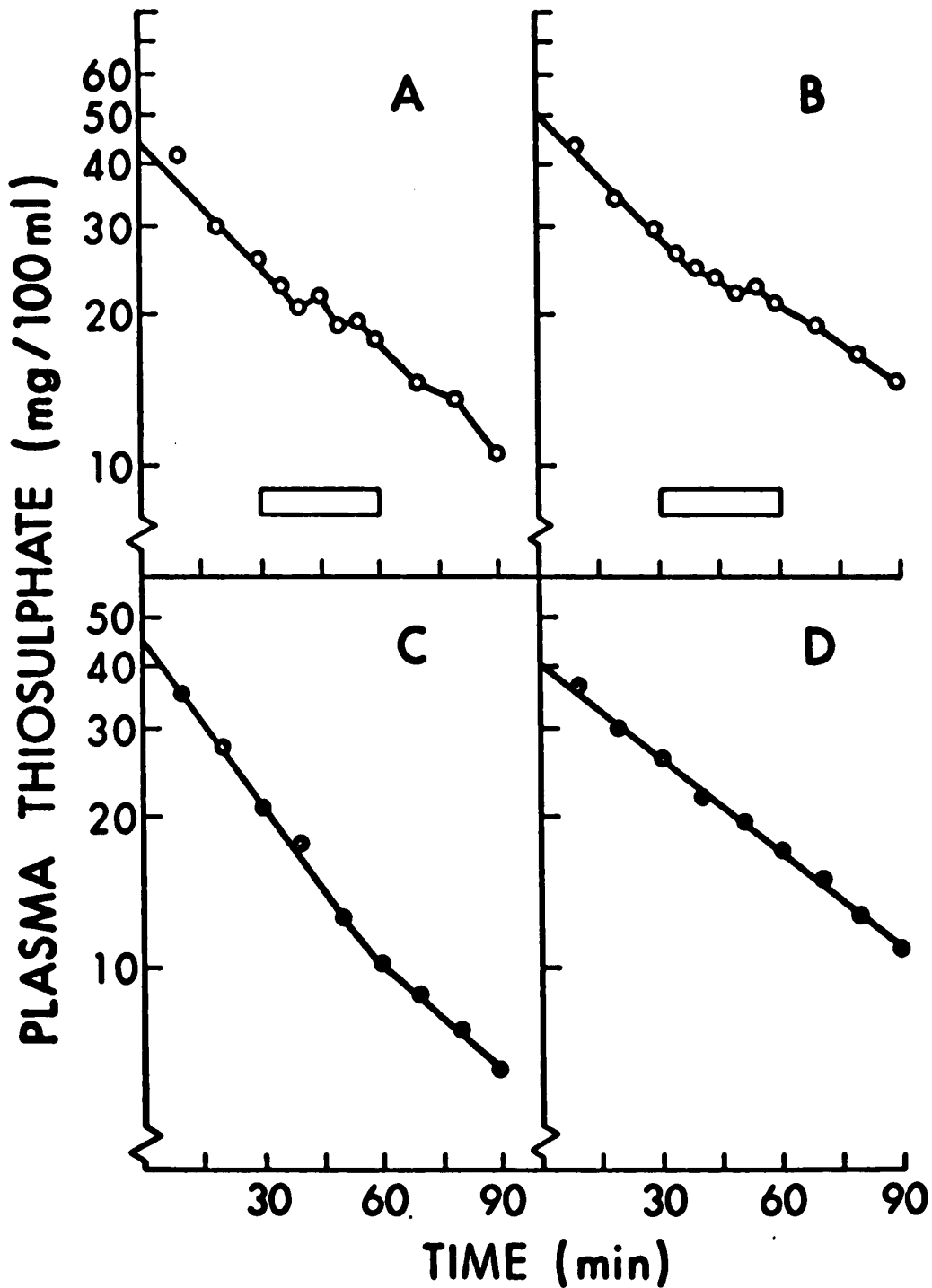
Figure 11. Semilogarithmic graphs of the plasma thiosulphate concentration v.s. time after injection during four trials with one Lincoln ewe. In A and B the sheep was allowed to eat between 30 and 60 minutes (open bar). In C and D the sheep was not fed during the trial.

increased heart rate) at a time when they were accustomed to being fed.

The decay curves obtained from individual sheep during eating trials were compared to the mean of the decay curves obtained in control trials. This undoubtedly gave rise to considerable errors in estimating thiosulphate space changes during eating because of the between-day variations in slope of the curves for individual sheep. The alternative approach was to extrapolate points on the individual decay curves during eating back to the intercept and thus estimate the change in extracellular fluid volume. The percent changes in the thiosulphate space at the end of the meal, estimated by extrapolation within individual decay curves are shown in Table 10. The latter method probably has less justification, particularily for estimating changes more than 60 minutes following injection, since in those trials in which the disappearance of thiosulphate did not follow a single exponential pattern, the rate of disappearance usually declined. If this happened during eating, it would inevitably and unjustifiably overestimate the fall in extracellular fluid volume. In Figure 11-B the rate of clearance following eating is obviously slower during the post-eating period than prior to eating. On the other hand, during the trial shown in Figure 11-A there was very little difference between the pre-eating and post-eating clearance rates. In some other individual eating trails the clearance rate was slightly greater after than before eating. Whatever the cause of the variation in the latter stages of the thiosulphate disappearance curve, it is clear that it is an unreliable predictor of the ECF volume more than 60 minutes after injection.

Mean values for the cumulative urinary excretion of thiosulphate

Table 10. Percent decrease in thiosulphate space at the end of the meal, estimated by extrapolation within individual decay curves and feed consumed per kg body weight per minute spent eating

Sheep	Trial	thiosulphate space (%)	Rate of eating (gm/kg/min)
312	1	21	0.108
	2	20	0.082
329	1	33	0.200
	2	27	0.124
	3	22	0.116
487	1	23	0.169
	2	27	0.171
	3	26	0.193
	4	22	0.179
466	1	22	0.113
	2	23	0.140
	3	29	0.162
	4	24	0.168

recorded during three control and three eating trials are presented in Figure 12. Although the absolute values in control experiments differ from those measured during the eating trials, there did not appear to be any marked difference in the pattern of excretion. Thiosulphate excretion rate, certainly did not appear to be reduced during and following eating as compared with controls. It was apparent, however,
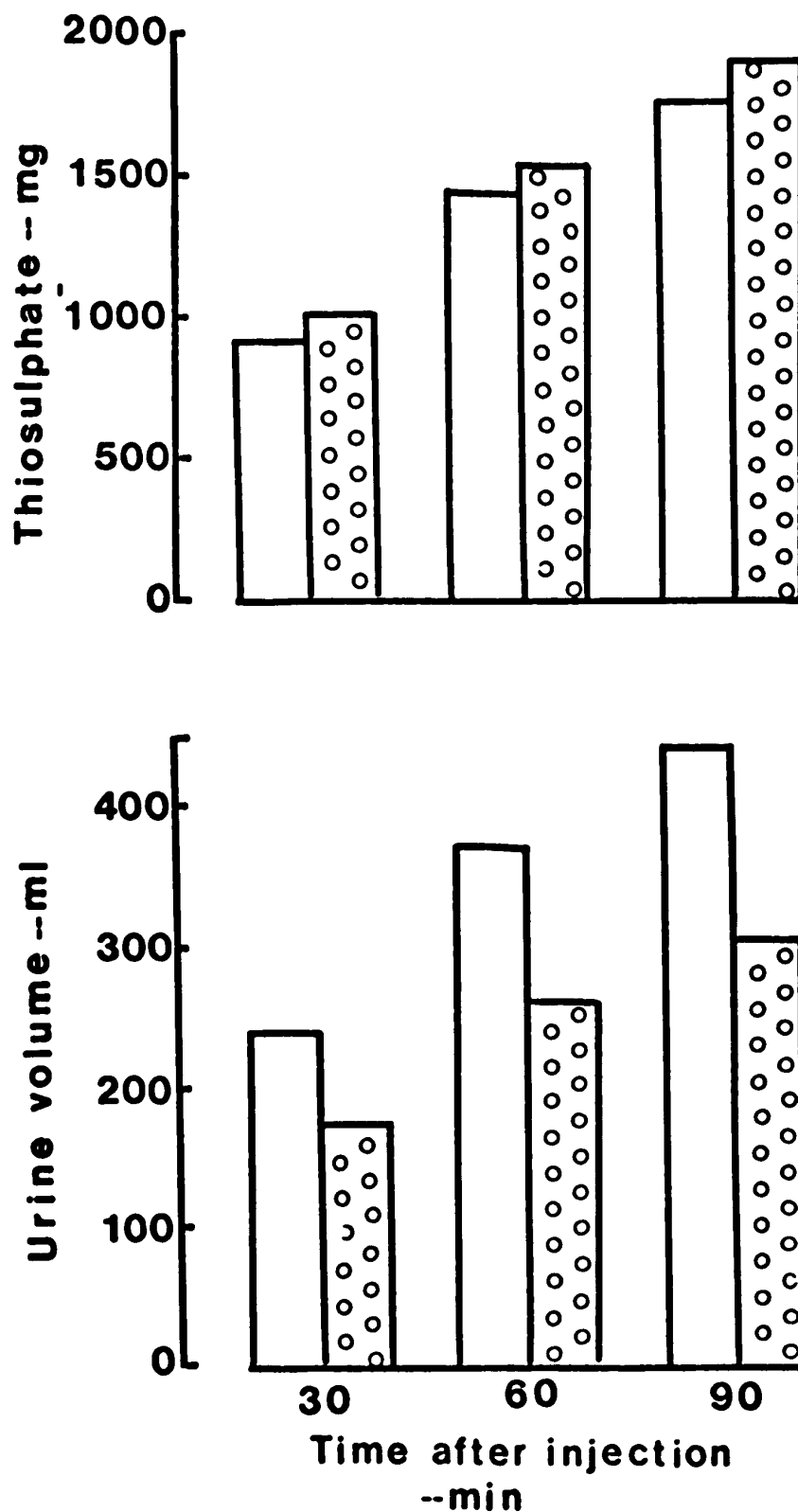
Figure 12. Cumulative urine volume and thiosulphate excretion during control experiments (open bars) and during experiments in which the sheep were fed between 30 and 60 minutes (stippled bars).

