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STUDIES ON THE DIGESTION OF RYEGRASS SPECIES AND THE REGULATION OF FOOD INTAKE BY SHEEP

A Thesis Submitted for the Degree of
Doctor of Philosophy
in the
Massey University of Manawatu

By

M. J. ULYATT

June 1964

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ACKNOWLEDGMENTS

It is a pleasure to acknowledge the guidance and help of my supervisors, Dr A. T. Johns and Professor A. L. Rae.

The author is indebted to Dr C.S.W. Reid, D.S.I.R., for his valuable help and criticism.

The assistance of Mr P. Vlieg, D.S.I.R., is also gratefully acknowledged.

The author wishes to thank the following members of D.S.I.R.: Dr R. W. Bailey, for his help with carbohydrate analyses of plant material; Dr R. W. Brougham, for discussion on agronomic aspects of the work; Mr A. C. Glenday, for assistance with statistical analyses; Miss M. Soulsby, for the photography; Messrs T. Brown, J. Balloch and J. Kook, for help in handling the animals; Mr J. Knowles and his farm staff; and Miss R. Fell, for typing this Thesis.

The writer also wishes to thank D.S.I.R. for granting leave on full salary.

PREFACE

The economy of New Zealand is dependent largely on the export of products of the livestock industry. While there has lately been an upsurge in secondary industry, a basic lack of raw materials and cheap labour curtails New Zealand's ability to compete economically with other countries as a manufacturing nation. If New Zealand's position as one of the world's leading agricultural exporters is to be maintained or expanded then increased primary production must be achieved. Such an increase is also necessary to maintain or improve the high standard of living of a rapidly growing internal population.

Three general factors are important when the problem of increasing livestock production is considered.

1. Nutrition
2. Breeding
3. Diseases and Pests

The interest of the author lies with nutrition but the other two factors are clearly important if animal production is to be improved. Breeding is important particularly in relation to raising fertility and producing types of animals that will give optimum returns in particular environments. It must be remembered however, that genetic improvement by selection of livestock, though essential, is usually slow because of the low heritability of many productive characteristics.

Pests and diseases can limit animal production, particularly at high stocking rates, so the prevention of epidemics and the eradication of persistent diseases such as footrot, brucellosis and tuberculosis must continue to be actively pursued.

At a symposium entitled, "Problems of Increasing Sheep Production", held in February 1964 by the New Zealand Society of Animal Production, it was evident that the most effective short-term method of increasing animal production would be by increasing stocking rate. This infers increases in both animal numbers and the food to support these. Thus to increase the carrying capacity of the present farming area there must be an increase in the quality and quantity of pasture produced. This can be achieved in two ways: by techniques of pasture management and by breeding improved strains of pasture plants. It must also be remembered that considerable advances may be obtained with improved management of existing strains of pasture plants.

In 1953 Melville stated that there are at least three criteria to be satisfied in providing ideal pasture for ruminants:

1. Ideally the pasture should be capable of providing the requirements of sheep and cattle for protein and carbohydrates.

2. It should provide those minerals and accessory food substances which are essential to animal health in sufficient amount and in a state of balance within the feed.

3. Under no condition should the total herbage contain potentially harmful compounds at a level which will cause animal disorders or a decline in production.

These criteria still apply but little progress has been made since this statement to implement them for the improvement of pasture species. For example, the causes of metabolic disorders such as bloat, hogget ill-thrift and ryegrass staggers, which are generally associated with rapidly growing pasture, are still unknown. The plant breeder has continued to select mainly for agronomic characters such as, greater productivity, seasonal spread of production, persistency, resistance to disease, time and uniformity of flowering, and to a lesser extent "palatability". Agronomic characters are important but the final criterion as to the value of a pasture plant selection must be in terms of animal production. However the animal nutritionist and biochemist must also share the blame for they have been unable to define clearly what is required by the animal. The plant breeder must be provided with a relatively simple index of the nutritive value to ruminants of a pasture species.

Such an index will remain difficult to define until more precise information is available on the fermentation reactions that the individual components of a plant undergo in the rumen, and on the efficiency with which the end-products of such fermentations are utilized by the animal for productive purposes.

The amount of pasture an animal can eat and the efficiency of utilization of this pasture will determine the nutritional welfare of the animal when factors such as pests, infectious disease, metabolic disorders and mineral imbalances are not limiting. This generalization is the basis of the work presented in this Thesis.

More specifically, the present work was commenced to try and elucidate the results of some earlier experiments, where a comparison was made of the nutritional value to sheep of short-rotation (S) and perennial (P) ryegrasses with and without white clover (C) (Rae, Brougham, Glenday and Butler, 1963; Barton and Ulyatt, 1963; Johns, Ulyatt and Glenday, 1963). In those experiments there were differences in the live weight gains of sheep grazing the pastures in the order: S + C were greater than P + C and S, which were approximately equal, while P produced the lowest gains. Dealing with the treatment extremes, S + C and P, it was found that although live weight gains were greatest

on S + C, rumen "fill" was greater on the P treatments. Further, rumens from S + C sheep had greater development of the mucosal papillae and contained a higher concentration of volatile fatty acids (VFA) than rumens from the P sheep. There was a lower proportion of acetic and higher proportions of propionic and butyric acids in rumen liquor from the S + C animals. Animals from the P + C and S pastures were intermediate in the above characteristics.

Three possible causes of the observed differences were considered likely:

1. That the end-products of the ruminal fermentation of S + C were utilized by the animals more efficiently than those from P.
2. That the intake of S + C was higher than that of P. This would infer a faster rate of flow of digesta through the digestive tracts of the S + C animals.
3. A combination of 1 and 2.

Experiments which were designed to test the above possibilities under grazing conditions are described in Chapter 2. Most of the published techniques for measuring rumen "fill" and the rate of flow of digesta through the digestive tract were developed under conditions of stall-feeding. In the present work methods for measuring these variables in grazing sheep were required. Lithium was suggested to the author as

a possible marker for this purpose (Chapter 4) but it was found unsatisfactory compared with the substance polyethylene glycol (PEG). The use of PEG as a marker under grazing conditions is considered in Chapter 1. The possibility that the VFAs absorbed from the rumen could cause a chemostatic regulation of food intake was studied in Chapter 3.

CHAPTER 1

THE USE OF POLYETHYLENE GLYCOL AS A MARKER FOR MEASURING RUMEN WATER VOLUME AND THE RATE OF FLOW OF WATER FROM THE RUMEN OF GRAZING SHEEP

INTRODUCTION

A large proportion of the energy requirements of ruminants is derived from fermentation in the rumen (Carroll and Hungate, 1954; Balch, 1958; Blaxter, 1962). Further, the major delay in the movement of digesta through the alimentary tract occurs at the rumen: movement of digesta in the intestines is similar in ruminants and non-ruminants (Lenkeit, 1930, 1932; Usselli, 1933; Columbus, 1936; Balch, 1950; Blaxter, 1962). Therefore the energy metabolism of the ruminant must be closely related to rumen volume and the rate of movement of digesta through the rumen.

Methods of measuring rumen volume and the movement of digesta through the alimentary tract will be reviewed separately.

1. Measurement of Rumen Volume

In this review the term "rumen volume" will be used in the widest sense to indicate any measure of reticulo-rumen capacity. Throughout this Thesis the reticulo-rumen will be known simply as the rumen. The weight or volume of rumen digesta is considered the important functional entity in studies of normal digestive processes in the rumen: this comprises solid plus liquid components and will be known as rumen "fill".

Rumen "fill" can be divided into many fractions which will be referred to specifically when necessary: the largest fraction is water which comprises approximately 85% of "fill".

Measures of rumen volume can be divided into two categories: those involving slaughter of the animals and methods of measuring volume in the live animal.

(a) Methods Involving Slaughter of the Animals

Direct measurement of rumen "fill" immediately after slaughter has been employed for many years (e.g. Mäkelä, 1956; Tayler, 1959; Johns et al., 1963).

Mäkelä (1956) described a method of measuring the potential capacity of the rumen. This was the amount of water required to fill an isolated rumen to a constant water pressure. A variation of this scheme was described by Warner, Flatt and Loosli (1956) who immersed the rumen in a container full of water, filled the rumen with water, and measured the water displaced from the container. When water is thus added to the rumen considerable stretching occurs, and the volume obtained is largely dependent on the pressure of water used (Mäkelä, 1956). This stretching was found by Johns et al. (1963) to limit the usefulness of the technique.

The main disadvantage of slaughter experiments

is that repeated measurements on the same animals cannot be made. However they do have value if large numbers of animals are available. This does not solve the problem, so in vivo methods have been evolved.

(b) Direct Removal of Rumen Contents from the Live Animal

With cattle prepared with rumen fistulae it is possible to remove the rumen contents completely and to return them after weighing (e.g. Schalk and Amadon, 1928; Campling, Freer and Balch, 1961).

Flatt, Warner and Loosli (1959) emptied the rumens of fistulated calves and then filled the rumens with water to a level 4 cm above the centre of the fistula plug. This procedure was repeated three times in rapid succession and the mean amount of water required was considered to be the rumen volume.

The above procedures are not feasible with sheep because of the large size of rumen fistula required. Such fistulae have been prepared in sheep (e.g. Ash, 1957), but the size of the fistula in proportion to rumen size leads to the possibility of impairment of normal rumen function.

(c) Use of Reference Substances

Rumen "fill" is commonly divided into water and dry matter (DM) fractions. If water volume can be estimated with a marker substance, and representative DM samples obtained, then the distribution volume of

any rumen DM constituent can be calculated. Most marker substances are therefore designed to measure rumen water volume.

Water can enter and leave the rumen in various ways: inflow is usually via the oesophagus in food, drinking water and saliva, but passage into the rumen across the rumen epithelium can occur. Outflow of water from the rumen may occur by passage through the reticulo-omasal orifice, by absorption across the rumen epithelium or by removal during rumen sampling.

A marker for measuring rumen water volume must fulfil the following criteria:-

(i) It must be inert, i.e., not absorbed from or metabolised in the rumen, or absorbed on the rumen epithelium.

(ii) It must be non-toxic to both the host animal and the symbiotic rumen micro-organisms.

(iii) It must have the same distribution volume as the substance being measured. In the case of water, the marker must be completely water soluble.

(iv) The marker must be adaptable for easy analysis.

The assumptions and mathematical relationships involved in using reference substances to measure rumen water volume have been detailed by Hydén (1960, 1961) but will be repeated here. Two important assumptions are made:

(i) That the volume of water in the rumen remains constant during the experiment.

(ii) That the flow of water through the reticulo-omasal orifice is continuous and the rate of flow is constant.

Hydén (1961) considered that if the animals had continuous access to food and water, and if the experiment was of long duration, then the assumptions would be valid.

The procedures used in estimating rumen water volume with markers will be outlined below. A known quantity of marker dissolved in a known quantity of water is added to the rumen contents via the rumen fistula. Administration by mouth or stomach tube has been tried (Smith, 1959), but there is always some doubt as to the exact site of addition of the marker. After the marker is added, sufficient time is allowed for it to mix completely with the rumen contents (about 1.5 hr with sheep). Samples of rumen liquor are then collected at pre-determined time intervals, the concentration of marker in each of them determined, and a marker dilution curve constructed. If the assumptions are correct this curve should be exponential; thus if the natural logarithm of marker concentration is plotted against time a straight line should result. Extrapolation of this straight line back to

zero time (usually by linear regression) produces an estimate of marker concentration at the time of dosing. Rumen water volume (V) can thus be calculated from the relationship $V = \frac{p}{C'' - C'}$

where p is the amount of marker administered and C' and C'' are marker concentrations before and after dosing. When a significant amount of water is dosed with the marker the rumen water volume (V') can be calculated from $V' = \frac{p - LC''}{C'' - C'}$

where L is the volume of water added.

It has been difficult to find a substance suitable as a marker for estimating rumen water volume because of the rigid criteria involved. A polyethylene glycol (PEG) marker with an average molecular weight of 4,000 was introduced by Sperber, Hydén and Ekman (1953) and appears to meet the requirements satisfactorily. PEG is water soluble and its distribution volume in rumen contents is approximately 95% of the total water (Hyden, 1961). PEG does not enter the intracellular water of undigested plant fragments and so measures the "free" water in the rumen. In other respects PEG fulfils the theoretical requirements of a marker for estimating rumen water volume: it is non-toxic, not absorbed and is not metabolised by rumen micro-organisms (Sperber et al.,

1953; Hydén, 1956b). In a paper published since the present work was completed Jacobson, Bondy, Broitman and Fordtran (1963) found that the water volumes of isolated loops of rat intestines estimated with PEG, were slightly higher than water volumes determined by direct measurement. Small amounts of PEG were either adsorbed on to the intestinal mucosa or were retained in the intervillous spaces. This retained PEG could be recovered if the intestines were washed out with water. Small losses in faecal recovery have been reported by Hydén (1956b) and Corbett, Greenhalgh, Gwynn and Walker (1958). Hydén (1956a) has developed a rapid and accurate turbidimetric method of estimating PEG which has been simplified by Smith (1959).

2. Measurement of the Movement of Digesta Through the Digestive Tract

Balch (1960) defined the rate of movement of digesta through the digestive tract in two ways. "The rate of passage is the rate at which undigested residues of a given meal pass a given point in the gut, or are eliminated in the faeces. In contrast the rate of flow of digesta expresses the rate at which the mixture of undigested residues from previous meals passes a given point in the gut or is eliminated in the faeces." These definitions consider digesta as a whole and do not account for the fact that various

fractions of the digesta pass through the alimentary tract at different rates which depend on characteristics such as solubility, particle size and specific gravity (Balch, 1950, 1960; Corbett, Greenhalgh and Florence, 1959; Campling and Freer, 1960; Hydén, 1960). Balch's (1960) definition must therefore be expanded to include the rates of passage and flow of various fractions of the digesta. In ruminants the major site of delay in the passage of digesta is at the rumen, so it is desirable to obtain measures of digesta movement through both the rumen and the remainder of the alimentary tract.

(a) A Technique Involving Slaughter of the Animals

A slaughter method of estimating the rate of flow of various food fractions through the rumen and the rest of the alimentary tract has been developed by a group of Finnish workers (Paloheimo and Mäkelä, 1952; Mäkelä, 1956; Paloheimo and Mäkelä, 1959; Mäkelä, 1960). Cows were fed at a constant intake level for 10 to 15 days, then slaughtered and the distribution volume of various fractions of the digesta in the rumen and alimentary tract determined. The time of retention of a food fraction was determined by the ratio:

$$\frac{\text{Amount of fraction in rumen (or in rest of alimentary tract}}{\text{Amount of fraction in daily food intake}}$$

This method is based on the two assumptions; that during uniform feeding, (i) inflow to and outflow from the rumen are equal, and (ii) rumen "fill" is constant. The method lacks the precision required in detailed physiological work.

(b) Direct Measurement Using Special Cannulae

Surgical methods for exteriorizing the flow of digesta in several parts of the digestive tract using re-entrant cannulae have been devised. Phillipson (1952) measured the flow of digesta from the abomasum to the duodenum of sheep in this way. Similarly Hogan and Phillipson (1960) measured the flow of digesta in the duodenum and in the terminal part of the ileum of sheep, and Singleton (1961) measured the flow of digesta in the duodenum of goats and sheep. This method has limitations under grazing conditions: the risk of infection is high, there are problems in measuring the flow and returning digesta to the digestive tract, and the cannulae are easily torn out.

(c) Marker Methods

Reference substances have been used for many years to follow the course of digestion in ruminants. As stated above various fractions of the digesta move through the alimentary tract at different rates. Thus the volume of distribution of any reference substance and its physical characteristics will determine what

fraction of the digesta it is associated with and the speed of its throughput.

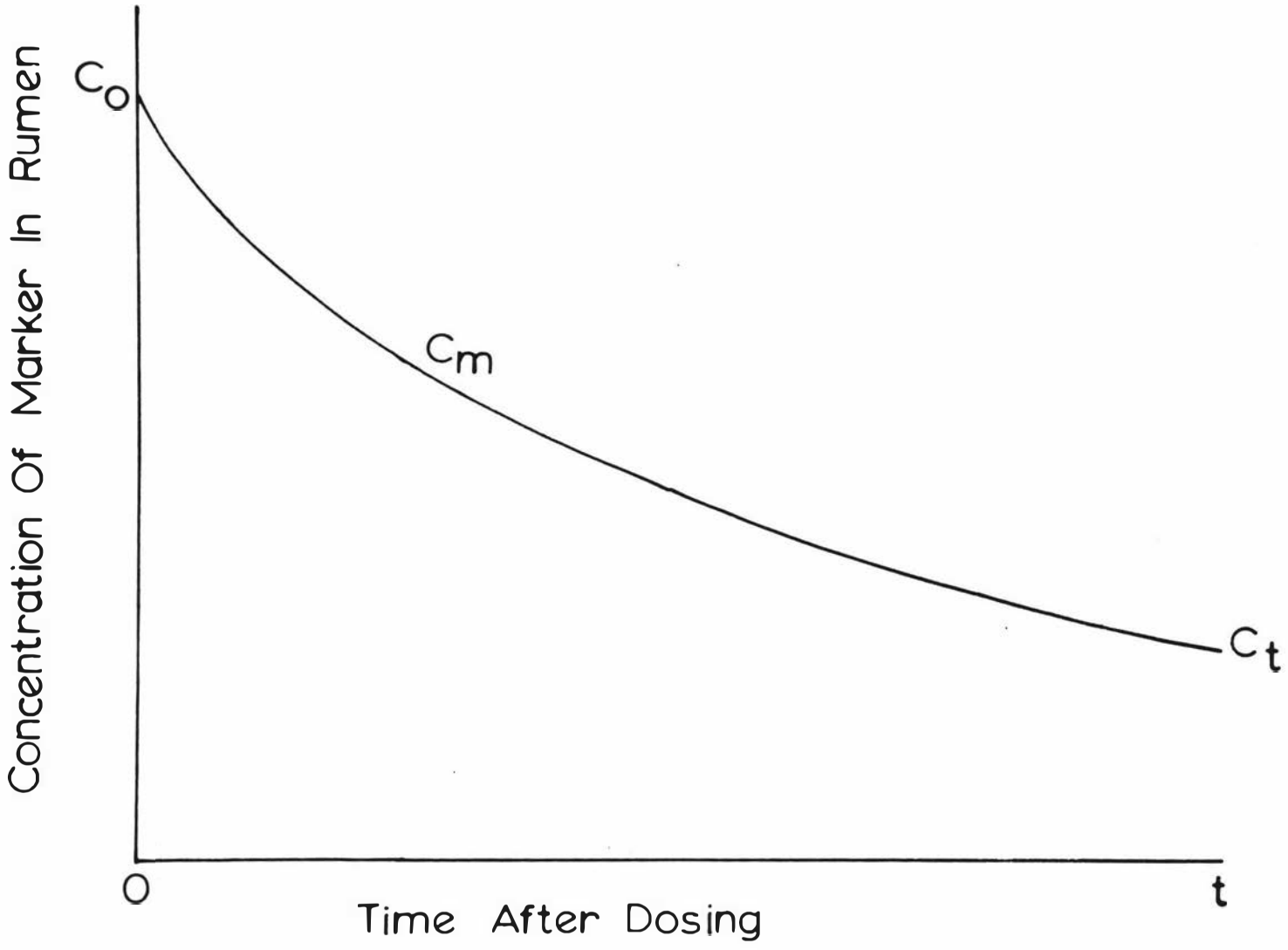
Many reference substances have been used for different purposes and with varying degrees of success. Examples are: silica (Wildt, 1874), ferric oxide (Knott, Murer and Hodgson, 1936), dyes (Coup and Lancaster, 1952; Cortin and Forbes, 1951), radio isotopes (Kane, Jacobson, Ely and Moore, 1953), pectin (Gray, 1947), titanium oxide (Lloyd, Rutherford and Crampton, 1955), rubber chips (King and Moore, 1959), polythene chips (Minson, Tayler, Alder, Raymond and Rudman, 1960), chromic oxide (Edin, Kihlén and Nordfeldt, 1944). Chromic oxide has been used extensively for estimating total faecal production in digestibility trials.