that urinary excretion rates of thiosulphate cannot account for the total clearance of thiosulphate based upon plasma clearance rates.

Although, it is not easy to separate the sources of error involved in estimating changes in extracellular fluid volume by the use of thiosulphate decay rates, values for thiosulphate space before, during and after eating were estimated by comparing the decay curves in eating trials with the control decay curves for each individual sheep. Mean values obtained by this method are presented in Table 11. In three sheep there was a significant decrease in thiosulphate space during eating.

Overall mean values for thiosulphate space are shown in Figure 13, together with values for plasma volume, hematocrit and total blood volume, to indicate the time course of the volume changes associated with eating.

Table 11. Comparison of mean thiosulphate space (litres) determined during and after eating with mean values determined before eating

Sheep	Before eating	During eating	After eating
312	13.28	10.04**	8.32**
329	12.82	13.10	14.97
466	14.01	12.50**	12.61*
487	12.40	11.39*	10.81
All Trials	13.05	11.96**	12.12

Means with asterisks differ significantly from the pre-eating mean.
* $p < 0.05$), ** ($p < 0.01$).

Changes in plasma volume and hematocrit occurred rapidly at the beginning of the meal. Although both plasma volume and total blood
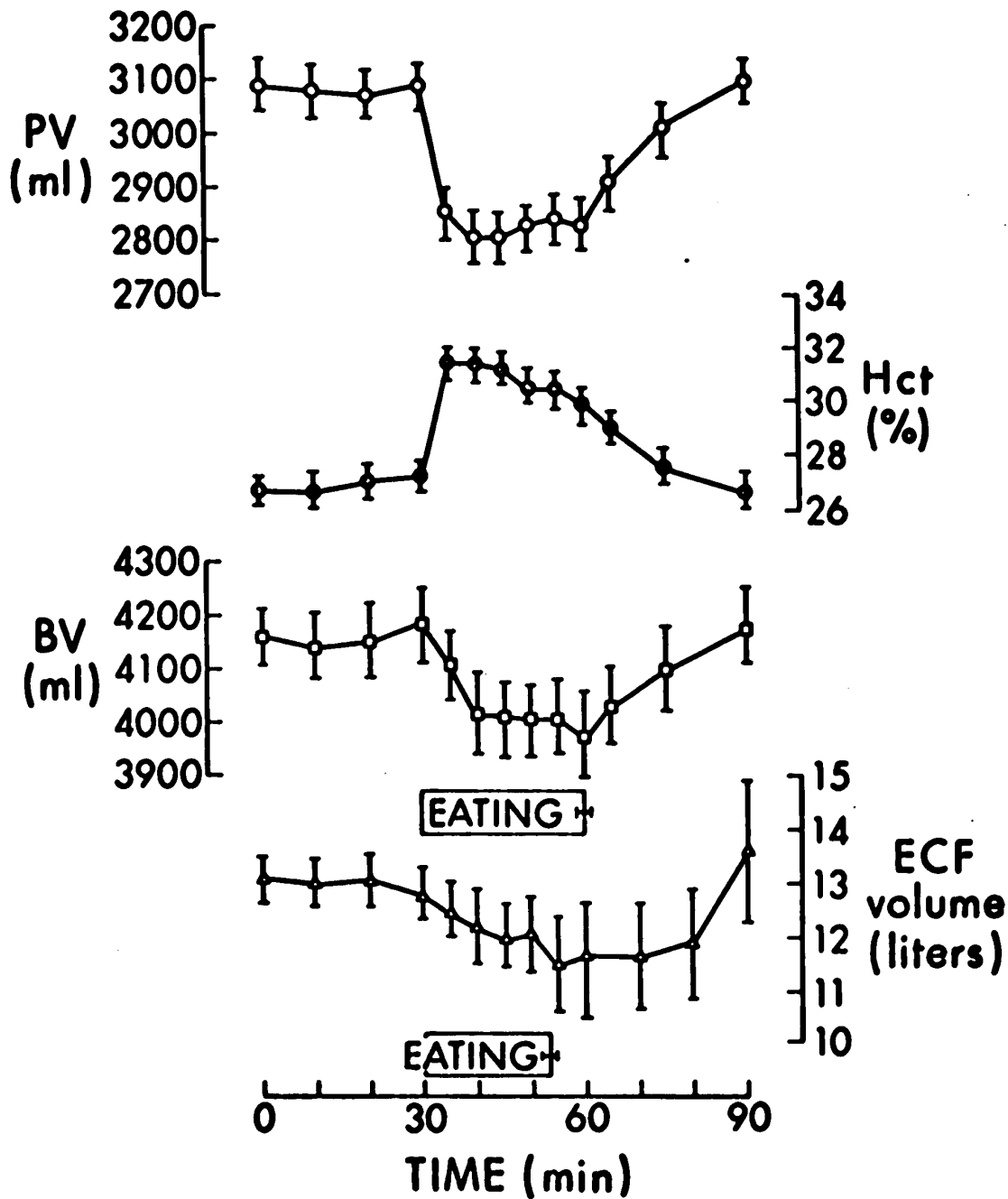
Figure 13. Time course of changes in plasma volume (PV), hematocrit (Hct), whole blood volume (BV) and extracellular fluid (ECF) volume during eating. Values for PV, Hct and BV are means (+SEs) of 25 trials. Values for ECF volume are means (+SEs) of 13 trials.

volume appeared to become stabilized rather quickly during the meal they did not begin to return toward pre-feeding levels until the sheep stopped eating.

Thiosulphate space decreased more gradually from about 13.0 litres to about 11.6 litres by the end of the meal. Recovery following the meal, likewise appeared to be rather slow. However, the standard errors attached to the post-feeding values are too large to permit a convincing interpretation.

Plasma Sodium and Potassium Concentrations

Mean values for plasma Na^+ and K^+ concentration determined in three Lincoln ewes and one wether before, during and after eating are presented in Table 12. There was an increase of approximately 10% in plasma Na^+ concentration during the meal but only a slight change in plasma K^+ concentration. Thus the plasma Na^+/K^+ ratio mainly reflected the increase in Na^+ concentration.

Table 12. Mean values ($\pm$SE) for plasma sodium, plasma potassium and plasma Na^+/K^+ ratio determined before, during and after eating

	Before	During	After
plasma Na^+ (mEq/l)	145.7 $\pm$ 1.4	160.2 $\pm$ 2.19	155.9 $\pm$ 1.9
plasma K^+ (mEq/l)	4.59 $\pm$ 0.13	4.62 $\pm$ 0.10	4.74 $\pm$ 0.14
plasma Na^+/K^+ ratio	32.0 $\pm$ 0.9	34.9 $\pm$ 0.7	33.2 $\pm$ 1.1
n	12	20	12

In the three ewes the Na^+ concentration increased abruptly during the meal while in the wether the Na^+ concentration increased more gradually throughout the meal. Two of the ewes plus the wether were

found to have low potassium content in the red blood cells while the
remaining ewe had high potassium red blood cells (Evans, 1954). The
high-K^+ ewe responded in a fashion similar to the other two with
respect to plasma electrolyte changes during eating, however, the
number of observations were too few to permit a meaningful comparison
among individuals and the data from all four sheep were included in
the mean values given in Table 12.

DISCUSSION

The objective of this research project was to obtain a comprehensive picture of changes that occur in the interrelated parameters of oxygen consumption, respiratory gas transport in the blood, volumes of circulating and extracellular fluids and cardiac performance of sheep during the activity of eating a single meal.

The increases of 60-70% in $\dot{V}_{O_2}$ and heart rate during eating in a thermoneutral environment in the present study were similar to the responses reported previously (Blaxter and Joyce, 1963; Young, 1966; Webster, 1967; Webster and Hays, 1968). The increased $\dot{V}_{O_2}$ during eating recorded in the present study was slightly smaller than the changes recorded by previous authors, however, this difference in response would be expected since the sheep in these experiments consumed less feed and usually at a slower rate. This is consistent with the results of Young (1966) who showed that a positive relationship exists between the rate of eating and the rate energy expenditure of sheep during the meal.

When the Suffolk wethers were fed during exposure to a mild cold stress of short duration, the increment $\dot{V}_{O_2}$ during eating was similar to that recorded during eating in a thermoneutral environment. The metabolic response to eating in this case was superimposed on the small metabolic increment induced by the mild cold exposure. During the trials when Suffolk wethers were shorn and exposed to -15°C (i.e. a more severe cold stimulus), $\dot{V}_{O_2}$, unfortunately, was not recorded. Thus it is not known whether the increased metabolic rate during eating can substitute for or is additive to the marked elevation in the heat production of sheep when they are exposed to a cold stress of

appreciable intensity.

It is generally assumed that the heat increment of feeding can be used to help maintain homeothermy in a cold environment (Kleiber, 1961) but detailed studies have not been made to see whether this also applies to the increased heat production during the activity of eating. Presumably this component could also contribute to the maintenance of body temperature in a cold environment.

The marked increase in blood P_{CO_2} during and following eating was undoubtedly due partly to an increase in tissue metabolism since $\dot{V}_{O_2}$ was also increased by about 60%. However, since both arterial and venous P_{CO_2} were increased it is apparent that pulmonary clearance of CO_2 was not increased in proportion to the CO_2 production. Blaxter and Joyce (1963) and Young (1966) have shown that pulmonary ventilation in sheep was often reduced during eating. This might be expected since the activities of swallowing and breathing cannot occur simultaneously. Eructation of rumen gas has been shown to increase during eating and this activity briefly interrupts pulmonary ventilation resulting in sudden decreases in blood P_{O_2} (Dougherty, 1965). Inhalation of CO_2 of rumen origin might also be expected to occur under these circumstances. In some trials a reduced arterial P_{O_2} was occasionally recorded during eating for an individual blood sample while the P_{O_2} of preceding and subsequent samples were near to the pre-feeding level. These isolated samples with a lowered P_{O_2} may have been due to a brief interruption in pulmonary ventilation due either to swallowing or eructation.

The decrease in blood pH during eating was obviously related to the increased blood P_{CO_2} in the present experiments. Stacy (1969) reported a significant decrease in blood pH of sheep 30 minutes after

feeding which he attributed to a loss of plasma bicarbonate into the saliva during eating. Although there must have been an increased transfer of bicarbonate from the plasma into the saliva during eating in the present experiments, the calculated plasma bicarbonate was shown to increase during and after eating inside the hood. When the sheep were fed outside of the hood there was a smaller increase in blood P_{CO_2} and the plasma bicarbonate either did not change or decreased slightly during eating. Since plasma bicarbonate increased during eating in the hood there was a greater tendency towards respiratory acidosis in the latter circumstance. But, since the pH of the blood is directly related to the level of bicarbonate at a given P_{CO_2} the results of these experiments do not necessarily disagree with the results of Stacy (1969). Instead, in both studies it is apparent that the levels of bicarbonate relative to the P_{CO_2} were decreased during eating, as indicated by the drop in blood pH. This altered relationship between arterial P_{CO_2} and plasma bicarbonate during eating is illustrated in Fig. 14. The venous-arterial difference in P_{CO_2} and in plasma bicarbonate were very closely related throughout the experiment, which reflects the role of bicarbonate as an intermediate in the uptake and release of CO_2 by the blood.

There was a significant decrease in MCHC during eating. The method used for determining hemoglobin is subject to a random error of plus or minus 5% but there is no reason to suggest that hemoglobin concentration would be underestimated during eating. Thus the changes observed in MCHC may reflect an increase in red blood cell size. Siggaard-Anderson (1961) reported a decrease in MCHC during acidosis in dogs, but the changes which he induced in blood pH were greater than the decrease recorded during eating in the present experiments.
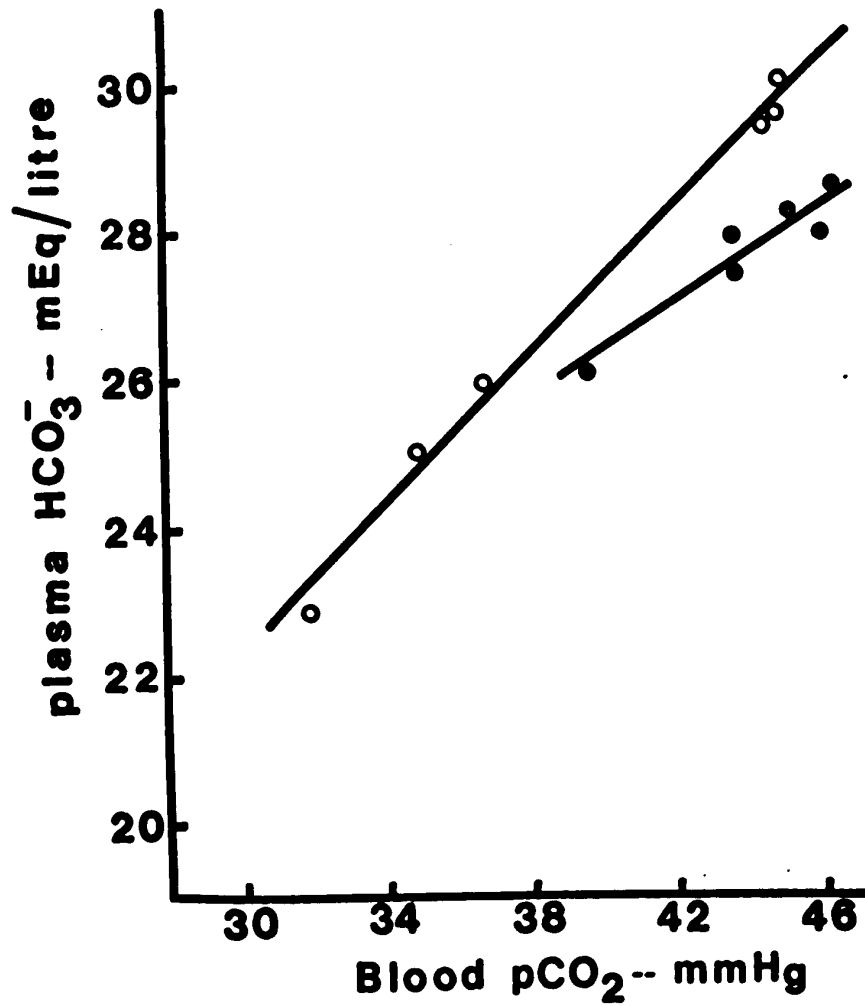
Figure 14. Relationship between mean plasma bicarbonate and P_{CO_2} in arterial blood when the sheep were eating (solid circles) and when they were not eating (open circles).

The method used in these studies for determining blood oxygen content indirectly from P_{O_2}, oxygen saturation and hemoglobin is undoubtedly less accurate in absolute terms than the direct volumetric determination using, for example, the "Van Slyke Technique". The oxygen dissociation curves assumed in this study may not give an accurate estimate of the oxygen saturation of sheep hemoglobin. However, the expected errors would be small relative to the changes observed in the present experiments. In spite of the errors involved this method does provide a reliable indication of the direction and relative magnitude of changes in the arterio-venous oxygen content during eating.

The cardiac output showed a remarkably small increase during eating, in comparison to the changes in $\dot{V}_{O_2}$ and heart rate. Because of the marked increase in hematocrit during eating the change in rate of plasma flow during the meal would be even less than the change in flow of the whole blood.

Although the absolute accuracy of measurement of cardiac output is uncertain, it is clear that cardiac output was not increased in direct proportion to the increased rate of tissue metabolism during eating. Thus, the increased oxygen supply to the tissues during eating was achieved, in part, by extraction of a larger proportion of the oxygen content of the blood.

Estimation of cardiac output by the continuous dye-dilution technique is subject to errors arising from recycling of the dye which prevents the establishment of a stable plateau concentration during the period of infusion. This factor alone makes it impossible to estimate Q from the dye concentration of a single sample of blood

drawn during the period of infusion. Moreover, since recycling occurred more quickly during eating, the total recycling component was larger when measurements were made during than before eating. The time to onset of recycling is, of course, a direct function of Q. Thus it is impossible to establish a uniform correction factor to account for recycled dye at a fixed time after the onset of infusion. This means that accuracy of the dye-dilution technique depends absolutely on the accuracy with which the initial rate of appearance of the dye in the circulation can be analysed and integrated. This, in conscious animals and with long sampling catheters presents serious problems. The present results, in which the recycling component was 24-30% of the total BSP level, clearly deny the original hypothesis that a stable plateau concentration of dye is established in the arterial outflow during the period of infusion. Although the sudden injection dye-dilution method (Zierler, 1962) partially avoids the recycling problem there is still the need for multiple, rapid sampling to define the dilution curve. For this reason the latter technique also is unattractive. The "direct Fick" method which predicts an average value for cardiac output would appear to be a more reliable and more practical choice for use with conscious animals.