A common method of determining the rate of passage of digesta in ruminants is based on staining a known proportion of the food with a fast dye and measuring the faecal excretion of stained particles. This method originated in the work of Lenkeit and Habeck (1930), Lenkeit (1930, 1932), Usuelli (1933) and Columbus (1936). In recent years the method has been used extensively. Most workers using this technique base their measurement of rate of passage on some aspect of the time of retention of the stained particles in the digestive tract. When cumulative percentages of

stained particles voided in the faeces are plotted against time a sigmoid curve is obtained. Unfortunately various workers have used different aspects of this curve to measure time of retention. Balch (1950) developed the concept that the time required for the excretion of 5% of the stained particles gave a measure of the time required for the food to pass through the whole alimentary tract, while the time required for the passage of 80% of the particles minus the time for 5%, gave an indication of the time of retention of the stained meal in the rumen. Brandt and Thacker (1958) used a similar system. Castle (1956) and Blaxter, Graham and Wainman (1956) estimated the mean time of retention of stained particles in the digestive tract. Shellenberger and Kesler (1961) compared these various methods and concluded that those measuring mean time of retention were slightly more reliable.

Hydén (1960, 1961) presented a method whereby the rate of flow of water from the rumen could be estimated using PEG as a marker. The mathematical relationships involved in this measurement have been given in detail by Hydén (1961) but the relevant parts will be repeated here. The criteria and assumptions stated above in using PEG as a rumen water volume marker still apply. When marker is introduced to the rumen it will leave via the reticulo-omasal orifice and the

Fig. 1 Rumen Marker Dilution Curve



marker dilution curve resulting (Fig. 1) can be described by the equation:

$$C_t = C_o e^{-kt} \qquad (1)$$

The change in amount of reference substance in the rumen is: $d(CV) = -CdF = -Cudt$ (2)

where C is the marker concentration, V is the rumen water volume at any moment, F is the total flow and u is the rate of flow. Assuming water volume remains unchanged during the experiment, equation (2) yields on integration

$$\text{Log}_e C = -\frac{ut}{V} + A$$

when $t = o$, $C = C_o$ and thus $A = \text{Log}_e C_o$, and $\text{Log}_e \frac{C_t}{C_o} = -\frac{ut}{V}$

Then equation (1) can be written

$$C_t = C_o e^{\frac{-ut}{V}}$$

Since the amount of water leaving the rumen per unit of time, $k = u/V$, the rate of flow is given by

$$u = kV \qquad (3)$$

When the dilution curve is converted to a semi-logarithmic plot of marker concentration against time, the concentration at time zero can be calculated by linear regression, and k is the regression coefficient.

The total flow of water from the rumen during an experiment is given by

$$F = ut = ktV \qquad (4)$$

In the majority of experiments the flow can be

estimated from the amount of marker which has disappeared from the rumen during the experiment and the average marker concentration. A more accurate method takes into account rumen water volume at the beginning and the end of the experiment. If the initial and final amounts of the marker in the rumen are C_oV_o and C_tV_t respectively, then the amount which leaves the rumen during the experiment is given by

$$z = C_oV_o - C_tV_t$$

By substitution and integration in equation (2) the following relationship is obtained:

$$u = \frac{z}{C_m t}$$

where C_m is the mean concentration of marker in rumen water during the experiment.

Total flow will then be

$$F = \frac{z}{C_m}$$

This formula is applicable to any shape of the marker dilution curve, and when this is represented by equation (1)

$$C_m = \frac{C_o - C_t}{\text{Log}_e\ (C_o/C_t)}$$

and

$$F = z\ \frac{\text{Log}_e\ (C_o/C_t)}{C_o - C_t} \qquad (5)$$

Application of equation (5) is limited by having to know rumen water volume at the beginning and end of an experiment. However, if flow is calculated simply

from the initial volume and the slope of the curve (equation 3), it will be underestimated if volume diminishes, and overestimated if volume increases during the experiment.

That the dilution curve in Hydén's (1961) work could be described by equation (1) does not necessarily prove that rumen water volume remains constant. If the ratio of inflow to water volume remains constant during an experiment, the semi-logarithmic plot will give a straight line whether volume increases, decreases, or remains unchanged.

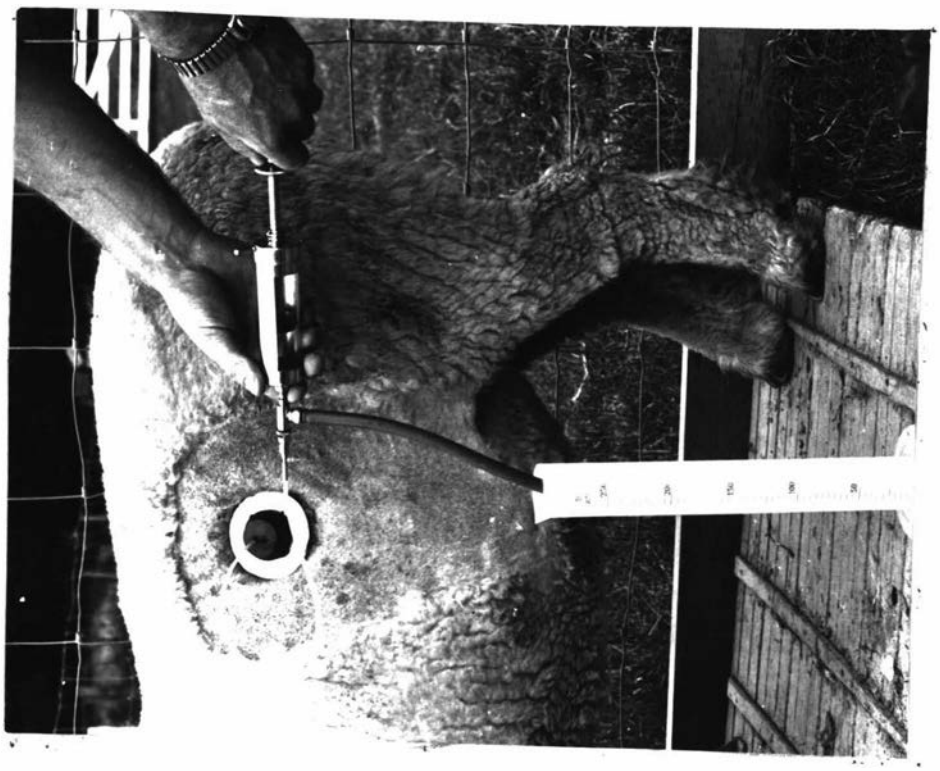
The above considerations apply to any marker that is used to determine the rate of flow of water from the rumen. Hydén (1961) used PEG in his studies and claimed considerable precision, but he did not present any statistical indication of how well his data fitted a linear relationship, nor did he indicate the size of error involved in measuring rumen water volume and flow rate with PEG.

In the present work methods were required to measure rumen "fill" and the rate of flow of digesta from the rumen in sheep grazing pasture. Slaughter techniques were not possible because repeated measurements were required on the same animals. The best available technique for measuring rumen water volume, and thus indirectly rumen DM volume, seemed to be the PEG method of Hydén (1961). However estimation of rate

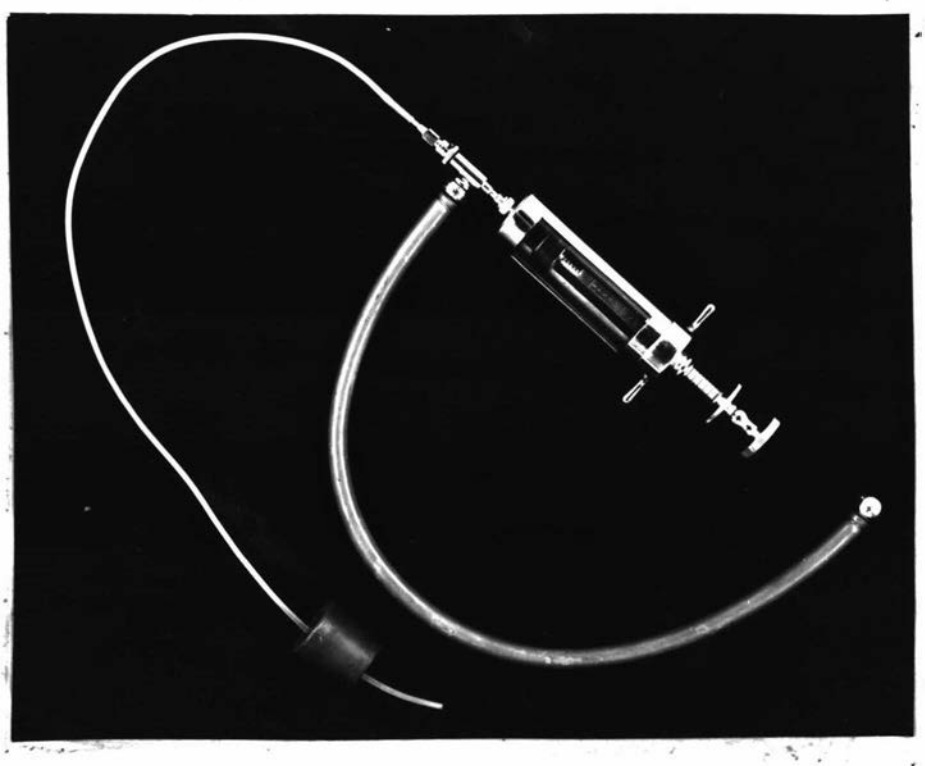
of flow of digesta presented greater difficulties. The stained food particle method is unsuitable for grazing sheep because stained grass would have to be fed as a marker, and the problem of recovering this in faeces is formidable. As PEG was being used to estimate rumen water volume it was decided to use it to measure the rate of flow of water out of the rumen. This latter measurement would also give an indication of the rate of flow of any substance soluble in water. It was realised that the assumptions involved in using this technique were of doubtful validity, and that the rate of flow of water cannot necessarily be equated with the rate of flow of digesta through the rumen or through the whole digestive tract. In fact Hydén (1961) could find no clear relationship between food intake and the rate of flow of water from the rumen.

In this chapter PEG is investigated as a marker for measuring rumen water volume and the rate of flow of water from the rumen in grazing animals. An effort has been made to check the validity of the assumptions, to determine the magnitude of error involved, and to find means of improving the technique.

Fig. 2. The Apparatus and Method of Dosing a Sheep with PEG Marker



(b) Dosing



(a) Apparatus

EXPERIMENTAL METHODS

Dosing and Sampling

Food was withheld from the sheep in the 2 hr prior to dosing with PEG each time rumen water volume and flow rate was measured. This was to enable the rumen contents to become more fluid than when the animals were fully fed, and to facilitate rapid mixing when the marker was added. The marker dose of approximately 10 g of PEG (mol. wt. 4,000) in 250 ml of water was administered via the rumen fistula by means of a 50 ml syringe fitted with a two-way valve and a long piece of polythene tubing (Fig. 2). At dosing the polythene tubing was introduced to the rumen and marker was injected into various areas by guiding the tubing. After dosing the sheep were immediately returned to pasture.

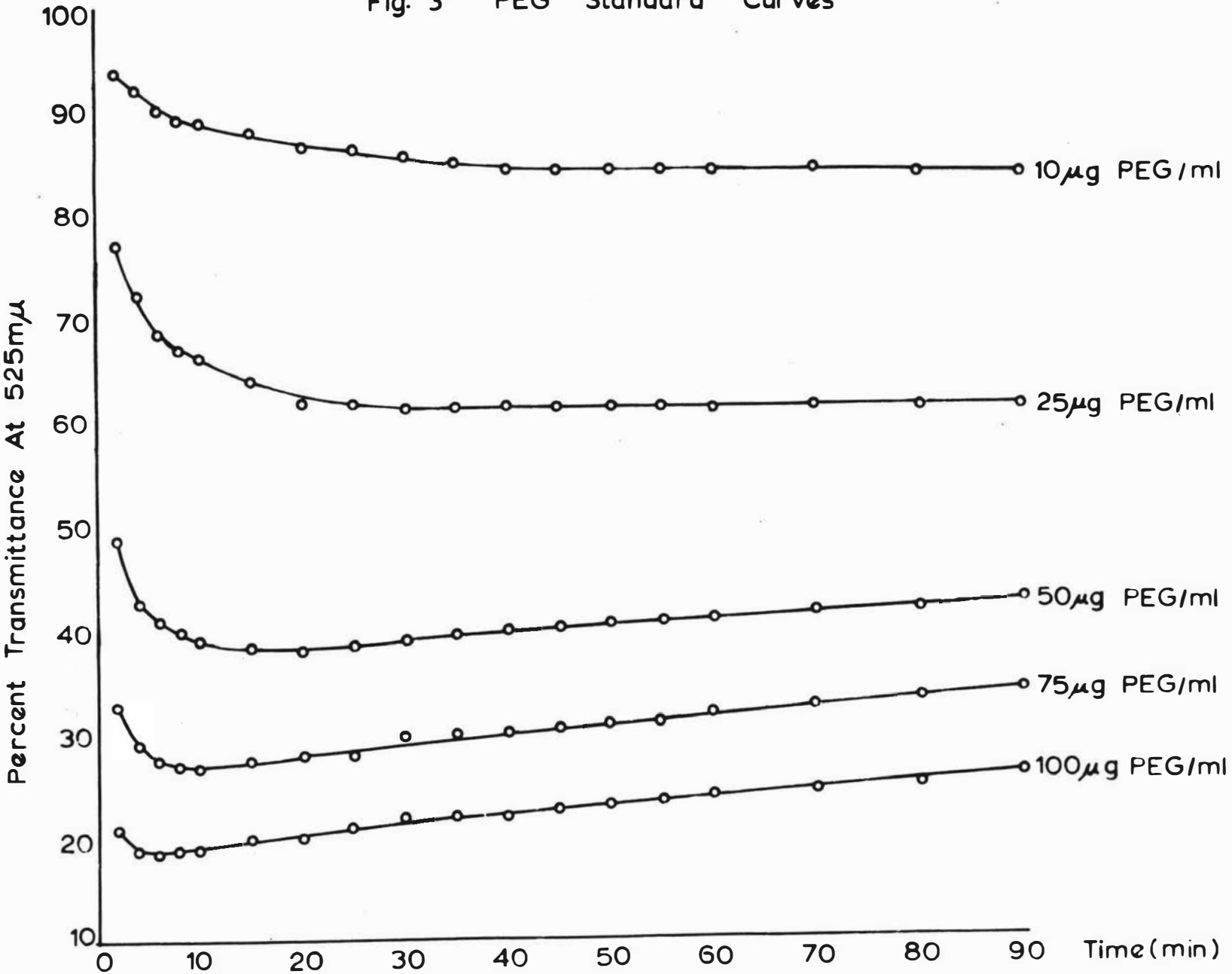
Samples of rumen contents were collected 1.5, 3, 6, 12 and 24 hr after dosing from a position immediately below the fistula, and the rumen liquor was strained through muslin to remove plant debris. Hydén (1961) has shown that this sampling position gives an average representation of marker concentration in various parts of the rumen.

Analytical

PEG in rumen liquor was determined by the turbidimetric method of Hydén (1956a) with some of the

modifications of Smith (1959). Strained rumen liquor containing PEG was centrifuged for 10 min at 1,000 x g to remove particulate organic matter. Up to 5 ml of this centrifuged rumen liquor (depending on PEG concentration) was added to a test tube graduated at 10 ml and clarified as follows. For up to 3 ml of rumen liquor, 1 ml of 0.3 N $Ba(OH)_2$ was added to the test tube followed by 1 ml of 5% (w/v) $ZnSO_4.7H_2O$ and 0.5 ml of 10% (w/v) $BaCl_2.2H_2O$. For 4 to 5 ml of rumen liquor the quantities of clarifying reagents were doubled. The tubes were then made up to the 10 ml mark with distilled water, shaken well, and allowed to stand for 5 min. Hydén (1956a) recommended filtering the clarified rumen liquor but in the present work this was unsatisfactory. Centrifugation at 15,000 x g for 10 min was adopted and after this no trouble with interfering substances was encountered. The centrifugation was followed by pipetting 0.5 to 5 ml of the supernatant (depending again on PEG concentration) into 10 ml Bausch and Lomb spectrophotometer tubes and these were made up to 5 ml with distilled water. Five ml of an aqueous solution of 30% (w/v) trichloroacetic acid and 5.9% (w/v) $BaCl_2.2H_2O$ (TCA) was then added to the tubes with a syringe pipette: this produced a turbidity with the PEG. The tubes were left for 20 min before reading the percentage transmittance in a Bausch

Fig. 3 PEG Standard Curves



and Lomb spectrophotometer at 525 mμ. If duplicates varied by more than 3% transmittance the analysis was repeated. PEG only gives a measurable turbidity in the range 10 to 100 μg/ml and for this reason care must be taken to ensure that all solutions analysed are within this range. Enough PEG had to be added to the rumen so that it could be detected in the 24 hr sample: for sheep on pasture 10 g of PEG was found to be a satisfactory dose. The time of 20 min required to develop average maximum turbidity was arrived at experimentally and differs from the times stated by other authors. A plot of percentage transmittance against time for various standard concentrations of PEG is shown in Fig. 3. Under the conditions applying in the present work the time taken to reach maximum turbidity (i.e., minimum transmittance) was dependent on PEG concentration, but 20 min was an average estimate of this time. Hydén (1956a) claimed that stable turbidity was reached after 5 min and Smith (1959) used 1 hr. However Smith obtained his time by adding the TCA solution to standards made up in distilled water and under these conditions a time of 1 hr was obtained in the present work. However, when the clarifying reagents were added to the standards, average maximum turbidity was developed at 20 min. Corbett et al. (1958) measured turbidity in a nephelometer immediately

after adding TCA. In the present work it was found that the nephelometer reading changed so rapidly that considerable error was introduced if a delay in reading occurred.

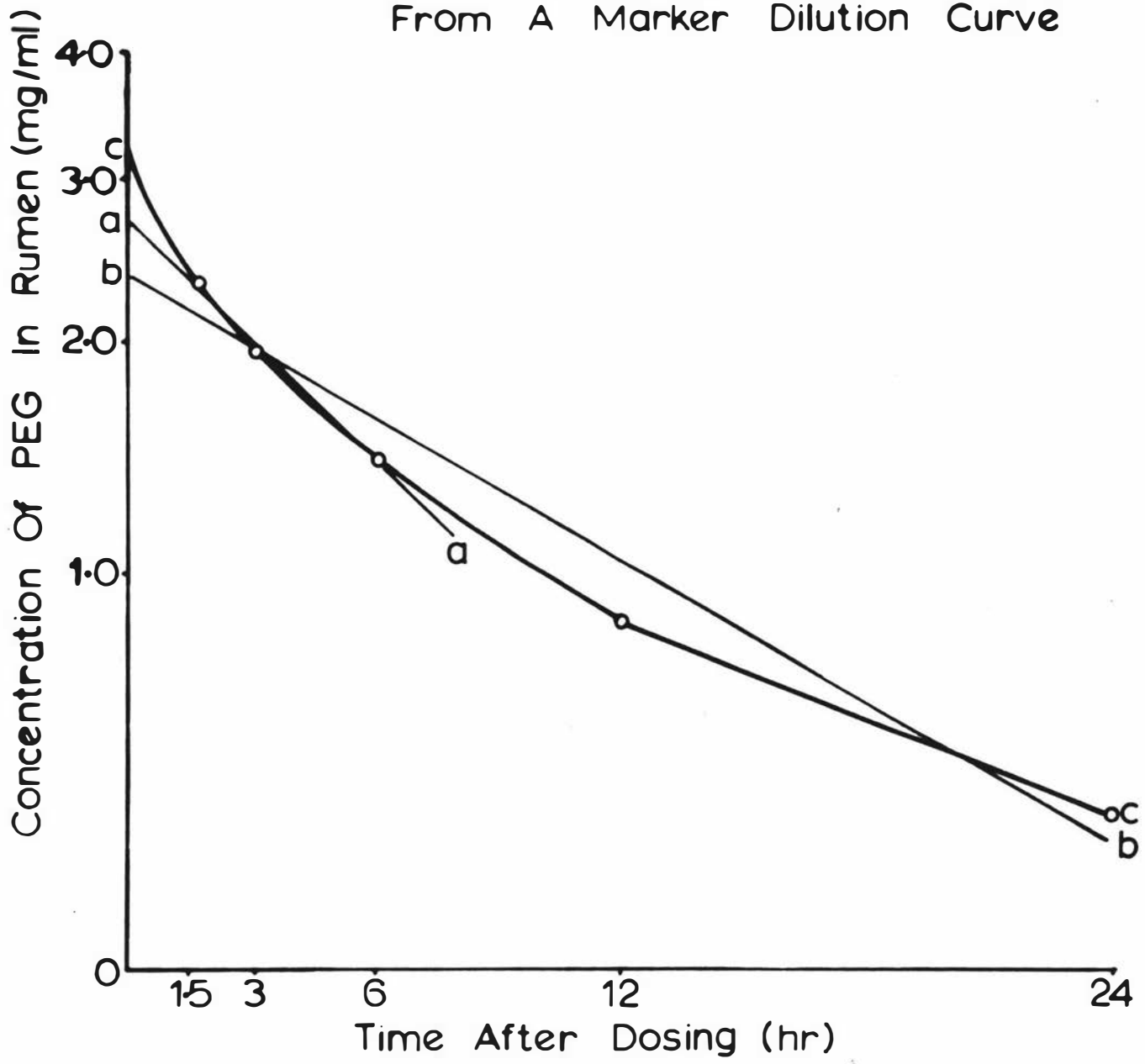
Incubation of PEG with Rumen Liquor

To check whether PEG was metabolised by rumen micro-organisms under the conditions of the present work, a known amount was incubated *in vitro* for 24 hr at 37^0 C with rumen liquor from pasture-fed sheep. Samples were removed at intervals through the incubation period and no loss of PEG was detected.

Source of Data

The data on rumen water volume and the rate of flow of water from the rumen used in this chapter were accumulated during the ryegrass strain trials described in Chapter 2. Five sets of data were obtained, each set comprising three consecutive measurements on each of six sheep. In sets 1 and 2 all animals were on the same treatment, while in sets 3, 4 and 5 three sheep were grazing short-rotation and three perennial ryegrass.

Fig. 4 Determination Of Initial Marker Concentration From A Marker Dilution Curve



RESULTS

1. Rumen Water Volume

If it is assumed that rumen water volume and the rate of flow of water through the reticulo-omasal orifice are constant over the experimental period, and that added marker is mixed rapidly, then the semi-logarithmic relationship between marker concentration and time will be linear. The initial marker concentration, and thus rumen water volume, can be estimated by linear regression. However inspection of the plots of the natural logarithm of concentration against time in the present work showed that in most cases the relationship was not linear but concave. Three ways of estimating initial marker concentration from this curvilinear plot are shown in Fig. 4.

(a) A straight line can be drawn through the first three points (a - a).

(b) All the points can be fitted by linear regression (b - b).

(c) The points can be fitted by quadratic regression (c - c).

This last method is equivalent to the assumption that the trend in flow rate shown between 1.5 and 24 hr started at time zero, which is in fact less restrictive than a straight line fit which assumes constant flow rate from zero time.

Table 1. Estimates of Rumen Water Volumes with their Standard Errors: Calculated from Linear and Quadratic Fits of the First Set of Data (ml)

Sheep No.	Type of Regression	Day		
		1	2	3
1	Linear	5658 ± 2413	5213 ± 2263	4037 ± 360
	Quadratic	3489 ± 342	3391 ± 516	3709 ± 460
2	Linear	5335 ± 2043	4949 ± 1405	4449 ± 973
	Quadratic	3942 ± 265	3747 ± 386	3621 ± 258
3	Linear	4230 ± 1139	4047 ± 992	3894 ± 843
	Quadratic	3974 ± 554	3638 ± 354	3876 ± 340
4	Linear	6332 ± 922	6336 ± 755	5419 ± 342
	Quadratic	4149 ± 590	4927 ± 1018	4170 ± 445
5	Linear	6378 ± 928	5714 ± 959	6113 ± 410
	Quadratic	5439 ± 358	4414 ± 238	5442 ± 369
6	Linear	3615 ± 1716	3380 ± 2044	3423 ±1004
	Quadratic	2215 ± 358	1953 ± 670	2664 ± 557

Table 2. Estimates of Rumen Water Volumes with their Standard Errors: Calculated from Linear and Quadratic Fits of the Data of Hydén (1961) (ml)

Day	Type of Regression	Sheep No.		
		1	2	3
1	Linear	4392 ± 345	4669 ± 183	4916 ± 273
	Quadratic	4506 ± 356	4874 ± 42	4880 ± 305
2	Linear	4458 ± 513	4545 ± 455	4839 ± 222
	Quadratic	4768 ± 508	4918 ± 385	5090 ± 146
3	Linear	3414 ± 158	5143 ± 623	5366 ± 268
	Quadratic	3521 ± 140	4598 ± 271	5307 ± 310
4	Linear	2620 ± 146	5199 ± 282	5209 ± 344
	Quadratic	2671 ± 154	5007 ± 301	5451 ± 355
5	Linear	3657 ± 456	5275 ± 595	6111 ± 242
	Quadratic	3366 ± 353	5945 ± 390	5915 ± 267

Estimates of initial marker concentration from both linear and quadratic regressions were calculated. Rumen water volumes estimated from the first set of data by both these methods are presented in Table 1: these data are typical of the whole series. The standard errors of estimate of initial marker concentration were calculated from both the linear and quadratic regressions and are presented in terms of rumen water volume in Table 1. The quadratic fit always gave a lower estimate of rumen water volume than did the linear regression because it gave a higher estimate of initial marker concentration. In most cases the quadratic regression gave a substantial reduction in the standard error. Similar results were obtained for the other four sets of data and it was obvious that use of quadratic regressions resulted in improved statistical precision in estimating initial marker concentration. For these reasons all estimates of rumen water volume presented in this Thesis were calculated from the quadratic fit of the marker dilution curve.

Hydén (1961) gave no estimate of the error involved in his measurements of rumen water volume, so standard errors of both linear and quadratic fits of data from his Experiment I (page 70) were calculated, and are presented in Table 2. There was no significant

Table 3. Components of Variance and Coefficients of Variation of the Rumen Water Volume Data from the Grazing Sheep

Components of* Variation	Group 1	Group 2	Components of* Variation	Group 3	Group 4	Group 5
s^2_s	846,646	316,251	s^2_t	1,927,090	588,117	763,963
s^2_d	8,944	-	s^2_{st}	46,235	1,017,364	-
s^2_{sd}	-	-	s^2_{dt}	-	1,707,133	-
s^2_m	201,421	228,146	s^2_{dst}	-	-	-
Coefficient of Variation per Sheep	26.9%	29.6%	s^2_m	2,572,937	482,699	971,411
			Coefficient of Variation per Sheep	228,743	436,411	392,014
				14.6%	27.2%	17.8%

* In the above table s = sheep, d = days, t = treatment and m = method.

Table 4. The Standard Errors of Mean Rumen Water Volume as a Percentage of the Overall Mean, for Various Numbers of Sheep per Treatment. Calculated from Data from Grazing Sheep.

Group	No. of Sheep per Treatment						
	2	4	6	8	10	15	20
1	19	13	10	10	9	8	7
2	21	15	12	10	9	8	7
3	10	7	6	5	5	4	3
4	19	14	11	10	9	7	6
5	13	9	7	6	6	5	4
Overall	17	12	10	9	8	6	5

improvement in accuracy from using a quadratic regression. Standard errors calculated from Hydén's data were generally lower than in the present work.

Components of variance for all five sets of data were obtained from an analysis of variance of rumen water volumes and are presented in Table 3. Coefficients of variation of individual rumen water volumes are also presented. The components of variance for all sets of data were pooled and this showed that 41% of the variance in rumen water volume was associated with sheep differences, 32% was due to day to day differences and 27% was intrinsic to the method of measuring rumen water volume. A pooled estimate of the coefficient of variation per individual rumen water volume was 24%.

A similar analysis was conducted on the rumen water volumes derived from the linear fitting of Hydén's (1961) data. In this case 62% of the variance was associated with sheep differences, 24% with the sheep x day interaction, and 14% was intrinsic to the method. The coefficient of variation per individual rumen water volume was 19%.

Standard errors of mean rumen water volume as a percentage of the overall mean, for various numbers of sheep per treatment are given in Table 4. At least eight sheep per treatment would be required to give standard errors less than 10% of the mean rumen water

volume. With this number of sheep, treatment differences of 30% would be statistically significant. Similar figures were calculated from Hydén's data where it was found that four sheep per treatment would give a standard error of less than 10% of the mean.

2. Rate of Flow of Water from the Rumen

The most accurate way of measuring the rate of flow of water from the rumen in short term experiments would be by equation (5) given in the introduction, where account was taken of initial and final rumen water volumes. In some cases in the present work it was not possible to detect PEG in the 24 hr sample so equation (5) could not be used. Therefore, flow rate of water from the rumen was calculated from initial rumen water volume and the average slope of the marker dilution curve in the first 12 hr after dosing. This slope was derived from the relationship:

$$k = \frac{\text{Log}_e (C_o - C_{12})}{12}$$

where C_o and C_{12}, the marker concentrations at times 0 and 12 hr, were calculated from the appropriate quadratic regression equation. This procedure assumes a straight line between 0 and 12 hr, which in most cases was incorrect. The method probably overestimates k over 24 hr because the rate of dilution of marker slows between 12 and 24 hr (Fig. 4).

Water flow from the rumen was calculated in two

ways:

(a) As a percentage of the water volume changing per hr (100 k), which gives a measure of the turnover of water from the rumen.

(b) By equation (3), where u = kV. This gives the absolute flow of water through the reticulo-omasal orifice in ml/hr.

In the current experiments with grazing sheep the mean flow as a percentage of the water leaving the rumen per hr was 22.5% (range 9.3 to 36.3%), while the mean absolute flow rate was 755 ml/hr (range 288 to 1,416 ml/hr). These figures were calculated from 90 observations. In Hydén's (1961) work, where the sheep were fed chopped hay, the mean flow as a percentage of the water leaving the rumen per hr was 6.4%, while the mean absolute flow was 300 ml/hr.

Some observations were made on factors affecting the marker dilution curve in grazing sheep.

(a) Grazing Behaviour

During the experiments the following approximate pattern of grazing behaviour was observed in the sheep:

8.00 AM - 10.00 AM	Removed from pasture prior to PEG dosing.
10.00 AM	Dosed with PEG and returned to pasture.
10.00 AM - 12.00 Noon	Intensive grazing.

Table 5. The Effect of Rain on the Third Day of Measurement, on Rumen Water Volume and Flow Rate in Grazing Sheep

Day	Variable	Sheep No.					
		1	2	3	4	5	6
1	Rumen Water Volume (ml)	4868	3766	4581	6926	8130	4111
	Flow Rate (a) Vol %	21.76	22.26	22.79	16.70	10.60	21.32
	(b) ml/hr	1059	838	1044	1157	862	876
2	Rumen Water Volume (ml)	4942	4454	3586	5183	7677	3878
	Flow Rate (a) Vol %	18.65	17.55	23.81	18.88	15.48	20.56
	(b) ml/hr	912	782	848	961	1068	789
3	Rumen Water Volume (ml)	2887	2490	1612	4873	3088	2172
	Flow Rate (a) Vol %	23.14	26.50	36.27	29.05	27.76	30.43
	(b) ml/hr	668	660	586	1416	857	661

12.00 Noon - 3.00 PM Grazing and resting.

3.00 PM - 6.00 PM Period of greatest grazing activity.

6.00 PM - 6.00 AM (approx). Intermittent grazing and resting.

6.00 AM - 8.00 AM Intensive grazing.

The period of greatest grazing activity (10.00 AM - 6.00 PM) coincided with the period in which marker was flowing most rapidly from the rumen (Fig. 4). Thus the grazing behaviour of the sheep could have contributed to the shape of the marker dilution curve.

(b) Starvation

If an animal was given only one feed per day, or if it was starved overnight, the flow rate of water from the rumen was rapid while food was available but decreased as the length of starvation increased. Similar results were described by Hydén (1961).

(c) The Effects of Rain

In one experiment steady rain was experienced on the third day of PEG dosing and sampling: this had a marked effect on both rumen water volume and flow rate (Table 5). Rumen water volume was reduced on the third day while the percentage of water leaving the rumen per hr was increased. In all cases but one the absolute flow rate of water from the rumen was decreased.

3. Relationship Between Absolute Water Flow and the Flow of Organic Matter

The current experiments provide little information on this important subject because the animals were grazing and no direct measurement of food intake could be made. However in three sets of data (numbers 3, 4 and 5) total faecal collections were made for 10 days prior to the marker experiments. There were no differences in pasture digestibility within each set of data (Chapter 2) so faecal organic matter was taken as a guide to intake. A correlation of 0.55 ($P < .05$) was calculated between mean absolute rate of flow of water and total faecal organic matter production for each sheep.

DISCUSSION

The substance PEG appears to fulfil the criteria required of a reference substance for measuring alimentary water and has been used as such by several workers (Corbett *et al.*, 1959; Smith, 1959; Gray, Jones and Pilgrim, 1960; Hydén, 1960, 1961; Oyaert and Bouckaert, 1961; Sutton, McGilliard and Jacobson, 1962; Weller, Pilgrim and Gray, 1962; Jacobson *et al.*, 1963). PEG is not toxic, not absorbed, not metabolised by rumen micro-organisms, its distribution volume in rumen digesta is approximately 95% of total water, and it can be accurately estimated if care is taken when clarifying the rumen liquor (Sperber *et al.*, 1953; Hydén, 1956a, 1956b, 1960, 1961; Smith, 1958, 1959). In the present work the chemically clarified rumen liquor was centrifuged at 15,000 x *g* for 10 min and no trouble with interfering substances was encountered after this procedure was adopted.

A small amount of a PEG dose was found to be adsorbed on or retained by the intestinal mucosa of rats by Jacobson *et al.* (1963). It is not known whether this occurs in the rumen and impairs the accuracy of PEG as a rumen water volume marker, but it might explain the small loss in faecal recovery noted by Hydén (1956b) and Corbett *et al.* (1958).

Although PEG appears to be a satisfactory marker

the main problems in its use are associated with theoretical aspects of the marker method of determining rumen water volume and flow rate. These problems would apply to any marker. The two assumptions, that rumen water volume and the rate of flow of water from the rumen remain constant over the experimental period, were not valid in the present work under grazing conditions. Rumen water volume changed from day to day (32% of the variation in rumen volume being associated with day differences), and the semi-logarithmic plot of the marker dilution curve was curvilinear indicating that flow rate changed during the day. Quadratic regressions were fitted to the data and these reduced the error involved in estimating initial marker concentration. In the current experiments the desire to measure water flow rate as well as rumen water volume probably reduced the efficiency of estimating the latter. If rumen volume alone were required the best procedure would be to collect samples at frequent intervals over the first 6 hr after dosing.

A major criticism of the present work must be that although the statistical error of estimating rumen water volume was reduced by fitting the marker dilution curves by quadratic regression, it is not known how accurately the marker technique measured actual rumen water volume. It was not possible to compare rumen

water volume estimated by the marker technique with a direct measurement of rumen water volume. There was no point in dosing PEG, obtaining a dilution curve, and then slaughtering the animal to obtain a direct measurement, because rumen water volume could change during the time lag. The only way to compare direct and indirect measures of rumen water volume is to empty the rumen of a live sheep through a rumen fistula, measure the water volume of the contents, add PEG to the contents and return them to the sheep, then measure the subsequent rate of removal of PEG from the rumen. This can be done with a cow but the Jarrett-type of rumen cannula in sheep is too small. Large rumen fistulae can be prepared in sheep but the physiological normality of rumen function in such preparations must be questioned. Thus an impasse is reached: there is no certainty that the PEG marker technique as used in the present work measured absolute rumen water volume. However the technique is probably adequate for comparative purposes such as in Chapter 2.

Rumen water volumes estimated in grazing sheep in the present work were compared with Hydén's (1961) estimates where the sheep were fed indoors under controlled conditions. In Hydén's work the semi-logarithmic relationship between PEG concentration and time was linear and initial PEG concentration was

estimated with greater precision than in the present work: the error intrinsic to the method was 14% for Hydén's work and 27% for the grazing sheep, while the coefficient of variation of an estimate of rumen water volume was 19% for Hydén and 24% for the present work. Reasons for the greater error involved in measuring rumen water volume in grazing sheep can be suggested:

(a) Environmental Variations

In an effort to approach "steady state" feeding conditions Hydén's sheep were housed indoors under continuous artificial lighting and with free access to food and water.

Under grazing conditions the sheep were subject to normal environmental fluctuations, and, as the observations on grazing behaviour showed, they did not spread their eating evenly through the day. This uneven grazing probably contributed greatly to the curvilinear nature of the semi-logarithmic plot of marker concentration against time.

Extremes of environment could have influenced the type of result obtained. For example, rain caused a reduction in rumen water volume and an increase in the turnover of water from the rumen (Table 5). Other environmental factors such as high winds or cold could cause variations in rumen water volume and flow rate measurements.

These results show that under grazing conditions more than one measurement of rumen water volume and flow rate must be made and that such measurements should be made in similar environmental conditions.