Since cardiac output did not increase in proportion to heart rate during eating, the calculated stroke volume decreased. The modest increase in cardiac output and progressive decline in stroke volume during eating are undoubtedly related to the fact that plasma volume, and often, the whole blood volume are reduced during the meal.

The drop in plasma volume observed during eating confirms the results of Blair-West and Brook (1969). The present study indicates, however, that the circulating volume of red cells increases during

eating and partially compensates for the plasma deficit. In several trials there was complete compensation so that total circulating blood volume remained approximately constant. The increased red cell volume is probably due to mobilization of stored red blood cells from the spleen, which in sheep has been shown to be an important storage site for red blood cells (Turner and Hodgetts, 1959). This is also consistent with the suggestion of Berzins (1969) that the rapid hematocrit change at the onset of eating in sheep is due partly to release of red cells from the spleen. The possibility that increased circulating red cell volume could be due in part to a net increase in size of the red cells cannot be ruled out. Although this is supported by the decrease in MCHC there is not other direct evidence to suggest that this might occur as a consequence of eating. An increase in the number of circulating erythrocytes would achieve a greater oxygen carrying capacity of the blood. This would inevitably permit oxygen consumption to increase to a proportionally greater extent than cardiac output.

It has been suggested that the relationship between heart rate and metabolic rate could be used as a basis for predicting energy expenditure from measurements of heart rate even during eating, provided that the oxygen pulse either remained constant during the measurement period or varied in a predictable fashion with heart rate (Webster, 1967). The present results suggest that, although a direct relationship between $\dot{V}_{O_2}$ and heart rate exists (Figure 15), the variability is too great in some individuals to give an accurate prediction of metabolic rate. In experiments with Lincoln ewes the oxygen pulse increased at the onset of eating and then declined progressively throughout the meal. The relationship between metabolic rate and
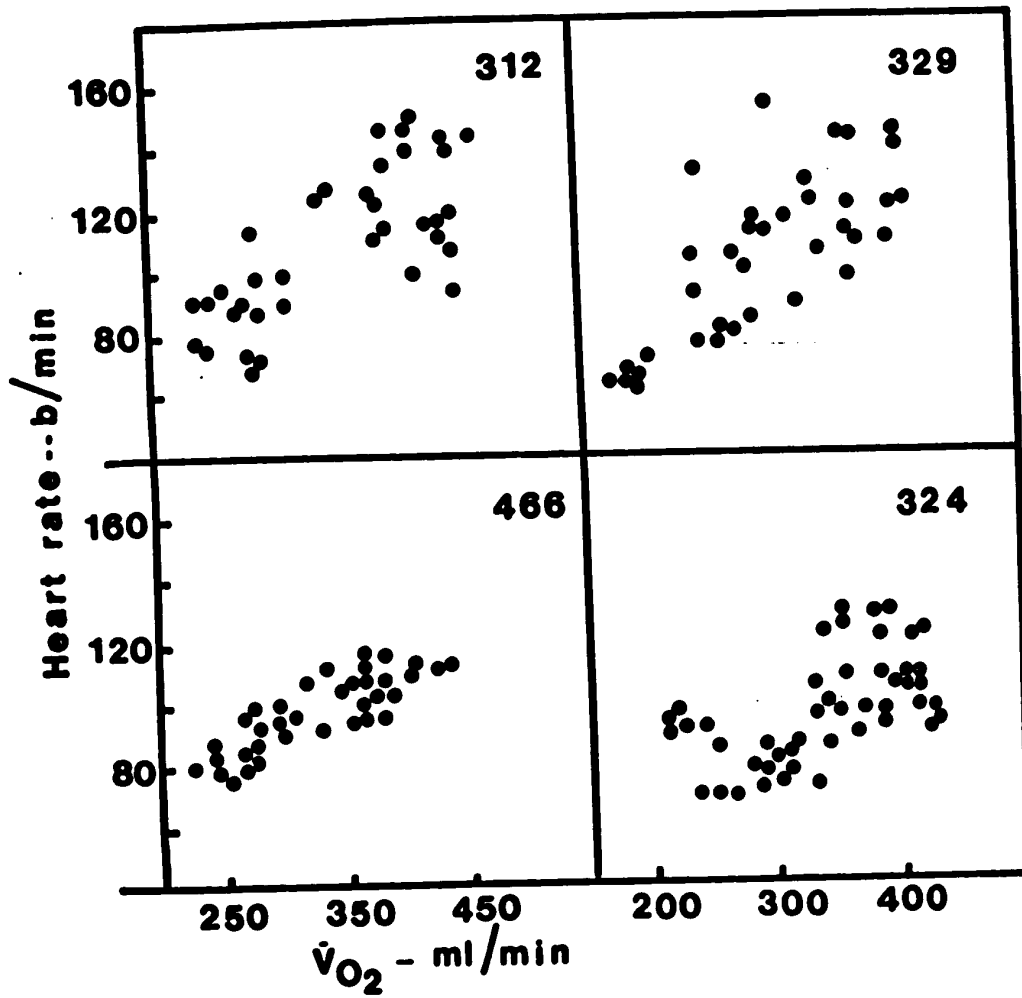
Figure 15. Relationships between heart rate and oxygen consumption in four Lincoln ewes.

heart rate during eating in the cold has not been reported previously.
The present results with Suffolk wethers indicate that the relation-
ship between $\dot{V}_{O_2}$ and heart rate is markedly altered when the sheep eat
in a cold environment and values for oxygen pulse showed marked
departures from the values determined at thermoneutral temperatures.
In general, these results suggest that oxygen pulse does not vary in a
predictable fashion with heart rate in all circumstances. This factor
would undoubtedly contribute to the large standard error associated
with individual regressions of heart rate on energy expenditure during
eating described by Young (1964).

The extreme tachycardia during eating in the cold also suggests
that the mechanisms which regulate metabolic rate may differ from those
which regulate heart rate during eating. In propranolol-treated
animals eating in the cold, heart rate increased to an average of 150
beats/minute. This increase was too large to be accounted for by
relaxation of parasympathetic tone since Hays (1968) showed that the
intrinsic rate of the pharmacologically isolated heart in sheep was
about 120 beats/minute. This suggests that the tachycardia during
eating in sheep is partly independent of the autonomic nervous system.

Blair-West and Brook (1969) showed that the renin-angiotensin
mechanism in sheep was activated at the onset of eating and they
attributed increases in blood pressure during eating to this homeo-
static mechanism. Krasney (1968) demonstrated that angiotensin in-
creased heart rate but decreased stroke volume of dogs in circumstances
where the blood pressure was mechanically prevented from rising
following administration of the drug. Hays and Webster (1971) showed
that angiotensin infusion could elevate heart rate in sheep after total

surgical and pharmacological autonomic blockage, and concluded that angiotensin may be partly responsible for the tachycardia of eating in sheep. Berzins (1969), in contrast to Blair-West and Brook (1969) showed that blood pressure in sheep did not change appreciably during eating except at the beginning of the meal when a transient increase occurred in association with excitement of the animal. Following the excitement of feed presentation, the tachycardia of eating "in comfort" presumably does not involve the sympathetic nervous system. In cold environments, on the other hand, there is evidence for increased activity of the sympathetic nervous system and increased catecholamine excretion in sheep (Webster, Hays, Heitman and Olynyk, 1969). Thus, during eating in the cold there may be an opportunity for expression of synergism between angiotensin and catecholamines in the regulation of heart rate. Such an interaction could account for the extreme tachycardia observed during eating in the cold in the present study.

Although there was considerable scatter, the change in heart rate appeared to be inversely related to plasma volume during eating (Figure 16). The marked increase in heart rate and decrease in stroke volume of the Lincoln ewes during eating tended to follow the drop in plasma volume and presented a picture very similar to that produced by Krasney (1968) following injection of angiotensin during blood pressure stabilization in dogs namely a positively chronotropic and negatively inotropic effect on the heart. Angiotensin formed as a consequence of the loss of plasma into the gut during eating might be expected to have a similar effect; i.e. exactly the type of changes in cardiac performance observed in these experiments.

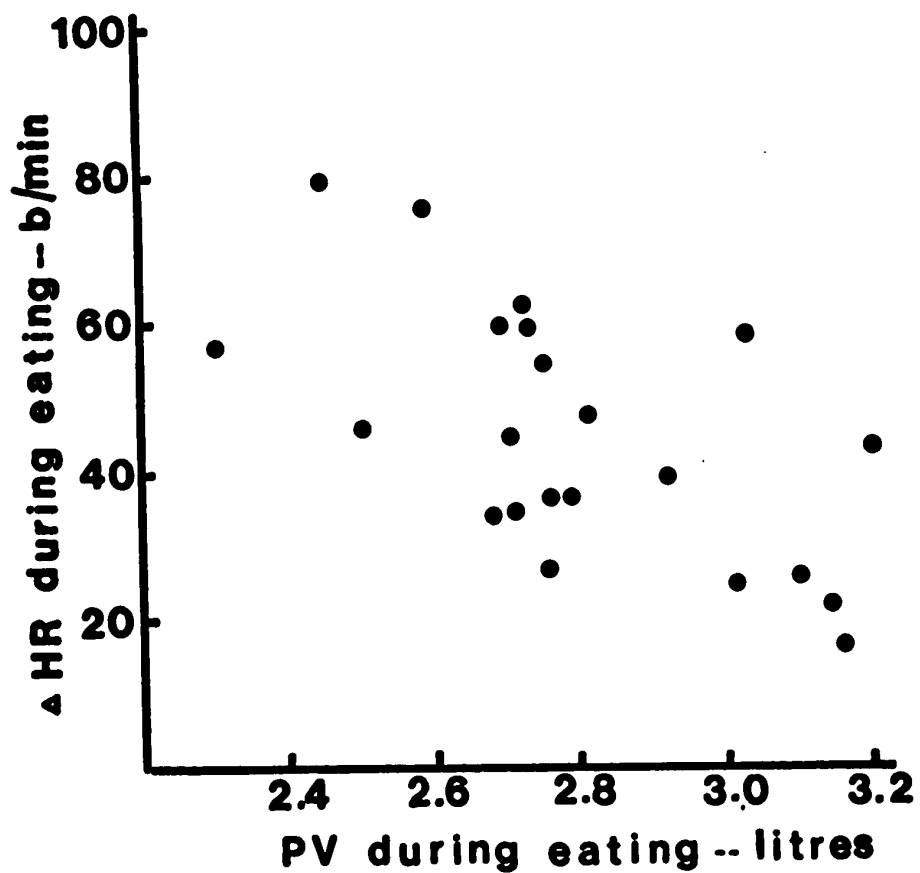The present study indicates therefore that the relationship

Figure 16. The relationship between the change in heart rate and the mean plasma volume in Lincoln ewes during eating.

between heart rate and metabolic rate during eating is somewhat fortuitous, the tachycardia resulting, in part, from the consequence of endocrine changes designed to maintain blood pressure during a period of reduced plasma volume.

Brockway and McEwan (1969) have made the alternative suggestion that continuous measurement of cardiac output would be more likely than heart rate to provide a precise estimate of metabolic rate. However, the present study also throws doubt on this assumption, since the cardiac output during eating had a poorer relationship to metabolic rate than did the heart rate in these circumstances. The usefulness of cardiac output to estimate metabolic rate would depend upon constancy of the arterio-venous oxygen difference throughout the experiment. It is doubtful that this would be a valid assumption in all circumstances although over a period of 24 hrs, transient fluctuations in Ca_{O_2} and Cv_{O_2} would probably have a small effect on the overall relationship between $\dot{V}_{O_2}$ and Q.

With the exception of estimates of right ruminal and omasal artery flow (Sellers et al., 1964) and jugular vein blood flow (Berzins, 1969) there have been few reports describing changes in blood flow during eating in ruminants and there would appear to be no previous estimate of cardiac output during this activity. Increases in blood flow in the above mentioned vessels during eating would be expected since the activity of salivary glands, jaw muscles and the reticulo-rumen is increased during eating. It could be inferred that a redistribution of blood flow is an important means of supplying those tissues which become more active during eating since the changes in cardiac output recorded in the present study were rather modest. This

is supported by the observation of Blaxter and Joyce (1963) that
vasoconstriction of the extremities of sheep often occurred in
association with feeding. Measurements of portal blood flow in sheep
(Bensadoun and Reid, 1962) and cardiac output in cattle (Waldern et al.,
1963) have been made at intervals during the post-feeding period. In
both of these studies the flow rates tended to increase to maximum
levels between 3 and 7 hours following feeding and the maximum flow
rates were associated with peak levels of VFA's in the rumen and port-
al blood. Thus, the events associated with digestion and absorption
of a meal may enhance blood flow to an extent as large or even larger
than that induced by the activity of eating. During the post-feeding
period, recovery of the plasma volume would help to provide for an
increased cardiac output at that time.

The decrease in plasma volume during eating as measured in these
experiments and by Blair-West and Brook (1969) does not provide a
true indication of the amount of fluid transferred from the body into
the gut, since plasma represents an interface between the total ECF and
the gut. This suggestion is supported by the rapid stabilization of
the plasma volume during eating. The ECF tends to change more gradu-
ally and declines progressively during the meal. Changes in the ECF
might also tend to underestimate the gross flow of fluid into the gut
since the ECF may be partly restored from intracellular water. The
intracellular water space was not estimated since techniques for
estimating total body water (Hix, 1959; Black et al., 1964) all would
appear to include water within the digestive tract and thus be mean-
ingless for purposes of this experiment. The sodium concentration and
osmotic pressure of saliva are similar to that of plasma (Kay, 1960).

This suggests that fluid loss via saliva into the gut would not cause marked increases in sodium concentration and osmotic pressure of the ECF and thus would not tend to draw much intracellular water into the ECF. The results of Stacy and Warner (1965) and Ternouth (1968) suggest that the Na^+ concentration and osmotic pressure of the plasma do not increase much during the initial 30 minutes of eating. This implies that eating may induce a state of temporary, reversible, Na^+ depletion rather than water depletion. In the present study, however, plasma Na^+ concentration increased, on average, about 10% during a thirty minute meal and in three out of the four animals the increase occurred abruptly at the onset of eating. The increased plasma sodium concentration might be due to an increased plasma protein concentration and its influence on the distribution of ions by virtue of the Gibbs-Donnon equilibrium (Bland, 1963). However, if this were the major factor influencing plasma cation levels during eating, then the plasma potassium concentration should have increased in a manner similar to that of sodium. This was clearly not the case, since potassium concentration hardly changed. This suggests that water was lost from the ECF at a faster rate than Na^+. It also seems probable that the osmotic pressure of the ECF was increased during eating since this is mainly determined by the concentration of sodium (Bland, 1963). Thus, the abrupt increase in plasma sodium concentration during eating would be an adequate stimulus for the release of ADH (reported by Stacy and Brook, 1965) even during the early stages of the meal.

The problems associated with estimation of the changes in ECF volume have already been mentioned in the Results section. Since

thiocyanate is concentrated several-fold in the saliva it could not be expected to give a reliable measure of changes in volume of the ECF during eating. If saliva were the only route by which fluid is lost from the ECF during eating then the plasma thiocyanate concentration would be expected to decrease during the meal. This was not observed in the present experiments and in some trials there was evidence of a slight increase in plasma thiocyanate concentration. These results suggest that some water may have been lost from the ECF by an extra-salivary route.

It was shown by Termouth (1968) and again in the present study that thiosulphate does not become concentrated in the saliva. This would make it a more suitable choice for estimating ECF changes during eating. During the eating trials there was a change in the decay curve which suggested a decrease in the thiosulphate space associated with eating. However, problems were encountered in attempting to place quantitative values on the changes. The main problem was a lack of consistency among trials in the slope of the decay curve. In some individuals the slope of the decay curve during the eating trials was slightly steeper than the slope of the controls. In this case the predicted change in thiosulphate space was greatly underestimated. In other cases the opposite situation prevailed and the predicted change in thiosulphate space during eating was greatly overestimated. These differences in slope of the decay curves, therefore, were not in any way related to the treatments and the reason for the differences is not clear. Although the errors caused by these differences in slope were large, they did appear to be random errors. Thus, comparison of the control and eating curves were used to describe the time course of

changes in ECF volume during eating.

Based upon a comparison of decay curves from control and eating trials, the decline in thiosulphate space of 1000-1500 ml during eating in the present experiments represents roughly 10% of the thiosulphate space, and is, therefore, similar to the change shown by Ternouth (1968). This volume of fluid is about twice as large as the expected increase in saliva secretion during eating. Stacy and Warner (1965) estimated saliva secretion rates of about 3 ml/gm of feed consumed during the meal and resting secretion rates of about 5 ml/minute. For a sheep that consumes 300 gm of hay during thirty minutes the extra saliva secreted as a consequence of eating would be roughly 700-800 millilitres. This suggests that saliva secretion alone could not account for the change in ECF volume during eating in the present study.

By extrapolation within the decay curve of individual eating trials it was shown that the ECF volume at the end of the meal was decreased by 20-33%. If the clearance rate of thiosulphate remained constant throughout the trial, this extrapolated value would give a fairly accurate estimate of the absolute change in the ECF volume. Although there was no apparent change in urinary excretion of thiosulphate that could be attributed to eating, there was evidence that the rate of clearance of thiosulphate was altered following eating. This was suggested by the observation of changes in the slope of the decay curve following eating. These changes in slope after the end of the meal did not occur consistently in one direction or another but appeared to be random. In those control trials in which departures from linearity occurred, the rate of clearance was usually decreased.