(b) Type of Feed

Hydén's sheep were fed on chopped hay so most of the "exogenous" water entering their rumens must have come from drinking water.

In the present studies the sheep were grazing pasture which would be of higher digestibility and water content than chopped hay. Thus any variations in grazing behaviour would be expected to have a greater effect on the amount of "exogenous" water reaching the rumen in grazing sheep. Further, the rate of flow of water from the rumens of pasture-fed sheep was three times as great as in Hydén's animals. These differences in rate of flow of water between the present work and Hyden's no doubt contributed to the greater error involved in measuring rumen water volume in pasture-fed sheep.

The finding that under grazing conditions at least eight sheep would be required per treatment to produce a standard error of less than 10% of mean rumen water volume (Table 4), has considerable practical importance. In Chapter 2 where only three sheep are used per treatment a difference of at least 45% between

treatment means would be required for the results to be statistically significant.

It is difficult to equate the rate of flow of water from the rumen with the flow of digesta through the digestive tract. Many of the substances passing through the reticulo-omasal orifice will be water soluble, but the flow of solid particles is dependent on physical characteristics such as particle size and specific gravity (Balch, 1960). A comparison of the rates of passage of chromium oxide and PEG through the digestive tracts of cows was made by Corbett et al. (1958, 1959). They found that the passage of PEG was faster than chromium oxide and concluded that PEG was probably associated primarily with the liquid fraction of the digesta and chromium oxide with the solid fraction. Hydén (1961) thought that increased food consumption should influence the rate of flow of water but he could provide no clear indication of a relationship. In the present experiments absolute flow of water from the rumen was related to faecal organic matter production and a correlation of 0.55 obtained. This suggests that there is some relationship between the rates of flow of water and dry matter through the digestive tract.

Initial marker concentration and the slope of the marker dilution curve are not determined independently. Further, the relationship between rumen water

volume and absolute flow rate is a part-whole one. Thus in the present study there is a mathematical relationship between both measures of water flow rate and rumen water volume. These variables require independent measurement to determine whether they are related physiologically.

SUMMARY

1. PEG was studied as a marker substance for estimating rumen water volume and the rate of flow of water from the rumen in grazing sheep.

2. Although PEG appeared to fulfil the requirements of a marker substance the main problems in its use were associated with theoretical aspects of the marker method: the assumptions that rumen water volume and flow rate are constant over the experimental period were not valid under grazing conditions.

3. With grazing sheep the semi-logarithmic plot of marker concentration against time was curvilinear and so was fitted by quadratic regression. This procedure increased the precision of estimating initial marker concentration.

4. The results from grazing sheep were compared with those of Hydén (1961) whose animals were fed chopped hay and kept under constant environment conditions. The error involved in measuring rumen water volume and flow rate in grazing sheep, which were subjected to normal environmental fluctuations, was higher than under the controlled conditions of Hydén (1961).

5. Problems associated with using PEG under grazing conditions are discussed.

CHAPTER 2

A COMPARISON OF THE NUTRITIVE VALUE TO GRAZING SHEEP OF PERENNIAL AND SHORT-ROTATION RYEGRASSES

Table 6. Treatment Means and Standard Errors for Carcass and Rumen Characteristics of Ewes Killed December 1960 (n = 8 ewes/treatment). (Compiled from Barton and Ulyatt (1963) and Johns et al. (1963)).

Item	P	P + C	S	S + C	S.E. of Mean	Significance Level
Liveweight at Slaughter (lb)	97.3	124.6	120.3	136.7	3.65	* *
Frozen Carcass Weight (lb)	40.9	59.2	61.2	69.1	2.42	* *
Water Weight (lb)	21.45	27.82	26.34	31.35	0.84	* *
Fat Weight (lb)	11.27	21.21	25.03	26.07	1.41	* *
Protein Weight (lb)	6.44	7.59	7.56	9.14	0.24	* *
Ash Weight (lb)	1.70	2.53	2.28	2.54	0.08	* *
Weight of Rumen Contents (lb)	11.7	10.5	8.1	8.1	0.6	* *
Volatile Fatty Acid Concentration in Rumen Liquor (mM/100 ml)	11.8	13.4	12.3	14.7	0.6	* *
Proportions of Individual Volatile Fatty Acids:						
Acetic Acid	74.5			61.4	2.2	* *
Propionic Acid	20.7			25.3	1.4	* *
n - Butyric Acid	4.8			12.3	1.6	* *

(* * = $P < .01$)

INTRODUCTION

In a series of experiments conducted at Paddock 16, Massey University, the nutritive value to sheep of short-rotation (S) and perennial (P) rye-grasses, with and without white clover (C), was examined (Rae, Barton and Ulyatt, 1961; Johns, 1962). Live weight gains were produced in sheep grazing these pastures in the order, S + C $>$ S $\simeq$ P + C $>$ P, and it was considered that there were two main effects: a difference between S and P; and a difference caused by the addition of C to each of the grasses (Ulyatt, 1960; Rae et al., 1963). There were differences in carcass weight, and also treatment variations in body composition, with the components of composition generally following carcass weight: S + C animals had the highest fat, water, protein and ash content and P animals the least (Barton and Ulyatt, 1963). The results of one experiment are summarized from Barton and Ulyatt (1963) and Johns et al. (1963) in Table 6. Johns et al. (1963) showed that there was a negative correlation between live weight and rumen "fill": the S + C animals grew faster than those on P yet had less rumen "fill". The concentrations of VFAs in rumen liquor were determined at slaughter in one experiment and found to follow a similar pattern to that in live weight, i.e., S + C $>$ P + C $>$ S $>$ P.

The clover effect on VFA concentration in rumen liquor was greater than the difference between the two grasses. The proportions of individual VFAs in rumen liquor from the treatment extremes were examined and S + C was significantly lower in acetic acid and higher in propionic and butyric acids than P. Rumen papillae size was determined visually and the same treatment order as in live weight was observed.

McLean, Thomson, Jagusch, and Iversen and Lawson (1964) at Lincoln College have been able to confirm the above results under South Island conditions.

In the review that follows the above observations will be considered in the light of present knowledge of the chemical composition of ryegrasses and white clover and their metabolism by the ruminant.

1. <u>The Effect of the Chemical Composition of Ryegrasses and Clover on the End-products of their Ruminal Digestion</u>

(a) <u>Carbohydrates</u>

Bailey (1964) has investigated the carbohydrate composition of S, P and C over several seasons. The major difference between P and S was in cellulose content: P was significantly higher than S. There was a trend towards higher soluble sugar content in S but this was not as marked as the cellulose difference. There were no significant differences between P and S

in other carbohydrate fractions. C was found to contain approximately the same level of sugars as the ryegrasses but considerably more water soluble polysaccharide and pectin. C also had lower hemicellulose and cellulose fractions than the ryegrass species. Thus the ratio of soluble to structural carbohydrate is much higher in C than in either P or S.

In the rumen, plant carbohydrates are fermented by micro-organisms at rates which depend largely on their solubility. In general, degradation of the insoluble carbohydrates produces predominantly acetic acid while fermentation of the soluble carbohydrates leads to proportionately more propionic and butyric acids (see Oxford (1958) for a review). There are reported instances where differences in the carbohydrate fractions of ryegrasses have been associated with differences in rumen VFA proportions (Armstrong, 1960; Tilley, Deriaz and Terry, 1960). A logical explanation of the differences in rumen VFAs found by Johns et al. (1963) can also be provided in this manner. The most pronounced effect on VFA concentration was caused by the addition of C (Table 6), which was not surprising in view of the high ratio of soluble carbohydrate to cellulose found in C compared to either of the ryegrass species.

(b) Lignin

Bailey (1964) found no significant differences in lignin between S and P. Lignin is not digested to any extent by the ruminant but high lignification of hemicellulose and cellulose could slow the rate of fermentation of these constituents.

(c) Nitrogenous Fractions

The nitrogenous fractions of the plant can be divided into non-protein nitrogen (NPN) and protein fractions. NPN is a fraction of great complexity and includes many substances of nutritional significance to the animal, such as peptides, amino acids, amines and nitrate. The NPN fraction of New Zealand ryegrass varieties has been shown to fluctuate markedly in apparent relation to seasonal and environmental influences (Johns, 1955; Bathurst and Mitchell, 1958; Butler, 1959; Bailey, 1964). Some components of the NPN fraction, particularly nitrate, can have toxic effects on ruminants when present in high concentrations (see Garner (1963) for a review).

Lyttleton (pers. comm.) found that the type of protein present in ryegrasses, as determined by electrophoresis, showed little seasonal variation.

The metabolism of nitrogenous compounds in the rumen has been reviewed recently by Lewis (1960) and McDonald (1962). The concentration of ammonia in

rumen liquor is an important guide to nitrogen metabolism in the rumen. It indicates the balance between production and removal: production by the degradation of feed protein and NPN, and removal by incorporation into microbial protein, by absorption, and by passage out of the rumen. In fact McDonald (1962) suggested that the nutritive value of dietary nitrogen was determined largely by the production and subsequent fate of ammonia. However the metabolism of nitrogenous compounds in the rumen is influenced by the solubility and type of protein and carbohydrate present in the feed (Lewis, 1960). For example, the presence of adequate soluble carbohydrate ensures that nitrate is reduced completely and rapidly to ammonia. If soluble carbohydrate levels are low nitrite may accumulate in the rumen and be absorbed into the blood stream, and this can lead to severe toxicity.

2. Utilization of the End-products of Ruminal Digestion of Ryegrasses and Clover

The calorimetric efficiencies of the VFAs for lipogenesis in sheep were determined by Armstrong and Blaxter (1957b), Armstrong, Blaxter, Graham and Wainman (1958) and Blaxter (1960). Efficiency of utilization for lipogenesis depended on molecular weight and was in the order: butyric > propionic > acetic. These workers also showed that the efficiencies of mixtures

of these acids for lipogenesis depended on the sum of the efficiencies of the acids comprising the mixture.

The differences in live weight gain observed in animals on the ryegrass strain trials could be explained on this basis. Digestion of S + C produces a higher ruminal concentration of VFAs and higher proportions of the more efficiently utilized propionic and butyric acids than did digestion of P (see Table 6).

3. The Significance of Rumen "Fill"

The nutritional significance of rumen "fill" is not fully understood but "fill" is known to vary with: body weight (Johns et al., 1963), pregnancy (Mäkelä, 1956, Graham and Williams, 1962), weight of internal fat depots (Mäkelä, 1956; Tayler, 1959), diet (Blaxter et al., 1956; Flatt et al., 1959; Campling et al., 1961; Johns et al., 1963) and lactational stress (Wyatt and Barton, 1964).

4. Intake and Rate of Passage of Food

The possibility that the higher live weight gains of sheep grazing S were due to a greater intake of that grass must be considered. A higher food intake by the S animals, which had less rumen "fill", would imply a faster rate of flow of digesta through their digestive tracts. McLean et al. (1964) could find no difference in intake between sheep grazing S and P but their measurement technique was not satisfactory.

Methods for measuring the feed intake of grazing ruminants have been reviewed by Reid and Kennedy (1956) and Raymond, Minson and Harris (1956) and are based on the relationship:

$$\text{Intake} = \text{Faecal Production} \times \frac{100}{100 - \text{Digestibility \%}}$$

Faecal production can be measured directly by bagging the animals or it can be estimated indirectly using an indigestible marker such as chromium oxide (Reid and Kennedy, 1956). The most commonly used method of determining the digestibility of grazed pasture is the faecal index method. Digestibility is estimated from the chemical composition of the faeces using regressions derived from indoor digestibility experiments. Faecal nitrogen (Lancaster, 1954) and chromogen (Reid, Woolfolk, Hardison, Martin, Brundage and Kaufmann, 1952) have been used regularly for this purpose. Numerous faecal index regressions have been published and these have shown that there are differences within the original relationship caused by season, plant species, region and other variables (Minson and Raymond, 1957). Further, Lambourne and Reardon (1963) estimated that approximately 75% of the error associated with estimating intake this way could be attributed to the faecal index regression. A better procedure, but one involving more work, is to conduct a digestibility trial indoors every time intake is to be measured in

the field. The error involved in assuming digestibility determined indoors is the same as that for the grazing animal is not likely to be as large as the error involved in estimating digestibility from a faecal index regression.

From the above considerations it is thought that four possibilities could account for the observed differences in production of sheep grazing S and P:

(a) That there was a greater intake of S than P;

(b) That S was utilised more efficiently than P;

(c) A combination of (a) and (b);

(d) That P could have contained toxic or growth inhibitory substances.

The latter possibility seems unlikely as guinea pigs were fed both grasses by Ulyatt (1962) and they showed greater live weight gains on P than on S. From this result it appeared that the observed differences in sheep production were associated with the ruminant mode of digestion.

As stated previously, the observed differences in animal characteristics may be partitioned into two major effects: a grass effect and a clover effect. It was considered that the basic difference in previous experiments was between P and S and that C was having an additive effect to each grass. Therefore in the experiments to be described the nutritive value to

sheep of only P and S was studied. An attempt was made to determine how much of the differences in live weight were due to food intake and how much were due to differences in the efficiency of utilization of the grasses.

Fig. 5 Experimental Area, K Block

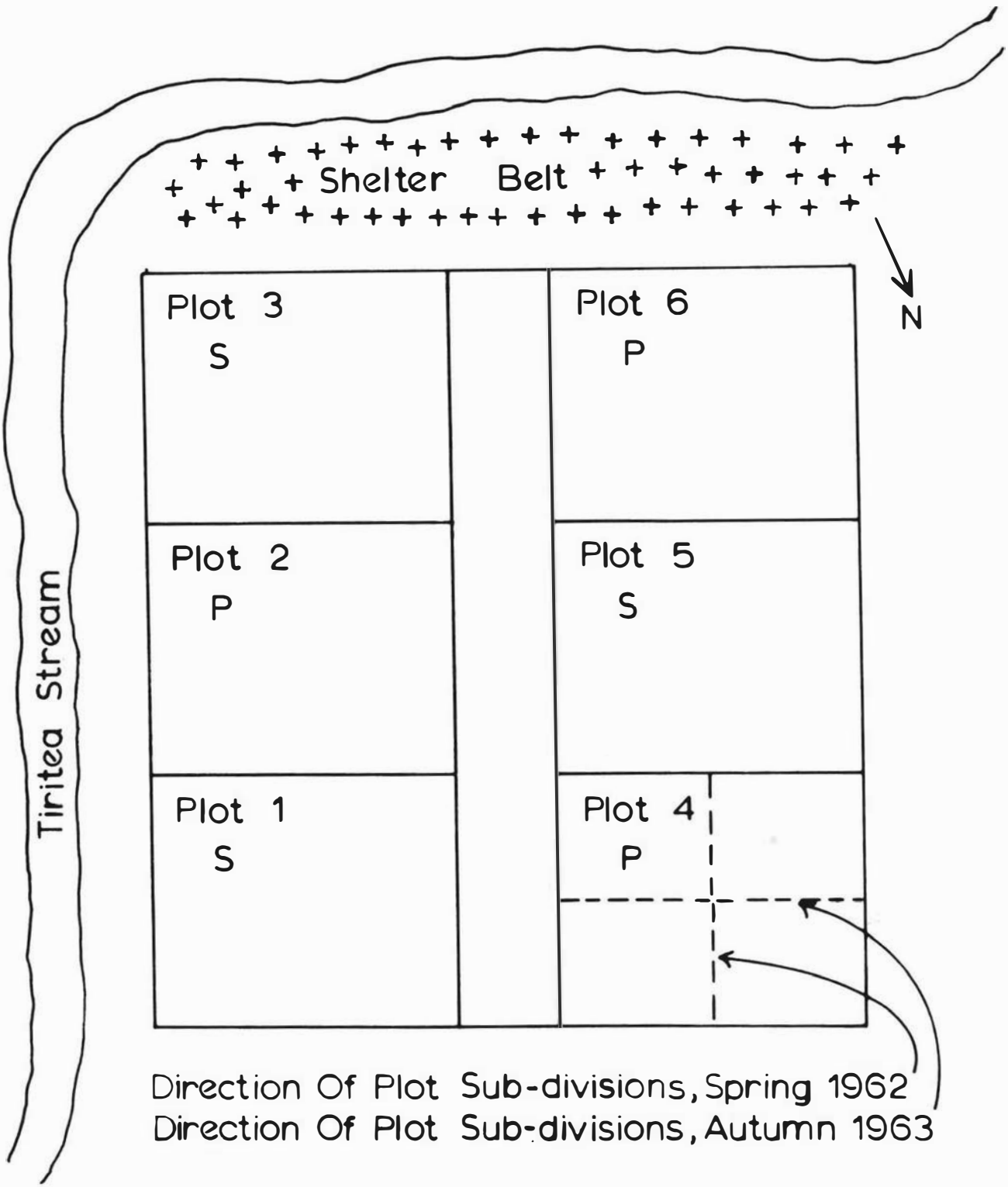


Table 7. Botanical Composition of K Block Pastures (DM Basis). (Each Figure is the Mean of Three Plots)

	Experiment 1		Experiment 2	
	S	P	S	P
Ryegrass Species	94.8	97.0	97.9	98.0
Poa Species	4.6	2.5	1.8	1.7
Bromus mollis	0.1	0.5	0	0
Other Species	0.5	Trace	0.3	0.3
Sample Date	16 October 1962		3 May 1963	

EXPERIMENTAL METHODS

1. Experimental Area

The experimental area of one acre, which was of a recent alluvial soil type, was situated at D.S.I.R., Palmerston North, and known as K Block. The area was cultivated in January 1962 and again in March 1962. Prior to sowing the area was topdressed with a mixture comprising 2 cwt superphosphate, 1 cwt blood and bone and 1 cwt sulphate of ammonia. The six individual plots were sown by hand on 19 March 1962 as shown in Fig. 5: plots 1, 3 and 5 with S at 30 lb per acre, plots 2, 4 and 6 with P at 40 lb per acre and the central race with P. Subdivision fences were erected soon after the grass had germinated. A second area of land adjacent to K Block was used as common grazing during the experiment. This land was permanent pasture of long standing, the dominant species being P and C.

The pastures on K Block were grazed or topped with a mower when necessary, and by September 1962 quite pure swards had been established as can be seen from botanical analyses figures in Table 7. As pure swards uncontaminated by clover were required for the experiments it was necessary to apply nitrogenous fertilizers to maintain soil nitrogen status. Sears (1949) claimed that clovers contributed the equivalent

Table 8. Fertilizer Applications During Experiments 1 and 2

Date	Fertilizer	Amount per Acre
26 May 1962	Nitrolime	2 cwt
26 May 1962	Muriate of Potash	1 cwt
18 June 1962	Sulphate of Ammonia	2 cwt
28 July 1962	" " "	1 cwt
29 August 1962	" " "	1 cwt
14 September 1962	Nitrolime	1 cwt
4 November 1962	Sulphate of Ammonia	1 cwt
9 December 1962	Nitrolime	2 cwt
11 January 1963	Nitrolime	1.25 cwt
28 February 1963	DDT Superphosphate	2 cwt
11 April 1963	Sulphate of Ammonia	1 cwt

of approximately 1 ton of nitrogenous fertilizer per acre per annum in a high producing pasture. In the present work it was decided to apply approximately 1 cwt of sulphate of ammonia or its equivalent per acre per month. Because of the importance of nitrogenous fertilizers to the results of these experiments, the rates of application for both experiments are detailed in Table 8.