It is very unlikely that any consistent interpretation can be placed
on these changes in the rate of clearance of thiosulphate more than
60 minutes after injection whatever the subsequent actions of the
sheep. The changes in extracellular fluid volume at the end of the
meal estimated by extrapolation within the decay curve would likely
overestimate the absolute amount of water which is lost from the
extracellular space.

Most theories of appetite regulation attribute satiety in rumin-
ants to the quantity and physical or chemical composition of food in
the gut (Baile, 1968). Ternouth and Beattie (1971), on the other hand,
have suggested that satiety in sheep may be partly related to changes
in ECF volume and plasma osmolarity consequent upon fluid shifts into
the gut at feeding time. Although in the present studies there was
a tendency for plasma volume changes to be correlated with the amount
of feed consumed (Figure 17), it cannot be said that a fall in plasma
volume of more than a certain degree induced satiety and the end of
eating. This is particularly true since plasma represents only a
portion of the total ECF.

The percent change in thiosulphate space by the end of the meal
appeared to be directly related to the rate of eating in the present
experiments. While there was very little relationship between the
pre-feeding thiosulphate space and feed consumed at the meal, the
thiosulphate space at the end of the meal was distinctly lower at
higher rates of intake. The relationship between the change in ECF
volume and rate of eating is presented in Figure 18. From these
experiments it cannot be said whether satiety is induced by changes in
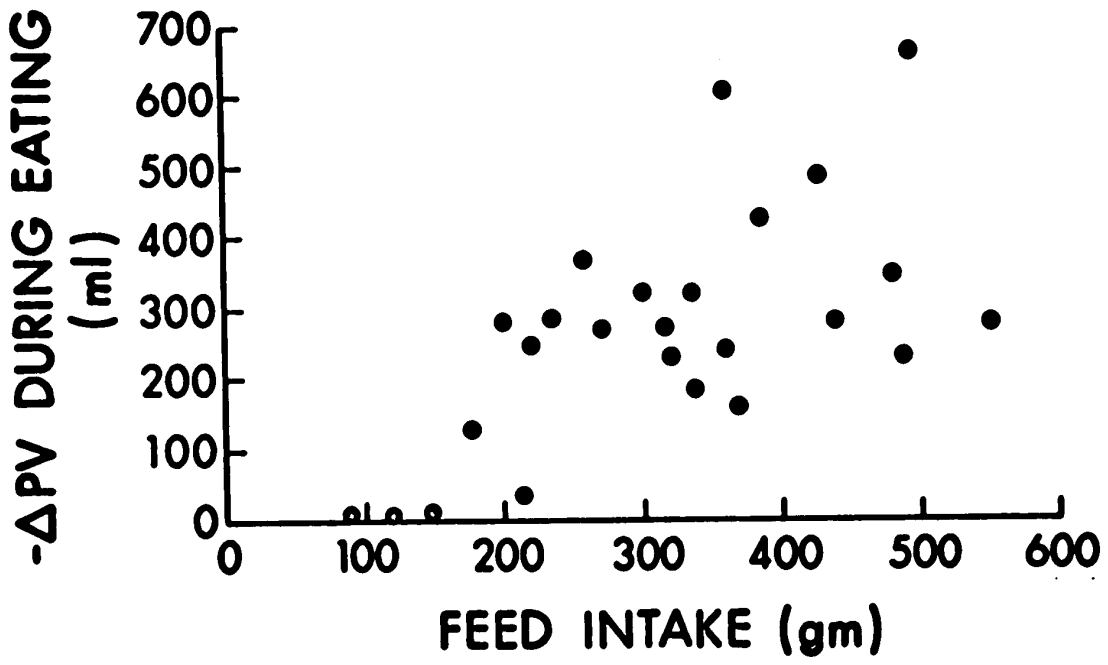the ECF volume. In these trials, other factors undoubtedly influenced

Figure 17. The relationship between the change in plasma volume (-ΔPV) during eating and the feed intake of Lincoln ewes at individual meals.
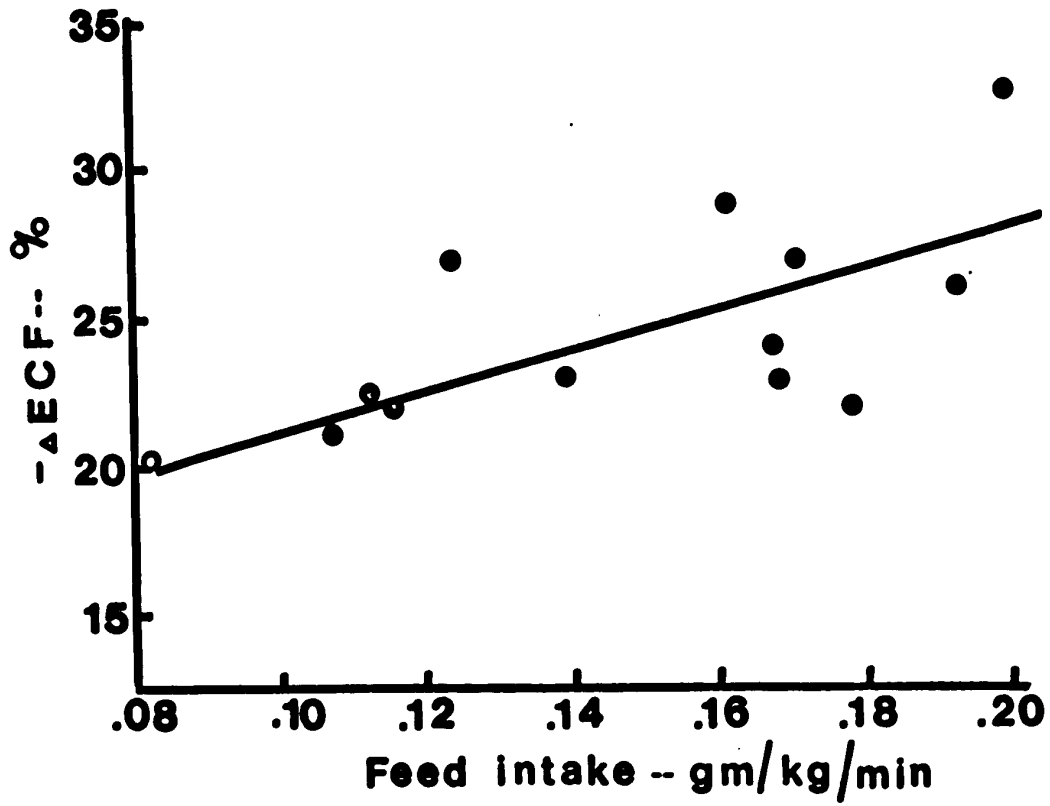
Figure 18. The relationship between the rate of eating and the percent change in extracellular fluid volume by the end of the meal in Lincoln ewes.

appetite, since the sheep ate less than they did on days in which they were not on trial. The results however, do confirm the large change in the ECF volume described by Ternouth (1968) and indicate that the decrease is related to the rate of eating. The present study also suggests that the very marked cardiovascular compensations that occurred during eating were a consequence not only of the increased energy expenditure but also, and predominantly, of the body fluid shifts.

If the changes in body fluids during a meal are important in determining satiety in sheep, these changes may have even greater implications for appetite regulation in cattle since the water turnover rate is faster in cattle than in sheep (Macfarlane and Howard, 1970). Cattle, therefore, would be influenced to a greater extent by water shortages or by other circumstances that result in dehydration.

Although exposure to a high environmental temperature with a higher rate of evaporative water loss is an obvious circumstance where body fluid status might act in determining satiety, it is also known that a sudden cold exposure can induce diuresis and result in a rapid loss of body water in several species (Cort, 1965). Short term cold exposure has been shown to result in haemoconcentration in cattle (Bailey, 1964) and in sheep (Mears and Groves, 1969). This type of stress may often be imposed on cattle kept out of doors, exposed to the Canadian winter. It is conceivable that sudden fluctuations in the outdoor temperature could cause transient changes in the water balance of ruminants and cause a temporary reduction in appetite. Most species of animals reduce their feed intake during the initial exposure to a reduced environmental temperature, a response that is usually attributed to "stress in general" (Mayer, 1967). However, this

response has not been explained in terms of specific satiety mechanisms.
Changes in water balance might partly account for this reduced appetite.

The marked change in the cardiac response of sheep during eating
in the cold in the present study indicates clearly that acute changes
in the environment influence the physiological changes associated with
eating. The observation that central blood temperature increases dur-
ing eating in ruminants (Ingram and Whittow, 1962; Mendel and Raghavan,
1963) suggests that eating may alter the temperature regulatory mech-
anisms of the animal. The fact that cardiac output did not increase
in proportion to the metabolic rate in the present study suggests that
blood flow becomes redistributed during eating with probably a larger
share being circulated to the alimentary tract (Sellers et al., 1964).
This would likely restrict peripheral blood flow and thereby interfere
with the distribution of body heat to the peripheral tissues. If
cardiac output were restricted as a consequence of the decrease in
available fluid in the ECF space during the process of eating the
circulatory demands of eating and the demand for optimum temperature
regulation in a warm environment could not be satisfied simultaneously.
Thermal polypnoea, another heat loss mechanism, is also reduced
during eating (Young, 1966). This may also contribute to loss of
appetite in a hot environment. During acclimatization to a warm
climate, the plasma and extracellular fluid volumes are expanded
(Macfarlane and Howard, 1970). This would appear to be a very
appropriate adaptation and would help to satisfy the conflicting de-
mands on the circulation induced by eating and thermoregulation in a
warm environment.

Although detailed studies have not been made on the interactions

between the physiology of eating and cold environmental temperatures
it is to be anticipated that conditions for eating are more favourable
in a cold environment provided that the temperature does not fluctuate
markedly. In a cold or cool environment the circulatory demands for
purposes of dissipating excess body heat would not be imposed. Apart
from the temporary shifts in body water already mentioned in regard to
acute cold exposure, the conditions for eating should be optimum in a
cold environment, however, there is a need for further study of the
processes related to eating and to cold acclimation.

Certain other factors that alter the nature of the physiological
changes during eating in ruminants require further investigation.
Young (1966) showed that the physical form of the ration influences
the energy cost of eating in sheep. Much information on the extent
to which feed processing can influence feed intake and energy expendi-
ture of .uminants on an ad lib regime could be obtained from a study
of the metabolic and physiological responses during eating and rumin-
ation. Management factors such as the frequency of offering feed may
influence the intake of young growing ruminants (Burt and Dunton,
1967). Ibrahim et al. (1970) reported that the intake of a straw-
based ration by cows was markedly increased when the ration was fed
from a continuous feeder, in small amounts, at 2 minute intervals
throughout the day and night. Although Blaxter et al. (1956) did not
observe any differences in heat production of sheep fed a constant
intake in one, two or four meals per day, detailed studies have not
been made on the relationship between ad lib feed intake and energy
expenditure when ruminants are fed at various frequencies. No studies
have been made of the energetic consequences of continuous or very

frequent feeding regimes or their relationship to satiety.

Blair-West and Brook (1969) reported that the plasma volume usually did not change during eating when the sheep were consuming fresh grass. This implies that the physiological responses to eating might be modified by the water content of the feed. Further research on the relationships between water and food and their influence on the physiological responses to eating and on appetite are required.

Consideration of the long term regulation of food intake may be outside the scope of this discussion since, in adult animals, it is probably related to the maintenance of fat stores in the body (Kennedy, 1961; Hervey, 1969). However, it is not known how the long-term mechanisms are integrated with the development of satiety at the end of individual meals. Since fat and water in the body tend to be inversely related, perhaps body fluid shifts associated with eating may also have relevance to long-term appetite regulation in ruminants.

Although the present study did not identify with certainty any specific determinant of satiety in sheep, the results emphasize the significance of the dynamic movements of body water in relation to the metabolic activity of eating and support the concept that the physiological changes associated with eating in ruminants (ingestion, salivation, etc.) are as likely to be involved in inducing satiety as are the physical and chemical consequences of the presence of food in the gut.

SUMMARY AND CONCLUSIONS

Recorded increases of 60-70% in oxygen consumption during eating in the present study are in agreement with previous estimates of the increased energy expenditure by sheep during individual meals. Exposure of sheep for 2 hrs to a mild cold stress did not alter the magnitude of this metabolic increment associated with eating.

The tachycardia that accompanied eating in sheep was shown to be markedly potentiated when the animals were fed during short-term exposure to a mild cold stress. In the presence of beta-adrenergic blockade with propranolol, there was still an increase in heart rate during eating to levels that were in excess of that which could be accounted for by the withdrawal of parasympathetic tone. It was concluded that a non-autonomic mechanism is partly responsible for the tachycardia during eating in sheep and that this component may act synergistically with the sympathetic system to potentiate the tachycardia during eating in the cold.

A detailed analysis of the cardiorespiratory changes that accompany eating in sheep indicated the manner in which an increased oxygen supply is provided to the tissues during the work of eating. The significant increase in hemoglobin concentration together with a relatively constant arterial blood P_{O_2} during eating markedly raised the oxygen carrying capacity of the blood. This was accompanied by a relative increase in the amount of oxygen extracted/100 ml blood in the systemic capillary bed. The increase in the arterio-venous difference in blood oxygen content and P_{CO_2} indicated that the cardiac output did not increase in direct proportion to the increase in oxygen consumption. The cardiac output determined by the direct Fick method

showed a moderate increase during th. meal and declined while the animals continued to eat. Stroke volume decreased progressively during the meal and began to recover after the animals stopped eating. A continuous dye-dilution technique was evaluated and shown to be unreliable for estimating cardiac output.

Blood pH decreased abruptly during eating in association with the marked increase in blood P_{CO_2} and a more moderate increase in plasma bicarbonate. Blood pH recovered slightly after the end of the meal but remained below pre-feeding values during the 30 minute post-feeding period.

Plasma volume was shown to decrease abruptly within 5 minutes after the onset of eating and stabilized quickly at a reduced level for the duration of the meal. The plasma volume recovered to the pre-feeding level within 30 minutes after the sheep stopped eating. Circulating red blood cell volume increased during eating and partially compensated for the decrease in plasma volume. Whole blood volume, therefore, did not decrease as much as plasma volume. The change in plasma volume during eating was related to the amount of feed consumed during the meal.

Problems associated with estimation of changes in the extracellular fluid volume have been discussed. Thiocyanate ion was concentrated about 12-fold in parotid saliva and 2-3 fold in mixed saliva. Thiocyanate disappearance is therefore of no value for estimating the time course of changes in the extracellular fluid volume during eating. Thiosulphate ion was not concentrated in the saliva but day to day variations in plasma decay curves introduced large errors when calculating changes in thiosulphate space during eating. The percent change

in thiosulphate space at the end of the meal showed a positive relationship to the rate of eating.

The increased plasma sodium concentration during eating confirms the suggestion that water is lost from the extracellular fluid during eating.

The significance of the marked shifts in body fluids during eating and their effect on cardiac performance has been discussed in relation to appetite in ruminants.

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APPENDIX

Table 13. Description of sheep used in the present study

Identification number	Breed	Sex	Average Body Weight (kg)
65	S	W	76
11	S	W	79
88	S	W	69
27	S	W	77
312	L	E	82
329	L	E	62
324	L	E	65
466	L	E	84
487	L	E	62

L - Lincoln   E - Ewe

S - Suffolk   W - Wether

Table 14. Mean values (±SE) for $\dot{V}_{O_2}$ and heart rate measured on Suffolk wethers before, during and after eating[1] at two environmental temperatures

Time (min)	Temperature °C	$\dot{V}_{O_2}$ (litres/hr)		Heart rate (beats/min)	
		+15	-15	+15	-15
0 - 30		21.4 ± 0.5	24.1 ± 1.1	61 ± 1	100 ± 14
30 - 60		33.7 ± 0.6	37.1 ± 1.2	94 ± 3	170 ± 17
60 - 90		36.6 ± 0.9	39.0 ± 1.6	104 ± 5	181 ± 12
90 - 120		31.5 ± 1.2	31.9 ± 1.2	80 ± 2	133 ± 13
120 - 150		29.4 ± 1.0	29.6 ± 1.0	76 ± 2	112 ± 12

[1] Sheep were allowed feed between 30 and 90 minutes.

Table 15. Mean values ±SE for blood pH, P_{CO_2} and plasma HCO_3 in Lincoln ewes at various times before, during and after eating[1]

Time (min)	Blood pH		Blood P_{CO_2} (mm Hg)		Plasma HCO_3 (mEq/litre)	
	a	v	a	v	a	v
0	7.475 ± .012	7.435 ± .008	31.9 ± 1.5	36.3 ± 1.4	22.9 ± 1.0	23.7 ± 0.9
15	7.477 ± .012	7.435 ± .010	34.8 ± 1.5	40.2 ± 2.2	25.1 ± 1.1	26.2 ± 1.4
30	7.470 ± .013	7.430 ± .011	36.7 ± 2.0	41.8 ± 2.8	26.0 ± 1.3	26.8 ± 1.5
35	7.441 ± .012	7.391 ± .013	39.6 ± 2.9	49.5 ± 3.6	26.1 ± 1.8	29.1 ± 2.0
40	7.423 ± .012	7.380 ± .013	43.7 ± 3.1	52.5 ± 4.3	28.0 ± 1.9	29.9 ± 2.1
45	7.419 ± .013	7.374 ± .012	45.0 ± 4.7	55.1 ± 5.1	28.2 ± 2.4	30.6 ± 2.4
50	7.414 ± .013	7.370 ± .013	46.5 ± 4.5	55.9 ± 5.5	28.6 ± 2.4	31.3 ± 2.6
55	7.422 ± .012	7.362 ± .014	43.6 ± 3.9	58.1 ± 5.8	27.5 ± 2.2	31.5 ± 2.8
60	7.413 ± .012	7.367 ± .014	46.0 ± 4.3	57.3 ± 5.6	28.1 ± 2.2	31.5 ± 2.8
65	7.445 ± .013	7.386 ± .014	44.7 ± 4.6	52.1 ± 4.8	29.6 ± 2.8	30.0 ± 2.4
75	7.450 ± .014	7.401 ± .014	44.6 ± 4.3	54.0 ± 5.3	29.7 ± 2.7	32.1 ± 2.8
90	7.450 ± .014	7.405 ± .013	45.0 ± 4.7	53.8 ± 5.3	30.0 ± 2.6	32.3 ± 2.7

[1] Sheep were allowed feed between 30 and 60 minutes.