In late December 1962 the S plots became badly infested with Argentine stem weevil (Hyperodes bonariensis). Neither irrigation nor spraying with Lindane controlled the infestation and all the grass on plot 3 and half that on plots 1 and 5 was destroyed. The S plots were therefore oversown on 28 February 1963 using a disc drill and a seeding rate of 60 lb per acre. The area was irrigated and the new grass became quickly established (see Table 7).

2. Animals

Adult Romney Marsh wethers with rumen fistulas prepared by the technique of Jarrett (1948) were used throughout the pasture grazing experiments. These animals were accustomed to frequent handling and were trained to wear harnesses. Non-fistulated Romney Marsh wethers were used for the digestibility trials. These sheep were trained to wear harnesses and to being confined in metabolism crates.

Fig. 6. The Holding Pen and Type of Harness Used in the Ryegrass Comparison

To facilitate handling of the fistulated sheep for rumen sampling and faecal collections, temporary pens, which utilized the plot gates, a light hurdle and a portable headstock, were erected in the central race of K Block (Fig. 6).

It was found necessary to erect an 18 in high single strand electrified wire round the perimeter of each plot to prevent the fistulated sheep from catching their cannulae on the netting fences. The area between the electrified wire and the permanent fence was sprayed with a long-acting herbicide, Vorox-SDA (Ivon Watkins Ltd.), to stop the animals foraging beneath the wire. This measure was very successful and completely eliminated the loss of cannulae during the experiments.

3. Experimental Design

(a) Experiment 1

On 27 August 1962 six fistulated wethers were introduced to a ryegrass and clover pasture for a period of common grazing. From 10 to 12 September rumen water volume and flow rate measurements were made on these animals using the polyethylene glycol (PEG) marker technique described in Chapter 1. The six animals were then divided randomly into two groups of three and introduced to K Block on 13 September: one group going to P and the other to S. During the following

7 weeks the sheep were rotationally grazed around the replications on K Block within their respective treatments. After 2 weeks of this period, each plot on K Block was subdivided with a temporary fence and for the remainder of the experimental period one half of each plot was grazed while the other half was mown to grazing height with a Dennis reel mower. During the last 3 weeks of the experimental period the fistulated sheep were harnessed and faeces were collected while at the same time a digestibility trial was conducted indoors on grass from the mown areas of K Block. On 23 and 25 October the fistulated sheep were starved overnight and put out to graze the following day when samples of rumen liquor were collected for fermentation studies. From 29 to 31 October the animals were dosed with PEG and rumen samples collected for rumen water volume and flow rate determinations. On 1 November rumen liquor samples were collected for estimating ammonia concentrations. This phase of the experiment ended on 2 November when harnesses were removed and a final live weight recorded.

Treatments were then reversed, the three sheep on P going to S and vice versa. The sheep continued to graze around the replications on K Block within treatments until 23 November when harnesses were again attached and faeces collected from 28 November to

7 December. It was not possible to conduct a parallel digestibility trial because the experimental area was too small to produce sufficient grass at this time of the year. Samples of rumen liquor were collected for ammonia determinations on 11 December. From 12 to 14 December the animals were dosed with PEG and rumen samples collected for rumen water volume and flow rate determinations. Live weights were measured regularly during the experiment. The experiment was finished on 15 December.

(b) Experiment 2

The fistulated sheep were grazed on a mixed ryegrass and clover pasture from 16 December 1962 until 12 April 1963. From 9 to 11 April they were dosed with PEG and rumen samples collected for rumen water volume and flow rate measurements. Six of these sheep were placed on K Block on 12 April: three on P and three on S. The six plots of K Block were again temporarily subdivided, this time in the opposite direction to the first experiment (Fig. 5). One half of each plot was mown to grazing height while the other halves were grazed in rotation by the experimental sheep. A digestibility trial was conducted indoors on grass from the mown areas from 7 to 11 May and the fistulated sheep on K Block were harnessed and faeces collected over the same period. From 15 to 17 May the fistulated sheep were

dosed with PEG and rumen samples collected for rumen water volume and flow rate determinations. Rumen fermentation studies were conducted on 14 and 23 May and on the latter date samples were also collected for rumen ammonia determinations. Live weights were recorded regularly and the experiment finished on 23 May 1963.

4. Digestibility Trial and Faecal Collection Techniques

A digestibility trial comprising 7 days pre-feeding, 10 days faecal collection and using three wethers per grass was conducted from 3 to 19 October 1962. The sheep were harnessed and equipped with faecal bags (Fig. 6) and were housed in individual pens which contained a feed bin and water tank.

The management of the mown areas of K Block was aimed at producing grass of grazing height, i.e., approximately 2 in long, for indoor feeding. In practice this became very difficult as there was insufficient area to provide enough grass at this height to satisfy the needs of the digestibility trial. The grass for feeding indoors was allowed to grow to a height of approximately 3 in and was thus not strictly comparable to the grass the fistulated sheep were grazing.

Grass for the digestibility trial was cut twice a day at 8.00 AM and 4.00 PM with a Dennis reel mower.

After cutting, the grass for each sheep was weighed into a tared plastic bag and the feed boxes replenished from this as required throughout the day. A sample of each cut of grass was dried in a forced draught oven at 90°C for 24 hr and the dried samples bulked for each grass over the 10 day trial period. Each day the whole feed refusal was dried and weighed. A refusal of 15% was aimed at.

Faecal collections from both the indoor and outdoor sheep were made three times daily, at 8.30 AM, 4.30 PM, and 8.30 PM, and the total wet faeces for each animal bulked for the day. Aliquots of 10% were weighed and dried at 90°C for 24 hr. The dried aliquots were bulked for each animal over the 10 day collection period.

A further 10 day faecal collection from the grazing sheep was made from 28 November to 7 December 1962. The procedures followed were the same as outlined above.

A second digestibility trial, again with three wethers per grass, was started on 1 May 1963. This trial was conducted in exactly the same manner as the first except that the sheep were housed in metabolism crates and only 7 days prefeeding followed by 5 days faecal collection was possible because of a shortage of grass.

Table 9. Diurnal Variation in the DM % of Rumen Contents

Sheep	Sampling Time			
	8.00 AM	1.00 PM	5.00 PM	10.00 PM
1	8.00	8.64	11.70	10.98
2	13.04	13.15	15.53	14.71
3	13.98	13.86	17.46	13.93

Dried, bulked, grass and faeces samples from both trials were finely ground in a Wiley mill and stored in tightly stoppered jars. To allow calculation of results on an organic matter basis, faeces and grass samples were ashed at 600°C for 4 hr. Faecal nitrogen was determined by a semi-micro Kjeldahl method using a selenium-potassium sulphate catalyst (Humphries, 1956).

When digestibility trial results were calculated a lag of 2 days was allowed between intake and faecal measurements in an attempt to account for the time taken for food to pass through the digestive tract.

5. Rumen Water Volume and Flow Rate Measurements

The procedures followed in using PEG as a marker have been described in Chapter 1.

6. Total Rumen Dry Matter Content

If it is assumed that rumen water volume remains constant during the day an approximation to total rumen DM content can be calculated from the relationship:

$$\frac{\text{DM\%}}{\text{Water \%}} = \frac{\text{Total rumen DM (g)}}{\text{Total rumen water (ml)}}$$

Diurnal variation in rumen DM % was checked using three sheep grazing a ryegrass-white clover pasture. Samples of rumen contents were collected from just below the fistula at 8.00 AM, 1.00 PM, 5.00 PM and 10.00 PM, and dried for 24 hr at 90°C. These results are shown in Table 9. There were significant differ-

ences between sheep ($P < .01$) and between sampling times ($P < .01$). Under the grazing conditions prevailing the highest rumen DM % was attained late in the afternoon. This was observed to coincide with the period of greatest grazing activity. In the present work a rumen sample was collected for PEG purposes at 4.15 PM each day, and so a DM sample was obtained at the same time. This allowed total rumen DM content to be estimated.

7. Rumen Fermentation Studies

(a) In vivo

Sheep were starved overnight prior to rumen fermentation studies. They were returned to pasture immediately after the first sampling at 9.00 AM and subsequent samples of rumen contents were collected at 11.00 AM and 3.00 PM. These samples were expressed through muslin to remove plant debris and the resulting rumen liquor was acidified with 10 N H_2SO_4 saturated with $MgSO_4$ in the proportions 1 ml of acid to 5 ml of rumen liquor. Six ml samples of acidified rumen liquor were steam distilled in a Markham apparatus and the distillate (80 ml) titrated against 0.1 N NaOH to give total VFAs. The distillate was then evaporated to dryness, redissolved in a few drops of distilled water, and the proportions of individual VFAs determined by gas-liquid chromatography using the method of James and

Martin (1952).

(b) In vitro

The Warburg-type fermentation apparatus described in Chapter 4 was used to compare S and P juices. Substrates were prepared the day before each experiment, by expressing juice from S and P with a "Protosaser" (E. H. Bentall and Co. Ltd.). The juices were cooled and kept at a temperature of 4°C until required. Experimental design in all cases was as follows:

Flask 1: 40 ml S rumen liquor plus 10 ml phosphate buffer.

Flasks 2 and 3: 40 ml S rumen liquor plus 10 ml S juice.

Flask 4: 40 ml P rumen liquor plus 10 ml phosphate buffer.

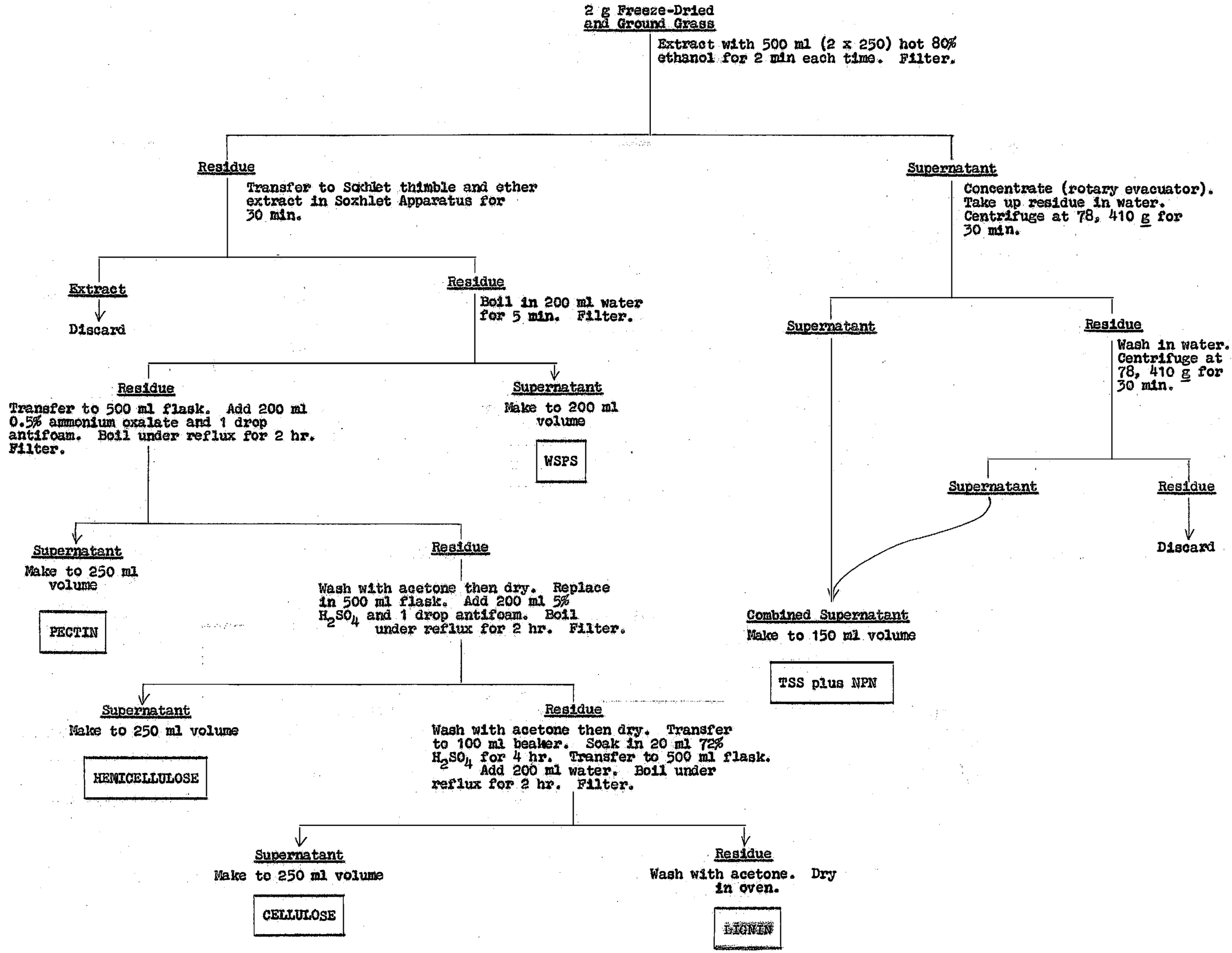
Flasks 5 and 6: 40 ml P rumen liquor plus 10 ml P juice.

The flasks were incubated in a water bath with continuous agitation for 2 hr at 37°C and gas production over this period was measured. At the end of each experiment 25 ml samples of the contents of each flask were acidified and stored for VFA analysis.

8. Ammonia in Rumen Liquor

Ammonia concentration in rumen liquor was determined by the method of McDonald (1958) using the micro-distillation apparatus of Conway (1957). No allowance was made for de-amination as this was found to be insignificant by Bryant (pers. comm.).

Fig. 7. Carbohydrate Analysis of Ryegrasses



9. Chemical Analysis of Ryegrass

All grass samples were freeze-dried and then ground through the 1 mm mesh of a Casella grain mill.

(a) Carbohydrate Fractions

The method of carbohydrate analysis was that of Bailey (1964) and involved a stepwise hydrolysis of the grass sample as indicated in Fig. 7. The analytical procedures used were as follows:

(i) Total Soluble Sugars (TSS). (Mono- and oligo-saccharides). Estimated by the method of Bath (1958). One ml of solution TSS (Fig. 7) was diluted to 100 ml with water and to 1 ml of this solution was added 6 ml of concentrated H_2SO_4. The resulting solution was heated on a boiling water bath for 6 min. Optical density was measured with a spectrophotometer at 322 mμ and compared with a standard solution of glucose.

(ii) Water Soluble Polysaccharides (WSPS). (Starch and fructosan). Estimated by the method of Bath (1958). Ten ml of solution WSPS (Fig. 7) was taken and diluted to 25 ml with water and 1 ml of this solution was put through the same procedure as TSS.

(iii) Pectin. Estimated by the method of Bath (1958). One ml of the pectin solution (Fig. 7) was put through the same procedure as TSS and compared

with a standard of galacturonic acid in a spectrophotometer at 301 mμ.

(iv) Hemicellulose. Method of Nelson (1944). Ten ml of the original solution (Fig. 7) was taken and neutralized with approximately 8 ml N NaOH using phenolphthalein as indicator. This neutralized solution was made to 100 ml with water and to 1 ml of this diluted solution was added with shaking 1 ml of mixed Nelson's reagent. This mixture was heated in a 25 ml graduated tube on a boiling water bath for 20 min and then 1 ml of arseno-molybdate solution was added with shaking. Optical density was measured in a spectrophotometer at 520 mμ and compared with a standard of glucose.

(v) Cellulose. Method of Nelson (1944). Ten ml of the solution indicated in Fig. 7 was neutralized with approximately 15 ml 2 N NaOH using phenolphthalein as indicator. This neutralized solution was made to 100 ml with water and 1 ml of the resulting solution put through the same routine as for hemicellulose.

(b) Lignin

Lignin was determined as a residue.

(c) Nitrogenous Fractions

(i) Total Nitrogen. This was determined by a semi-

micro Kjeldahl method using a selenium-potassium sulphate catalyst. Because of the presence of large amounts of nitrate in the grass samples a pre-reduction was carried out using the reduced iron method described by Humphries (1956).

(ii) Non-protein Nitrogen. This analysis was carried out on the water soluble fraction of the alcohol extract of the grass sample (Fig. 7). Nitrogen was determined after a Kjeldahl digestion following a pre-reduction of nitrate as above.

(iii) Protein Nitrogen. This was determined indirectly as the difference between total nitrogen and non-protein nitrogen.

(iv) Nitrate Nitrogen. Nitrate was determined on a water extract of the dried grass by the phenol-disulphonic acid method of Johnson and Ulrich (1959) with no precautions being taken for the presence of excess chloride.

Table 10. Chemical Composition of Ryegrasses, Experiment 1

Sample	Date	Carbohydrates (% DM)					Lignin (% DM)	Nitrogenous Fractions of DM			
		Total Soluble Sugars	Water Soluble Polysaccharide	Pectin	Hemi-cellulose	Cellulose		NPN (mg/100 g)	NO_3-N (mg/100 g)	Protein-N (%)	Total N (%)
SE	15 October 1962	10.50	0.28	1.35	9.70	15.20	3.30	556	232	3.58	4.24
SL		10.50	0.24	1.42	9.40	15.00	4.10	758	378	3.67	4.43
P		9.75	0.27	1.65	9.40	18.30	2.20	498	177	3.59	4.09
S	12 November 1962	11.70	0.45	1.29	9.98	8.61	0.72	1306	358	3.28	4.59
P		12.38	0.94	1.94	12.50	16.80	2.92	1011	237	2.66	3.67
S	26 November 1962	12.75	1.07	1.65	13.04	11.03	1.24	639	163	3.00	3.64
P		12.00	1.12	1.62	12.76	12.37	1.26	545	106	2.97	3.51
S	3 December 1962	12.15	1.09	1.30	13.04	11.90	0.98	565	92	2.96	3.52
P		10.88	0.87	1.30	12.76	12.68	1.64	600	103	3.43	4.03
S	10 December 1962	15.08	1.27	1.42	12.56	11.62	3.28	525	88	3.21	3.73
P		10.65	[illegible]	1.65	11.38	11.64	3.60	525	65	3.42	3.94

RESULTS

Experiment 1

1. Weather

The weather during Experiment 1 was not that normally experienced in the spring in this locality. Rainfall during the experiment is compared with the average of 30 years, below:

Month:	Aug.	Sept.	Oct.	Nov.	Dec.
Rainfall 1962 (in):	4.83	1.81	5.73	3.47	2.79
30 year average rainfall (in):	3.43	2.64	3.56	2.09	3.51

September was unusually dry and October, when the digestibility trial and first faecal collection took place, was abnormally wet.

2. Chemical Analysis of the Ryegrasses

Samples of grass were collected regularly throughout the experimental period and an analysis of carbohydrate and nitrogenous fractions is presented in Table 10. The samples of 15 October 1962 coincided with the period of the first digestibility trial, while samples on 3 and 10 December 1962 were taken at the time of the second faecal collection.

There was little difference between the grasses with regard to TSS until the December samplings when S was higher than P. WSPS and hemicellulose showed no consistent differences between grasses throughout the

sampling period. There were no quantitatively significant differences in the pectin fractions. P was higher than S in cellulose for the early samples but this difference gradually declined until the grasses were the same on 10 December 1962. At the time of the digestibility trial there were differences between the grasses in cellulose but not in TSS, while during the second faecal collection the reverse situation existed.

There was no obvious trend in lignin between the two grasses.

Marked differences were observed in some of the nitrogenous fractions. NPN was higher in S for the early samples but by the last two samplings the two grasses were approximately the same. Nitrate was more variable and in all cases except 3 December 1962 when S was higher than P. Nitrate content in both grasses was high for the early samples but declined during the experiment. The extremely high NPN and nitrate figures for 12 November 1962 were probably due to the fact that samples were collected 3 days after the pastures had been topdressed with nitrolime.

At the time of the digestibility trial and faecal collection in mid October a palatability problem existed on the S plots. Sheep would not graze these plots readily and large areas, which were distinct from urine patches, were not touched. On 15 October 1962 two

Table 11. Live Weights and Live Weight Gains in Experiment 1 (1b)

Sheep	Before Treatment Switch				After Treatment Switch		
	Treat-ment	Initial Live Weight	Live Weight 1 November 1962	Live Weight Gain	Treat-ment	Live Weight 15 December 1962	Live Weight Gain
BW	P	129.5	146.0	16.5	S	151.0	5.0
BR	P	97.0	116.0	19.0	S	127.0	11.0
BB	P	98.5	110.5	12.0	S	121.5	11.0
				Mean 15.8			Mean 9.0
GW	S	108.5	114.0	5.5	P	117.5	3.5
GR	S	100.0	111.5	11.5	P	116.0	4.5
GB	S	102.5	102.0	- 0.5	P	118.0	16.0
				Mean 5.5			Mean 8.0

samples of S were taken; the first, labelled SE in Table 10, was a random sample of all the plots while the sample SL was taken solely from the long grass that was not being eaten. It can be seen in Table 10 that there were no differences between these samples in carbohydrate constituents but that the NPN and nitrate fractions were higher for the unpalatable long grass. For comparison, samples of S and P were obtained on 24 October 1962 from an adjacent area which had not been heavily topdressed, but where each of the pure grasses were grown in association with white clover. The nitrate nitrogen concentrations for S and P were 100 and 36 mg per 100 g dried grass respectively. This indicated that the grasses on the experimental plots of K Block were extremely high in nitrate.

3. Live Weight

Live weights and live weight gains for each of the grazing sheep are given in Table 11. Before the treatment switch the P sheep gained more than those on S while after the switch gains were about equal.

4. Digestibility Trial, Faecal Production, and Intake

All intake and faecal production data were checked to determine whether treatment effects could be improved by removing variation due to animal size by dividing by live weight or some function of it. This type of transformation did not reduce variability so it

Table 12. Digestibility Trial Data, October 1962. (Data are Totalled from a 10 Day Collection Period).

Sheep	Treatment	OM Intake (g)	Faecal Organic Matter (g)	Total DOM (g)	Digest. % (OM)	Feed/Faeces	Faecal N % (OM Basis)
P1	P	12875	2238	10637	82.6	5.76	4.73
P2	P	13174	2358	10816	82.1	5.59	4.64
P3	P	11099	2177	8922	80.4	5.10	4.54
Mean		12383	2258	10125	81.7	5.48	4.64
S1	S	10778	1956	8822	81.8	5.52	4.56
S2	S	11813	2343	9470	80.2	5.04	4.72
S3	S	11218	1923	9295	82.8	5.83	4.79
Mean		11270	2074	9196	81.6	5.50	4.69

Table 13. Faecal Collection from Grazing Sheep. October 1962. (Data are Totalled from a 10 Day Collection Period)

Sheep	Treatment	Faecal OM (g)	Faecal N % (OM Basis)
BW	P	2477	4.49
BR	P	2281	4.26
BB	P	1724	4.35
Mean		2161	4.37
GW	S	1610	4.49
GR	S	2190	4.50
GB	S	1725	4.34
Mean		1842	4.44

was not used.

Results of the digestibility trial conducted in October 1962 are presented in Table 12. There were no differences between the grasses in digestibility, feed to faeces ratio or faecal nitrogen percentage. Organic matter (OM) intake, digestible organic matter (DOM) and faecal organic matter (FOM) were all slightly higher for the P sheep.

The results of the faecal collection from the outdoor fistulated sheep which were obtained at the same time as the digestibility trial are presented in Table 13. FOM was again slightly higher for the P animals. There was no difference between the treatments in faecal nitrogen percentage, indicating that the digestibility of both grasses was the same. Both FOM and faecal nitrogen percentage were slightly lower than for the digestibility trial animals.

One of the aims of the digestibility trial was to allow an estimate of the intake of the grazing animals to be made. Digestibility was assumed to be the same both indoors and outdoors and the intake of the grazing sheep was calculated as the product of their mean daily FOM output and feed to faeces ratio. This gave mean OM intakes of 1184 and 1014 g per day for the P and S sheep respectively. The validity of assuming digestibility to be the same indoors and out-

Table 14. Faecal Collection from Grazing Sheep, December 1962. (Data are Totalled from a 10 Day Collection Period)

Sheep	Treatment	Faecal OM (g)	Faecal N % (OM Basis)
BW	S	3380	3.47
BR	S	3608	3.43
BB	S	3016	3.15
Mean		3335	3.35
GW	P	2801	3.18
GR	P	2717	3.15
GB	P	3166	3.32
Mean		2895	3.22

Table 15. Rumen Water Volume and Flow Rate Data. Sheep Means for the Period 10 September 1962 to 12 September 1962 Inclusive.

Sheep	Treatment	Rumen Water Volume (ml)	Total Rumen DM Content (g)	Water Flow Rate From the Rumen	
				% Volume /hr	ml/hr
BW	Ryegrass plus white clover pasture	2587	474	28.5	735
BR		2091	382	29.8	623
BB		3036	426	19.1	580
Group means		2571 ± 159	427 ± 32	25.8±1.3	646 ± 38
GW	Same as above	2635	555	25.5	660
GR		3129	575	25.7	796
GB		1460	185	22.0	322
Group means		2408 ± 159	438 ± 32	24.4±1.3	593 ± 38
Probability level:					
(i)	Between days	NS	NS	NS	NS
(ii)	Sheep within groups	$P < .01$	$P < .001$	$P < .05$	$P < .01$
(iii)	Between groups	NS	NS	NS	NS

Table 16. Rumen Water Volume and Flow Rate Data. Sheep Means for the Period 29 October 1962 to 31 October 1962 Inclusive.

Sheep	Treatment	Rumen Water Volume (ml)	Total Rumen DM Content (g)	Water Flow Rate From the Rumen	
				% Volume /hr	ml/hr
BW	P	3837	580	24.4	934
BR	P	4201	677	20.5	851
BB	P	4416	613	15.9	741
Treatment means		4197 ± 350	624 ± 28	20.2±0.6	842 ± 27
GW	S	2717	363	19.6	532
GR	S	3134	510	17.7	552
GB	S	2682	296	13.4	344
Treatment means		2844 ± 350	390 ± 28	16.9±0.6	476 ± 27
Probability level:					
(i)	Between days	NS	NS	NS	NS
(ii)	Sheep within treatment	NS	NS	$P < .001$	$P < .025$
(iii)	Between Treatment	$P < .025$	$P < .001$	$P < .01$	$P < .001$

Table 17. Rumen Water Volume and Flow Rate Data. Sheep Means for the Period 12 December 1962 to 14 December 1962 Inclusive

Sheep	Treatment	Rumen Water Volume (ml)	Total Rumen DM Content (g)	Water Flow Rate From the Rumen	
				% Volume /hr	ml/hr
BW	S	3432	507	25.4	864
BR	S	5913	725	17.4	1019
BB	S	4610	621	21.1	964
Treatment means		4652 ± 500	618 ± 33	21.3±0.5	949 ± 32
GW	P	2399	403	27.3	650
GR	P	3232	500	21.2	674
GB	P	1955	288	31.1	586
Treatment means		2528 ± 500	397 ± 33	26.6±0.5	637 ± 32
Probability level:					
(i)	Between days	NS	$P < .05$	$P < .001$	NS
(ii)	Sheep within treatment	NS	$P < .06$	$P < .001$	NS
(iii)	Between treatment	$P < 0.025$	$P < .001$	$P < .001$	$P < .001$

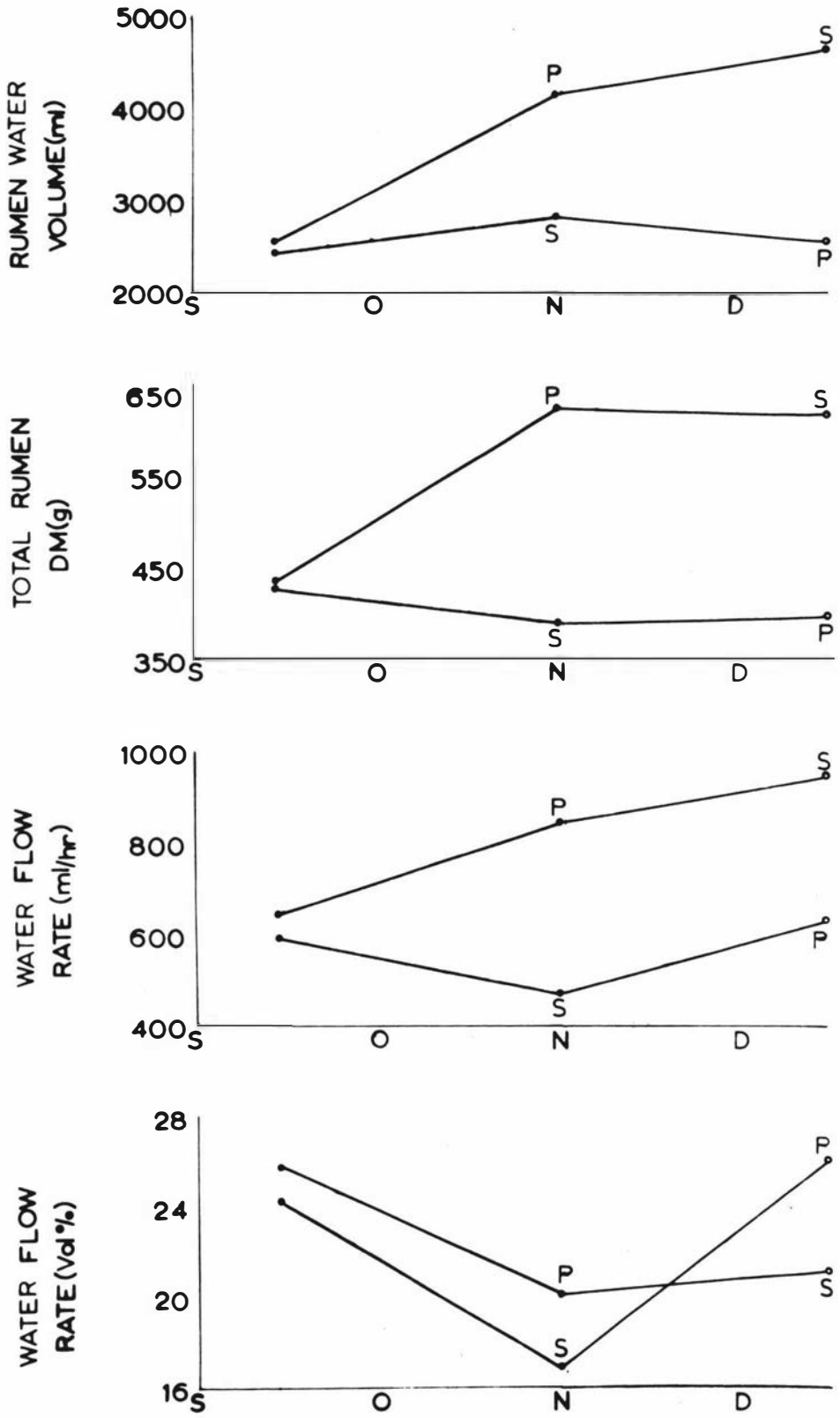
doors can be criticised in regard to the lower faecal nitrogen percentage of the outdoor animals (compare Tables 12 and 13). This may mean that the grazing sheep were selecting food of a slightly different digestibility to the indoor sheep (Lambourne and [illegible], 1962). In the present work it was considered that any such error was of minor importance in a comparison of the intakes of S and P by sheep.

Results from the faecal collection after the treatments had been switched are given in Table 14. FOM production was higher for the sheep grazing S. There was little difference in faecal nitrogen percentage between treatments, however the overall level was much lower than in the October digestibility trial indicating that digestibility of the grasses was lower in December.

5. Rumen Water Volume and Flow Rate

Data on rumen water volume, total rumen DM and the flow rate of water from the rumen for the period of common grazing in September and for treatment measurements in October and December are presented in Tables 15, 16 and 17 respectively. For each of the variables presented in these tables statistical tests of differences between measurement days, between sheep within treatment groups and between treatments are shown. Treatment effects are also presented

FIG. 8 ESTIMATES OF RUMEN "FILL" AND THE FLOW RATE OF WATER FROM THE RUMEN, SPRING 1962.



diagrammatically in Fig. 8.

The data on rumen water volume and total rumen DM was plotted against live weight in all cases as suggested by Johns et al. (1963). There was no significant relationship and no advantage was gained in this particular set of data by dividing the measures of rumen "fill" by live weight or some function of it.

For the September measurements, where the sheep were on common grazing, the only significant effects were between sheep within groups (Table 15).

In October after the animals had been on the ryegrass trial plots for 7 weeks there were significant treatment effects on all variables. Rumen water volume and total rumen DM were both significantly larger for the P animals. The percentage of rumen water volume leaving the rumen per hr decreased on both grasses when compared to September: values for the P animals were significantly higher than those on S. The absolute flow rate in ml per hr increased for P and decreased for S animals from September, and by the end of October the treatments were significantly different (Fig. 8). With both measures of water flow rate there were significant differences between sheep within treatments.

The variables were estimated again in December after the treatments had been switched for 6 weeks

Table 18. Volatile Fatty Acids from the Rumen Liquor of Grazing Sheep, 24 October 1962.

Sheep	Treatment	VFA Concentration (mM/100 ml)	VFA Proportions (%)			Total VFA* in Rumen (mM)
			Acetic	Propionic	Butyric	
BW	P	14.63	61.3	23.1	15.6	561
BR	P	14.70	55.2	26.2	18.6	618
BB	P	15.12	64.9	24.5	10.6	668
Mean		14.82	60.5	24.6	14.9	616
GW	S	16.06	55.7	28.0	16.3	436
GR	S	15.79	58.4	25.4	16.4	495
GB	S	15.46	56.2	25.2	18.6	415
Mean		15.77	56.8	26.2	17.1	449

* Calculated as the product of mean rumen water volume and VFA concentration.

Table 19. Volatile Fatty Acids from the Rumen Liquor of Grazing Sheep, 26 October 1962

Sheep	Treatment	VFA Concentration (mM/100 ml)	VFA Proportions (%)			Total VFA* in Rumen (mM)
			Acetic	Propionic	Butyric	
BW	P	9.22	62.2	22.8	15.0	354
BR	P	9.97	57.2	26.8	16.0	419
BB	P	11.35	62.2	23.0	14.8	501
Mean		10.18	60.5	24.2	15.3	425
GW	S	11.12	58.8	25.4	15.8	302
GR	S	12.96	57.7	26.3	16.0	406
GB	S	10.28	55.8	28.5	15.7	276
Mean		11.45	57.4	26.7	15.8	328

* Calculated as the product of mean rumen water volume and VFA concentration.

(Table 17, Fig. 8). The rumen water volumes of animals that had been on P before the switch continued to increase but at a reduced rate when switched to S. The rumen water volumes of animals that were switched from S to P declined. Total rumen DM remained virtually unchanged after the switch and the treatments remained significantly different. There were also significant day and sheep effects in total rumen DM. The percentage of water leaving the rumen per hr increased in animals on both treatments after the switch, but the values for sheep switched from S to P increased more rapidly than those going from P to S, and by December there were significant treatment, sheep and day effects. Absolute flow rate increased on both treatments after the switch and there were significant treatment differences in December.

6. Rumen Fermentation

Samples were available for analysis only for October.

(a) In vivo

The results of two separate samplings are shown in Tables 18 and 19. Both tables show that a slightly higher concentration of VFAs was found in rumen liquor from S sheep than from P sheep. Rumen liquor from S sheep contained a lower proportion of acetic and higher proportions of propionic and butyric acids than that

Table 20. The Results of *in vitro* Fermentations on 24 October 1962

Substrate	Final pH	Gas Evolved (ml)	Total VFA (mM/50 ml)	VFA Proportions (%)			Total Individual VFA Production (mM)		
				Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
P	6.72	39.0	3.96	56.5	27.4	16.1	2.237	1.085	0.638
Buffer	7.12	22.0	2.08	56.9	23.4	19.7	1.184	0.487	0.410
Net Production		17.0	1.88				1.054	0.598	0.228
S	6.65	54.5	5.13	50.0	31.5	18.5	2.565	1.616	0.949
Buffer	7.15	23.5	2.35	50.9	26.6	22.5	1.196	0.611	0.529
Net Production		31.0	2.78				1.369	1.005	0.420

Table 21. Concentration of Ammonia in Rumen Liquor, Experiment 1

Date		Sheep					
		BW	BR	BB	GW	GR	GB
1 November 1962	Treatment	P	P	P	S	S	S
	NH_3-N (mg/100 ml rumen liquor)	39.26	34.11	29.57	32.93	55.16	26.82
	Treatment means		34.31			38.38	
11 December 1962	Treatment	S	S	S	P	P	P
	NH_3-N (mg/100 ml rumen liquor)	35.11	47.60	45.47	46.98	46.42	47.60
	Treatment means		42.73			47.00	

from P animals. Total VFAs in the rumen were calculated and they reflected differences in rumen water volume.

(b) In vitro

Results of an in vitro fermentation of the grass juices are shown in Table 20. The fermentation of juice from S produced more gas, a higher VFA concentration, a lower proportion of acetic and higher proportions of propionic and butyric acids than the fermentation of P juice. Quantitatively the total amount of acid produced per flask was higher for the S treatment.

7. Concentration of Ammonia in Rumen Liquor

Results of ammonia determinations on rumen liquor are detailed in Table 21. There were no marked differences in treatment either before or after the switch, however both treatments had increased by approximately 8.5 mg per 100 ml by December.