Table 16. Mean values (+SE) for hematocrit, hemoglobin concentration, and MCHC in Lincoln ewes at various times before, during and after eating[1]

Time	Hct	Hb	MCHC
(min)	(%)	(gm/100 ml)	(gm/100 ml)
0	24.8 ± 0.8	8.57 ± .21	34.7 ± 0.6
15	24.1 ± 0.8	8.72 ± .21	36.4 ± 0.5
30	24.0 ± 0.8	8.50 ± .20	35.6 ± 0.7
35	27.6 ± 0.8	9.26 ± .24	33.6 ± 0.5
40	27.6 ± 0.8	9.40 ± .23	34.2 ± 0.5
45	27.9 ± 0.9	9.47 ± .24	34.1 ± 0.5
50	27.7 ± 0.7	9.40 ± .24	34.0 ± 0.6
55	27.7 ± 0.9	9.42 ± .20	34.1 ± 0.6
60	27.4 ± 0.9	9.33 ± .22	34.3 ± 0.6
65	25.9 ± 0.8	8.93 ± .18	35.0 ± 0.7
75	24.6 ± 0.8	8.49 ± .16	34.8 ± 1.1
90	24.5 ± 0.8	8.44 ± .16	34.8 ± 0.7

[1] Sheep were allowed feed between 30 and 60 minutes.

Table 17. Mean values (±SE) for blood P_{O_2}, O_2 saturation and C_{O_2} in Lincoln ewes at various times before, during and after eating[1]

Time	P_{O_2} (mm Hg)		O_2 satn (%)		C_{O_2} (ml/100 ml)	
(min)	a	v	a	v	a	v
0	71.6 ± 2.0	34.8 ± 1.3	91.3 ± 0.7	54.5 ± 2.1	10.5 ± 0.2	6.2 ± 0.3
15	68.3 ± 2.0	33.8 ± 1.3	89.8 ± 1.0	52.3 ± 2.4	10.5 ± 0.2	6.1 ± 0.3
30	68.5 ± 2.3	33.3 ± 1.3	89.5 ± 1.1	51.2 ± 2.5	10.2 ± 0.3	5.8 ± 0.3
35	69.1 ± 2.2	31.2 ± 1.1	89.0 ± 1.0	44.2 ± 2.2	11.3 ± 0.3	5.6 ± 0.3
40	67.7 ± 2.9	31.1 ± 1.0	86.0 ± 2.1	43.2 ± 2.2	11.2 ± 0.4	5.5 ± 0.3
45	67.7 ± 2.5	31.2 ± 1.2	87.1 ± 1.5	42.7 ± 2.1	11.4 ± 0.4	5.5 ± 0.3
50	66.6 ± 1.8	30.1 ± 1.4	87.0 ± 0.7	40.5 ± 2.7	11.3 ± 0.3	5.2 ± 0.4
55	69.6 ± 2.2	30.1 ± 1.3	88.4 ± 1.0	39.4 ± 2.4	11.4 ± 0.2	5.1 ± 0.3
60	66.7 ± 2.6	29.7 ± 1.3	86.3 ± 1.4	38.9 ± 2.4	11.1 ± 0.3	5.0 ± 0.3
65	70.8 ± 2.8	32.0 ± 1.4	89.2 ± 1.6	44.9 ± 2.6	10.9 ± 0.3	5.5 ± 0.3
75	68.2 ± 2.2	30.5 ± 1.4	88.6 ± 1.1	42.6 ± 2.7	10.3 ± 0.3	5.1 ± 0.3
90	70.4 ± 2.8	32.5 ± 1.4	88.9 ± 1.6	46.9 ± 2.7	10.3 ± 0.3	5.5 ± 0.3

[1] Sheep were allowed feed between 30 and 60 minutes.

Table 18. Mean values (±SE) for oxygen consumption ($\dot{V}_{O_2}$), heart rate (HR), cardiac output (Q), stroke volume (SV) and oxygen pulse measured in Lincoln ewes at various times before, during and after eating[1]

Time (min)	$\dot{V}_{O_2}$ (ml/min)	HR (beat/min)	Q (1/min)	SV (ml/beat)	O_2 pulse (ml/beat)
0	246 ± 11	81 ± 3	6.09 ± .25	77 ± 4	3.17 ± .12
15	256 ± 10	80 ± 3	6.06 ± .34	76 ± 4	3.23 ± .11
30	254 ± 11	77 ± 4	6.06 ± .34	76 ± 3	3.20 ± .11
35	374 ± 13	102 ± 2	6.92 ± .53	68 ± 5	3.68 ± .14
40	390 ± 7	110 ± 3	7.26 ± .45	66 ± 4	3.60 ± .15
45	395 ± 12	116 ± 4	7.06 ± .36	61 ± 3	3.46 ± .16
50	391 ± 10	119 ± 4	6.84 ± .45	58 ± 4	3.34 ± .15
55	377 ± 12	121 ± 5	6.14 ± .35	51 ± 3	3.17 ± .14
60	371 ± 13	120 ± 6	6.26 ± .34	53 ± 3	3.13 ± .12
65	334 ± 17	105 ± 5	6.32 ± .31	63 ± 4	3.23 ± .18
75	283 ± 13	95 ± 4	5.48 ± .33	59 ± 4	3.04 ± .17
90	288 ± 9	92 ± 6	6.01 ± .31	67 ± 4	3.19 ± .17

[1] Sheep were allowed to eat between 30 and 60 minutes.

Table 19. Mean values (+SE) for plasma volume, hematocrit and whole blood volume measured in Lincoln ewes at various times before, during and after eating[1]

Time(min)	PV (ml)	Hct (%)	BV (ml)
0	3097 ± 44	26.7 ± 0.5	4163 ± 58
10	3080 ± 46	26.7 ± 0.5	4141 ± 65
20	3074 ± 44	27.1 ± 0.5	4157 ± 67
30	3088 ± 41	27.2 ± 0.5	4186 ± 67
35	2852 ± 49	31.4 ± 0.6	4104 ± 66
40	2801 ± 42	31.4 ± 0.6	4014 ± 71
45	2804 ± 47	31.2 ± 0.5	4006 ± 69
50	2827 ± 47	30.6 ± 0.5	4005 ± 70
55	2839 ± 50	30.4 ± 0.5	4010 ± 79
60	2829 ± 52	29.9 ± 0.6	4971 ± 79
65	2911 ± 51	28.9 ± 0.5	4030 ± 75
75	3010 ± 56	27.6 ± 0.6	4100 ± 81
90	3104 ± 42	26.8 ± 0.6	4185 ± 67

[1] Sheep were allowed feed between 30 and 60 minutes.

Table 20. Mean values (+SE) for thiosulphate space of Lincoln ewes [1]
measured at various times before, during and after eating [1]

Time(min)	Thiosulphate space (litres)
0	13.1 ± 0.4
10	12.9 ± 0.4
20	13.1 ± 0.5
30	12.8 ± 0.4
35	12.5 ± 0.5
40	12.2 ± 0.7
45	12.0 ± 0.6
50	12.0 ± 0.7
55	11.5 ± 0.9
60	11.6 ± 1.1
70	11.6 ± 1.0
80	11.9 ± 1.2
90	13.7 ± 1.4

[1] Sheep were allowed feed between 30 and 60 minutes.

Table 21. Mean values for plasma thiocyanate concentration (mg/100 ml) measured at various times after injection during control trials and during trials in which the sheep were fed at 30, 60 and 120 minutes after injection

Time after Injection(min)	Control	Sheep fed at		
		30 min	60 min	120 min
10	9.00	8.60	9.00	8.60
15		8.00		
20	7.50	7.50	8.00	7.90
25		7.40		
30	7.40	_7.20_	7.60	7.60
35		7.20		
40	7.00	7.20	7.30	
45		6.70		7.20
50	6.85	6.90	7.10	
55		6.80		
60	6.60	_6.85_	_6.80_	6.80
65			7.25	
70	6.50	6.65	7.10	
75			6.90	6.65
80	6.45	6.60	6.85	
85			6.70	
90	6.30	6.50	_6.75_	6.60
100	6.35		6.55	6.50
120	6.20	6.35	6.65	_6.40_
125				6.50
130				6.50
135			6.50	6.30
140				6.15
145				6.20
150	6.15		6.55	_6.20_
165				5.80
180	6.05	6.20		5.65