Experiment 2

1. Weather

Normal summer and autumn weather prevailed during the second experiment. Rainfall data is shown below:

Month :	Feb.	March	April	May
1963 rainfall (in):	3.86	2.06	2.62	3.69
30 year average rainfall (in) :	3.01	2.64	3.20	3.59

Table 22. Chemical Composition of Ryegrasses, Experiment 2

Sample	Date	Carbohydrates (% DM)					Lignin (% DM)	Nitrogenous Fractions of DM			
		Total Soluble Sugars	Water Soluble Polysaccharide	Pectin	Hemi-cellulose	Cellulose		NPN (mg/100 g)	NO_3-N (mg/100 g)	Protein-N (%)	Total N (%)
S	6 March 1963	19.13	0.58	1.59	11.88	14.38	3.15	703	215	3.49	4.19
P		4.50	0.67	2.24	12.88	19.88	4.97	783	228	2.64	3.24
S	8 April 1963	10.69	0.70	1.45	9.13	10.63	3.87	794	252	3.73	4.53
P		6.56	0.66	1.91	10.88	13.63	6.23	594	114	3.61	4.20
S	7 May 1963	14.44	0.68	1.06	9.13	12.50	2.07	503	90	3.53	4.03
		12.00	0.48	1.76	8.00	12.13	5.47	656	99	3.82	4.47
S	12 May 1963	11.06	0.70	1.09	9.13	12.63	3.52	629	156	3.68	4.31
P		9.38	0.80	0.86	9.88	13.50	5.09	646	97	3.73	4.38

Table 23. Live Weights and Live Weight Gains, Experiment 2 (lb)

Sheep	Treatment	Initial Live Weight	Live Weight at 23.5.63	Live Weight Gain
BW	S	162.0	163.5	1.5
BB	S	130.0	135.5	5.5
GR	S	124.0	130.0	6.0
				Mean 4.3
BR	P	142.5	147.0	4.5
C	P	126.5	137.0	10.5
W	P	112.5	125.0	12.5
				Mean 9.2

Table 24. Digestibility Trial Data, May 1963 (Data are Totalled from a 5 Day Collection Period).

Sheep	Treatment	OM Intake (g)	Faecal OM (g)	Total DOM (g)	Digestibility % (OM)	Feed/Faeces	Faecal N% (OM Basis)
P1	P	5273	975	4298	81.5	5.41	5.19
P2	P	4632	952	3680	79.5	4.87	4.80
P3	P	6278	1199	5079	80.9	5.24	4.43
Mean		5394	1042	4352	80.7	5.17	4.81
S1	S	7256	1677	5579	76.9	4.33	4.81
S2	S	7220	1458	5762	79.8	4.95	4.82
S3	S	7185	1329	5856	81.5	5.41	4.84
Mean		7220	1485	5766	79.4	4.90	4.82

2. Chemical Analysis of the Ryegrasses

An analysis of the carbohydrate and nitrogenous fractions of samples of P and S collected between March and May 1963 is presented in Table 22. The last sample was collected prior to the rumen water volume and flow rate determinations in May.

In general the TSS content of S was greater than that of P, whereas the pectin, hemicellulose and cellulose content of S was lower than P. There was a trend for these differences to decline during the experimental period.

The lignin content of P was higher than that of S throughout the sampling period.

NPN declined over the experimental period and no obvious trend between S and P was observed. Nitrate nitrogen followed a similar pattern to NPN.

3. Live Weight

Live weights and live weight gains for the experimental period are given in Table 23. The gains were small but on average the P sheep gained twice as much as the S sheep.

4. Digestibility Trial, Faecal Production and Intake

Results from the digestibility trial of May 1963 are given in Table 24. OM intake, FOM and DOM were higher for the S animals. Estimates of OM digestibility were on average slightly higher for the P

Table 25. Faecal Collection from Grazing Sheep, May 1963. (Data are Totalled from a 5 Day Collection Period)

Sheep	Treatment	Faecal OM (g)	Faecal N % (OM Basis)
BW	S	1325	4.06
BB	S	1081	4.60
GR	S	1044	4.11
Mean		1150	4.26
BR	P	1414	4.39
C	P	1019	4.06
W	P	1191	4.26
Mean		1208	4.25

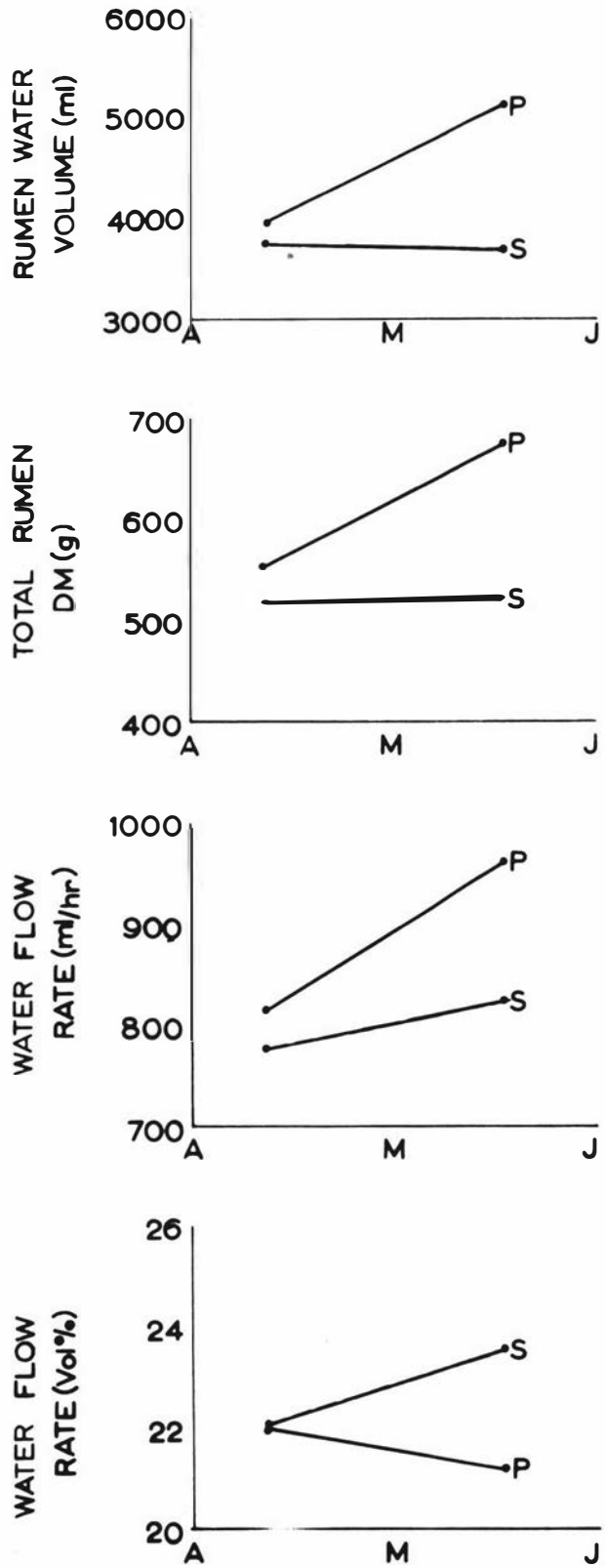
Table 26. Rumen Water Volume and Flow Rate Data. Sheep Means for the Period 9 April 1963 to 11 April 1963 Inclusive.

Sheep	Treatment	Rumen Water Volume (ml)	Total Rumen DM Content (g)	Water Flow Rate From the Rumen	
				% Volume /hr	ml/hr
BW	Ryegrass plus white clover pasture	3530	491	24.7	869
BB		3770	488	22.3	841
GR		3829	575	18.9	718
Group means		3710 ± 122	517 ± 19	22.0±0.5	776 ± 21
BR	Same as above	4415	607	22.3	981
C		5098	742	16.9	855
W		2277	303	27.2	612
Group means		3930 ± 122	551 ± 19	22.1±0.5	816 ± 21
Probability level:					
(i)	Between days	NS	NS	$P < .05$	NS
(ii)	Sheep within groups	$P < .001$	$P < .001$	$P < .001$	$P < .001$
(iii)	Between groups	NS	NS	NS	NS

Table 27. Rumen Water Volume and Flow Rate Data. Sheep Means for the Period 15 May 1963 to 17 May 1963 Inclusive

Sheep	Treatment	Rumen Water Volume (ml)	Total Rumen DM Content (g)	Water Flow Rate From the Rumen	
				% Volume /hr	ml/hr
BW	S	4232	601	21.2	880
BB	S	3570	496	22.1	760
GR	S	3259	462	27.6	826
Treatment means		3687 ± 286	520 ± 34	23.6±1.0	822 ± 55
BR	P	5661	768	21.5	1178
C	P	6276	819	18.0	929
W	P	3367	440	24.1	775
Treatment means		5115 ± 286	676 ± 34	21.2±1.0	961 ± 55
Probability level:					
(i)	Between days	$P < .001$	$P < .001$	$P < .001$	NS
(ii)	Sheep within treatment	$P < .025$	$P < .01$	$P < .05$	NS
(iii)	Between treatment	$P < .01$	$P < .01$	NS	NS

FIG. 9 ESTIMATES OF RUMEN "FILL" AND THE FLOW RATE OF WATER FROM THE RUMEN, AUTUMN 1963.



treatment and this is reflected by the feed to faeces ratios. Faecal nitrogen percentage was the same for both treatments.

Results of the faecal collection from the grazing fistulated sheep are presented in Table 25. There were no differences between the animals grazing S and P in FOM or faecal nitrogen percentage. Faecal nitrogen percentage was slightly lower than for the digestibility trial animals. If the feed to faeces ratios obtained in the digestibility trial are assumed to apply to the grazing animals, then mean OM intakes of 1127 and 1249 g per day can be calculated for the sheep grazing S and P respectively.

5. Rumen Water Volume and Flow Rate

Rumen water volume and flow rate data for the autumn experiment are presented in Tables 26 and 27. Treatment differences are indicated diagrammatically in Fig. 9.

During the period of common grazing in April (Table 26) there were significant differences between animals for all the measurements. There was also a significant difference between days in the percentage of the rumen water volume changing per hr.

After a period of approximately 5 weeks on treatment all measurements showed some divergence (Fig. 9). There were significant treatment, sheep and day effects

Table 28. Volatile Fatty Acids from the Rumen Liquor of Grazing Sheep, 13 May 1963

Sheep	Treatment	VFA Concentration (mM/100 ml)	VFA Proportions (%)			Total VFA* in Rumen (mM)
			Acetic	Propionic	Butyric	
EW	S	14.85	61.8	23.7	14.5	628
BB	S	17.80	62.4	24.2	13.5	635
GR	S	17.31	57.8	27.5	14.7	564
Mean		16.65	60.7	25.1	14.2	609
BR	P	16.84	60.8	25.7	13.5	953
C	P	15.93	63.5	23.8	12.7	1000
W	P	16.32	60.3	25.4	14.3	549
Mean		16.36	61.5	25.0	13.5	834

* Calculated as the product of mean rumen water volume and VFA concentration.

Table 29. Volatile Fatty Acids from the Rumen Liquor of Grazing Sheep, 23 May 1963

Sheep	Treatment	VFA Concentration (mM/100 ml)	VFA Proportions (%)			Total VFA* in Rumen (mM)
			Acetic	Propionic	Butyric	
BW	S	16.16	58.1	24.3	17.6	684
BB	S	14.08	55.1	28.9	16.0	503
GR	S	16.28	59.8	24.0	16.2	531
Mean		15.51	57.7	25.7	16.6	576
BR	P	14.38	56.8	27.0	16.2	814
C	P	18.14	60.1	24.9	15.0	1138
W	P	16.49	59.7	25.9	14.4	555
Mean		16.34	58.9	25.9	15.2	836

* Calculated as the product of mean rumen water volume and VFA concentration.

Table 30. The Results of *in vitro* Fermentations on 14 May 1963

Substrate	Final pH	Gas Evolved (ml)	Total VFA (mM/50 ml)	VFA Proportions (%)			Total Individual VFA Production (mM)		
				Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
P	6.46	61.3	5.65	62.6	23.4	14.0	3.54	1.32	0.79
Buffer	6.90	21.5	3.61	66.7	18.6	14.7	2.41	0.67	0.53
Net Production		39.8	2.04				1.13	0.65	0.26
S	6.60	60.8	5.68	64.8	23.5	11.7	3.68	1.33	0.66
Buffer	7.00	20.0	3.25	66.6	20.0	13.4	2.30	0.58	0.37
Net Production		40.8	2.43				1.38	0.75	0.29

Table 31. The Concentration of Ammonia in Rumen Liquor, Experiment 2 (23 May 1963)

	Sheep					
	BW	BB	GR	BR	C	W
Treatment	S	S	S	P	P	P
NH_3-N (mg/100 ml rumen liquor)	43.26	40.74	45.64	53.62	71.05	63.98
Treatment means		43.21			62.88	

on rumen water volume and total rumen DM. Neither measure of water flow rate from the rumen showed significant treatment effects. There were significant sheep and day effects on the percentage of rumen water volume changing per hr.

6. Rumen Fermentation

(a) In vivo

The results of two separate samplings are shown in Tables 28 and 29. There was little difference in the concentration of VFAs in rumen liquor collected from animals on both grasses, and no differences in VFA proportions. Total VFAs in the rumen reflected differences in rumen water volume and were higher for the P treatment.

(b) In vitro

Results from the in vitro fermentation of P and S grass juices are presented in Table 30. There was little difference between treatments with S producing more total VFA, a higher proportion of acetic acid and less butyric acid than P.

7. Concentration of Ammonia in Rumen Liquor

Results of ammonia determinations on rumen liquor are presented in Table 31. Samples from the P animals were higher than those from the S animals.

DISCUSSION

In the experiment conducted in October 1962 VFA and rumen "fill" data were similar to the earlier work of Johns et al. (1963); yet live weight was in the reverse order to that shown by Rae et al. (1963), with the sheep grazing P gaining more than those grazing S. A possible explanation was that food intake of the S animals was low compared to those on P, an explanation that is supported by the calculations of OM intake and the rates of flow of water from the rumen. This situation was thought to be due to a loss of palatability of S in October, associated with the high concentrations of NPN and nitrate in this grass (Table 10).

Under normal environmental conditions there is a seasonal variation in nitrogenous compounds in the soil, particularly in regard to nitrate and ammonium ions (Butler, 1959; Simpson, 1963). Over the summer such compounds accumulate in the soil and with the onset of autumn rains they become available as plant nutrients and so facilitate the autumn "flush" of pasture. These nitrogenous compounds are leached out of the soil during winter and in the spring their concentration is normally lower than in the autumn. In the present experiment unusual environmental conditions were experienced in that there was a warm dry September

followed by an abnormally wet October. A similar environmental situation was reported in Palmerston North in 1957 by Butler (1959) who indicated that a spring "flush" pasture high in nitrate resulted and sheep grazing it showed checks in live weight gain. It was significant that at the time of the present experiment, animals grazing S and P pastures on paddock 16 of Massey University showed no treatment differences in live weight gain. The animals on S subsequently grew faster once the spring period had been passed (Rae, pers. comm.). Under New Zealand conditions ryegrass varieties have shown a tendency to accumulate nitrate, particularly when warm rains follow dry periods (Butler, 1959). The safe limit of nitrate for ruminants is generally recognized as 200 mg of nitrate nitrogen per 100 g dry feed (Garner, 1963) and any ration containing excess of this must be considered a potential source of nitrate intoxication. In the present experiment where there were high applications of nitrogenous fertilizers as well as a season favouring the accumulation of nitrogenous compounds in the ryegrass, the S, particularly the unpalatable sample, contained higher than the safe nitrate level. While factors such as the concentration of soluble carbohydrate in the grass will determine the course of nitrate metabolism in the rumen, the possibility of subclinical nitrate intoxication

cannot be excluded. However, none of the symptoms of nitrate poisoning described by Garner (1963), such as methaemoglobinaemia or impairment of rumen motility were observed. It is thought more likely that some other nitrogenous fraction of the feed such as an organic nitro- or amino- compound, suggested by Butler (1957, 1959), could have caused the unpalatability. This possibility is strengthened by the observation that during the period of low palatability the animals were reluctant to eat S and sniffed at the grass when selecting their feed. It seemed likely therefore, that the lower intake and live weight gains of animals on S in the October experiment could have been due to an unpalatability caused by the accumulation of unusually high amounts of nitrogenous substances during abnormal environmental conditions.

After the switch of treatments in early November 1962, live weight gains were similar in the sheep grazing S and those grazing P. Data on faecal nitrogen percentage suggested that the grasses were of similar OM digestibility but that this was lower than in October. The production of FOM suggested a higher food intake of S than P. This latter result might have been implied from the chemical composition of the grasses (Table 10), where by December the ratio of soluble to structural carbohydrates was higher for S than P.

However the switch of treatments did not bring about the expected reversal of results. The rumen "fill" and water flow rate results after the switch (Fig. 8) were difficult to interpret. There was no consistent pattern in any of these variables and the conclusion was that the rumens were affected by previous nutritional history, i.e., there were carry-over effects from the high NPN and nitrate period. In this connection, it should be noted that ryegrass samples collected in November were still high in nitrogenous components and it was not until December that normal concentrations were attained. This might suggest that the high NPN content of S did have some effect on animal health and that the palatability differences between P and S may not have been the sole cause of the poor live weight gains of the S animals in the October experiment.

There did not seem to be any complicating factors such as palatability differences occurring in the autumn experiment, yet live weight gains were again in favour of the P animals. The similarity between P and S in OM digestibility and chemical composition in May 1963, as well as the similarity in VFA data from sheep grazing these grasses, suggested that there was little difference in their quality to the sheep. It appeared that the higher live weight gain of animals grazing P could be explained by their higher OM intake

of this grass. A complicating factor, however, was that sheep fed S during the digestibility trial had higher OM intakes than those fed P (Table 24). No explanation is offered: it is thought unlikely that this result was due to differences between fistulated and non-fistulated sheep.

The concentration of ammonia in rumen liquor only showed differences between treatments in May 1963 when rumen liquor from P sheep was higher than that from S sheep. As McDonald (1962) pointed out, ammonia is probably the key nitrogenous metabolite in the rumen and accumulation of ammonia in the rumen could be due to three causes: unusually high amounts of nitrogenous compounds in the feed; impaired absorption; or poor utilization of ammonia for synthetic purposes by the rumen micro-organisms. In the present work P was not abnormally high in nitrogenous compounds in May nor did it contain a significantly lower soluble carbohydrate fraction than S. Thus from the data presented it is difficult to see why rumen ammonia concentrations in the P sheep were high. A possible explanation may be obtained with reference to Fig. 9 where the percentage of water leaving the rumen per hr was lower for the P animals. Thus a unit of P rumen liquor was available for rumen microbial attack for a longer period than a unit of S. Another unconfirmed possibility was that

there were differences in the solubilities of P and S protein.

In the present work it was hoped that the fermentation of grass juices *in vitro* might give an indication of the fermentation of the ryegrasses in the rumen. However, expression of grass juices by physical means releases only substances soluble in the plant sap and the structural hemicellulose and cellulose fractions are not included. While this procedure is adequate in situations such as those described by Boda and Johns (1962) where the processes studied were associated with the plant juices, it was inadequate in the present circumstances where the ratio of soluble to structural carbohydrate was of considerable importance (Bailey, 1964).

The results of the present experiments are unusual in the sense that the great weight of evidence from earlier work, conducted as it was with large numbers of animals and over several years, suggested that higher live weight gains should be expected on S than on P (Rae *et al.*, 1963; Barton and Ulyatt, 1963; Johns, *et al.*, 1963; McLean *et al.*, 1964). However some points that are pertinent to the earlier studies have emerged:

1. Situations can exist where sheep grazing P show greater live weight gains than those on S. In this

respect S appears to be more susceptable to environmental changes in nitrogenous compounds than P.

2. There were higher live weight gains for animals grazing P than those grazing S in both the October and May experiments. This was in spite of the VFA proportions being theoretically orientated towards increased animal production on S in the October experiment. It is interesting to note that in the work of Johns et al. (1963) the proportion of acetic acid in rumen liquor from P-fed sheep was 74.5% (Table 6) whereas in the present work the highest figure recorded from the P animals was 64.9%. This is further evidence that the conditions of earlier work were not repeated in the present experiments.

It is considered that any explanation of the differences between S and P solely in terms of VFA utilization by the sheep might be an over-simplification and intake level may have been a major factor contributing to the live weight differences observed in earlier work.

3. The significance of the differences in rumen "fill" found in the present work needs careful evaluation. In both the spring and autumn experiments rumen "fill" followed the earlier work of Johns et al. (1963) and was larger on the P than S treatments, yet in the present work live weight gains were in the reverse order.

Evans (1964) carried out strength tests on samples of the same ryegrass analyzed by Bailey (1964) and these were found to correlate well with cellulose content: P which was consistently higher than S in cellulose also had the higher breaking strength. Other factors such as the extent of lignification, the proportion of sclerenchymous tissue and the structural orientation of the cellulose fibres may contribute to the strength difference (Evans, 1964). In the October experiment P was significantly higher than S in cellulose (Table 10) while in May P was consistently higher than S in lignin (Table 22). Further, total rumen DM, of which structural carbohydrate is a large fraction, was significantly higher for the P animals in both the October and May experiments. Such strength differences could mean that P was harder than S to break down in the rumen, by digestion and chewing, to a particle size that was readily passed through the reticulo-omasal orifice (Balch, 1950, 1960). P residues would thus tend to stay longer in the rumen than those from S. This would imply that S is broken down and passed through the rumen more rapidly than P. However this does not explain why the rumen "fills" of the S sheep should be smaller. In other words, what stops the S animals eating to repletion of their rumens? A feasible explanation is that the fermentation

of S produces metabolites which are absorbed and cause a chemostatic limitation of intake. There were only minor differences in the concentrations and proportions of VFAs found in rumen liquor from P and S sheep so there was no indication of a higher production of metabolites from the fermentation of S. However, results obtained from samples of rumen liquor must be treated with caution as the concentration and proportion of VFAs obtained are only the balance of formation over absorption. As the relative rates of absorption of the indivdual acids vary with pH of the rumen contents, the actual difference in amounts of acids available to sheep on the two ryegrasses could be greater than recorded here (see Annison and Lewis (1959) and Dobson (1960) for reviews on absorption from the rumen).

An account of an investigation into the possibility of chemostatic regulation of food intake by VFAs is given in Chapter 3.

Much of the evidence presented in this chapter demonstrated that the experimental conditions obtained did not reproduce those in the earlier work of Rae et al. (1963), Barton and Ulyatt (1963) and Johns et al. (1963). Yet within the current experiments several interesting aspects of the nutrition of sheep grazing pasture were encountered.

SUMMARY

1. Experiments were conducted on the nutritive value to grazing sheep of S and P in spring 1962 and autumn 1963.

2. Characteristics such as: live weight, OM digestibility, OM intake, rumen water and DM contents, rate of flow of water from the rumen, concentration and proportion of VFAs in rumen liquor and the concentration of ammonia in rumen liquor, were measured in sheep grazing both grasses, and were considered in relation to the chemical composition of the grasses.

3. The experimental conditions of earlier work were not reproduced and in both the spring and autumn experiments P-fed sheep had greater live weight gains than S-fed sheep.

4. In both experiments P-fed sheep had larger rumen "fills" and OM intakes than the S-fed sheep. The grasses were similar in OM digestibility. There was little difference between P-fed and S-fed sheep in the concentrations and proportions of VFAs found in rumen liquor. In the autumn experiment the concentration of ammonia was higher in rumen liquor from P-fed than S-fed sheep.

5. In the spring experiment a loss of palatability was encountered on S and this was thought to be associated with the NPN fraction of the grass.

6. The results are discussed in relation to those of earlier ryegrass comparisons.

CHAPTER 3

THE EFFECTS OF INTRARUMINAL INFUSIONS OF VOLATILE FATTY ACIDS ON THE REGULATION OF FOOD INTAKE IN SHEEP

INTRODUCTION

Much of the research into food intake regulation of mammals has been carried out with monogastric animals and orientated towards medical science. This review will briefly outline the present state of knowledge on monogastric animals before considering the specialized field of ruminant food intake regulation.

1. Current Knowledge on the Regulation of Mammalian Food Intake

(a) The Role of the Nervous System

Since the nervous system co-ordinates visceral and somatic activities of the animal it would be expected to play a major role in the regulation of food intake. There is much experimental support for this contention: for recent reviews see Brobeck (1955, 1960a, 1960b) Andersson and Larsson (1961a) and Anand (1961).

Anand (1961) postulated three levels of neural control over food intake:

(i) The lowest level is by feeding reflexes which operate through the spinal cord and brain stem.

(ii) The next highest level is at the hypothalamus. Two areas of the hypothalamus appear to be concerned (Anand and Brobeck, 1951; Larsson, 1954; Brobeck, 1955; Wyrwicka and Dobrzecka, 1960). The first is in the region of the ventro-

medial nuclei where stimulation produces hypophagia and lesions produce hyperphagia and obesity. This area has been called the "satiety centre". The second area known as the "feeding centre" is in the lateral hypothalamus where stimulation results in hyperphagia and lesions cause aphagia.

(iii) The highest level of nervous regulation of food intake is ascribed to cerebral control. Anand (1961) reviews evidence for higher levels of control and concludes: "This reviewer believes that the basic urge of 'hunger' (which is facilitatory for feeding behaviour) and the mechanism of 'satiety' (which is inhibitory for feeding behaviour) are located in the hypothalamus; the cerebral influences are regarded as mainly of a discriminative character and have been designated as 'appetite'."

Thus there is reason to suggest that regulation of food intake is mediated through the nervous system. In monogastric animals much interest has focussed on elucidating the nature of the stimuli which signal the nervous system in response to the ingestion of food. Several theories have been elaborated and the main ones will be briefly considered below. Before doing this it would be helpful to define the type of response under

discussion. Two types of intake regulation are possible: short-term regulation which adjusts day to day energy balance, and a long-term mechanism which acts to maintain body weight over a long period of time. This review will deal mainly with short-term food intake regulation.

(b) The Nature of the Stimuli Involved in Food Intake Regulation

(1) Chemostatic Theory. The post-prandial rise in blood concentration of various metabolites has been suggested as a possible mechanism of intake regulation.

The most widely discussed theory is the "glucostatic" theory of Mayer (1953, 1955). Mayer suggested that the circulating level of blood glucose regulated intake through gluco-receptors which he postulated exist in the satiety centre of the hypothalamus. Van Itallie, Beaudoin and Mayer (1953) proposed that arterio-venous differences in glucose gave a better correlation with intake than did blood glucose concentration. In this scheme the animal commences eating when arterio-venous differences in glucose are low and stops eating when differences are high. The glucostatic theory is not universally accepted (Grossman, 1955; Anand, 1961).

Other metabolites have been suggested as intake regulators such as non-esterified fatty acids (Van Itallie and Hashim, 1960) and serum amino acids (Kellinkoff, Frankland, Boyle and Greipel, 1956; Sanahuja and Harper, 1962).

(ii) Thermostatic Theory. Brobeck (1948) proposed that, "animals eat to keep warm and stop eating to prevent hyperthermia". Strominger and Brobeck (1953) elaborated this by proposing that the specific dynamic action of the food increases the heat stress of the body as a whole, and signals the need to stop eating. These authors considered that factors such as the interaction of the animal with its environment are of importance in food intake regulation.

Magoun, Harrison, Brobeck and Ranson (1938) and Andersson and Larsson (1961b) have shown that the hypothalamus is sensitive to temperature changes.

(iii) Lipostatic Theory. Bruce and Kennedy (1951) and Kennedy (1953) suggested that the circulating concentration of certain unspecified metabolites acts on the hypothalamus to prevent a surplus of energy intake over expenditure. They considered that the amount of fat in the depots could influence the concentration of these metabolites.

Mayer (1955) went further and suggested that the glucostatic mechanism provided short-term intake regulation while the lipostatic mechanism provided long-term control.

(iv) Regulation by the Gastro-intestinal Tract. Various workers have suggested that sensory receptors in the mouth, pharynx, oesophagus and stomach are important factors in the regulation of intake (Janowitz and Grossman, 1949a; Share, Martymuik and Grossman, 1952; Grossman, 1955; Quigley, 1955; Anand, 1961).

(v) Effect of Water Balance on Food Intake. Changes in water distribution between the body and the lumen of the digestive tract have been implicated in the control of food intake (Adolph, 1947; Strominger, 1947; Brobeck, 1955; Andersson and Larsson, 1961a).

Although many mechanisms have been suggested to provide stimuli to the nervous system for the regulation of food intake it appears that a multiple factor theory such as proposed by Janowitz and Grossman (1949b) is more realistic. Brobeck (1955) has summed up the situation very nicely: "satiety occurs following the ingestion of any type of diet, no matter what its composition and irrespective of whether the products of its digestion are amino acids and peptides, or mono-

saccharides, or fatty acids, glycerol, and neutral fats. Since there is this variety of changes brought about by feeding, it is not surprising that more than one of them can provide signals to the regulator, nor is it remarkable that no one hypothesis has proved to be entirely satisfactory."

2. The Regulation of Ruminant Food Intake

When considering ruminant intake regulation, special features of the ruminant such as the modification of the stomach into rumen, reticulum, omasum and abomasum, and the magnitude of the microbial fermentation taking place in the rumen must be taken into account.

This review will be concerned with the intake regulation of the physiologically normal animal eating a ration to which it is accustomed. The term palatability has frequently been used in ruminant nutrition and by definition it involves the subjective assessment of its food by the animal. Palatability will not be included in this review nor will the situation where the animal can exercise preference. Thus voluntary intake is defined as the amount of a specified food eaten during a period of time when this food is offered ad lib.

Previous work on ruminant intake regulation has been thoroughly reviewed by Blaxter (1950, 1958, 1962), Krüger and Müller (1955), Mäkelä (1956), and Balch and

Campling (1962).

The nervous systems of monogastic animals and ruminants are similar (Habel, 1956; Bell, 1960; Comline and Titchen, 1960). Satiety and feeding centres have been demonstrated in the hypothalamus of ruminants (Larsson, 1954; Wyrwicka and Dobrzecka, 1960). Little work has been carried out on the sensory stimuli involved in the regulation of feeding in ruminants.

For the present review past work on ruminant intake control has been divided into two main topics: the reduction of rumen load theory and the chemostatic theory.

(a) Reduction of Rumen Load Theory

That ruminants will eat more of a good quality than of a poor quality roughage has been recognised for many years (Wright, 1929; Huffman, 1939; Lehmann, 1941; Blaxter, 1950, 1958; Crasemann, 1953; Crampton, 1957). This is the reverse of the situation in monogastric animals which eat to a constant energy intake (Blaxter, Wainman and Wilson, 1961; Kleiber, 1961).

Lehmann (1941) recognised that the bulk of feed consumed by ruminants must have some effect on intake when he claimed that they eat to a constant ballast. Ballast was defined as non-digestible organic matter and according to this hypothesis animals would eat less of a poor quality roughage which contained more ballast.

Although Lehmann's idea was criticised by Fissmer (1941), [redacted] (1953) and Mäkelä (1956), these workers realised that some attribute of rumen "capacity" was of importance in the regulation of roughage intake. This was intake regulation in a physical sense: the "capacity" of the rumen provided an upper limit. Mäkelä (1956) has reviewed in detail the question of bulk in ruminant nutrition.

The regulation of voluntary intake of roughages has been the subject of detailed investigation in recent years by three main groups: Blaxter and his associates in Scotland (Blaxter et al., 1956; Blaxter et al., 1961; Blaxter and Wilson, 1962a, 1962b), Campling and co-workers in England (Campling and Balch, 1961; Campling et al., 1961; Campling, Freer and Balch, 1962; Freer, Campling and Balch, 1962; Freer and Campling, 1963; Campling, Freer and Balch, 1963) and Crampton's group in Canada (Crampton, 1957; Crampton, Donefer and Lloyd, 1960; Donefer, Lloyd and Crampton, 1963). While the approaches of these groups have been different they have come to essentially the same conclusion; namely, that the amount of roughage eaten is closely associated with the rate at which food is broken down in the rumen. According to the above workers the voluntary intake of roughages by ruminants appears to be determined by three factors:

(i) The capacity of the rumen.

(ii) The rate of break down of the feed.

(iii) The rate of passage of digesta through the digestive tract.

According to this theory an animal would eat to the repletion of its rumen, and the time taken to reduce rumen load to the point where hunger recurred would depend on the rate of break down of the food and rate of flow of digesta. The latter two variables would be largely determined by food quality, and on a high quality roughage rumen load would be reduced more rapidly and the animal would be able to eat more frequently than on a low quality roughage.

It follows that any factor that would affect the above three variables would in turn influence the amount of food eaten. Thus the intake of low quality roughages can be improved by the addition of nitrogenous compounds (Campling et al., 1962; Balch and Campling, 1962; Hemsley and Moir, 1963), certain minerals (Blaxter, 1962), long chain fatty acids (Hemsley and Moir, 1963) or yeast (Blaxter, 1962). These substances would aid microbial digestion and speed the rate of break down of the roughages. On the other hand substances which depress digestion such as antibiotics cause reductions in intake (Bell, Whitehair and Gallup, 1951; Oyaert, Quin and Clark, 1951). Further, the

grinding and pelleting of roughages increases intake by reducing particle size and increasing the rate of passage of digesta from the rumen (Balch and Campling, 1962; Campling et al., 1963; Minson, 1963).

It must be emphasized that the above work was carried out with roughages such as hay, chaff, and dried grass and there are many situations encountered with ruminants where the rumen load theory does not seem to apply. Depressed intakes have been reported on very high quality diets such as concentrates (Weir, Meyer, Garrett, Lofgreen and Ittner, 1959; Brent, Richardson, Tien and Menzies, 1961; Donefer et al., 1963; Freer and Campling, 1963), and also on silages (Dodsworth and Campbell, 1952; Sykes, Converse and Moore, 1955; Thomas, Moore, Okamoto and Sykes, 1961).

Little is known concerning how factors involved in the reduction of rumen load signal the nervous system to regulate feeding. Anand (1961) stated that the basic urge in feeding behaviour is "hunger". In other words, it is "satiety" and not "hunger" that is regulated. If this postulate is applied to the present considerations it would be the upper and not the lower limit to roughage intake that is regulated. The upper limit to roughage intake may be physical repletion of the rumen and in this respect there are reports of stretch receptors in the reticulo-rumen wall (see Bell, 1960; Comline and Titchen, 1960).

(b) Chemostatic Theory

In ruminants, chemostatic regulation has been suggested by Freer and Campling (1963) and Donefer et al. (1963) to explain depressed intakes on concentrate rations and Johns (1961) has considered it in relation to the intake of ryegrass species.

Manning, Alexander, Krueger and Bogart (1959) tested whether the glucostatic theory of Mayer (1953) was operative in ruminants by infusing varying amounts of glucose intravenously to sheep. They observed no intake depressions. Similar negative results were obtained by Tribe and Gordon (1950) and by Dowden and Jacobson (1960).

Alternative metabolites would be the volatile fatty acids (VFAs) which are absorbed from the rumen in large amounts. It is pertinent to discuss briefly here feed factors that influence the production of VFAs in the rumen. The chemical or physical nature of the feed eaten can determine:

(i) The amount of VFAs produced.

(ii) The proportions of individual VFAs produced.

(iii) The rate of passage of digesta through the digestive tract.

These factors are inter-related. Carbohydrate composition of the feed plays a major role in determining the end-products of ruminal fermentation. Structural carboh-

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drates, such as hemicellulose and cellulose, are degraded slowly in the rumen and acetic acid is the main fermentation product. Soluble carbohydrates, such as sugars, starch and fructosan, are rapidly fermented in the rumen with the formation of relatively more propionic and butyric acids than from structural carbohydrates. Thus the higher proportion of soluble to structural carbohydrate in the feed, the more rapid is the fermentation and relatively higher proportions of propionic and butyric acids are produced. This type of effect can be modified by treatment of the feed by cooking or grinding to a small particle size (see Johns, 1961; Minson, 1963). Grinding increases the rate of passage of food particles from the rumen; the soluble carbohydrate is rapidly fermented, but the structural carbohydrate spends less time in the rumen and because of this relatively more propionic and butyric acids are produced.

Essig, Garrigus and Johnson (1962) fed mixtures of VFAs and their salts to sheep and found a progressive decrease in food intake as the total amount of acid in the diet was increased. Additions of VFAs and lactic acid to either the food or into the rumens of dairy heifers were found by Thomas et al. (1961) to depress intakes of hay or silage. The effects of intravenous infusions of various metabolites, including the VFAs,

on the food intakes of identical twin cows were studied by Dowden and Jacobson (1960). Only acetic and propionic acids depressed intake. The significance of this propionic acid effect by intravenous infusion must be questioned because of the low concentration of this acid normally present in peripheral blood. A more acceptable procedure would have been to infuse the acids into the rumen. This was done by Rook, Balch and Campling (1960) and Rook, Balch, Campling and Fisher (1963), who infused 3,500 kcal of acetic, propionic and butyric acids intraruminally to heifers. Only acetic caused a significant reduction in intake although small depressions were obtained with the other two acids. Blaxter (1962) also reported that intraruminal infusions of acetic acid can depress intake. Montgomery, Schultz and Baumgardt (1963) infused acetic, propionic, butyric and lactic acids intraruminally to cows at dose rates that were calculated to equal one half of the 24 hr production figures for the acids in the rumen. Significant reductions in the intake of hay were noted with acetic and butyric acids but not with propionic or lactic acids. Against the above evidence, Holder (1963) found that intravenous infusions of acetate to sheep, which resulted in higher than normal post-prandial blood acetate levels, did not affect food intake.

(c) Other Possible Mechanisms in Ruminant Intake Regulation

It is not suggested that the reduction of rumen load and chemostatic mechanisms are the only ones regulating food intake in ruminants. However there is limited direct evidence of other mechanisms that might be operating. There is indirect support for Brobeck's (1948) thermostatic theory because it is recognised that ruminants eat less at high and more at low ambient temperatures (Brody, 1945; Blaxter, 1962). Evidence for oropharyngeal regulation is limited (Balch and Campling, 1962). Hormonal influences might be implicated, e.g., Hutton (1963) has shown that lactating cows will eat more than their dry identical twins.

3. The Metabolism of Volatile Fatty Acids and Glucose in Sheep, with Respect to their Possible Role in Intake Regulation

The main interest of the present chapter is whether the VFAs absorbed from the rumen can cause a chemostatic regulation of food intake. To understand any role of the VFAs in intake regulation it is necessary to consider their metabolic fate in the ruminant.

Under maintenance conditions a 50 kg sheep absorbs materials with a heat of combustion of approximately 1,350 kcal per 24 hr, a large proportion of this being as VFA (Blaxter, 1960). Carroll and Hungate (1954)

FIG.10 A SCHEME OF METABOLISM OF THE VOLATILE FATTY ACIDS AND GLUCOSE IN RUMINANTS

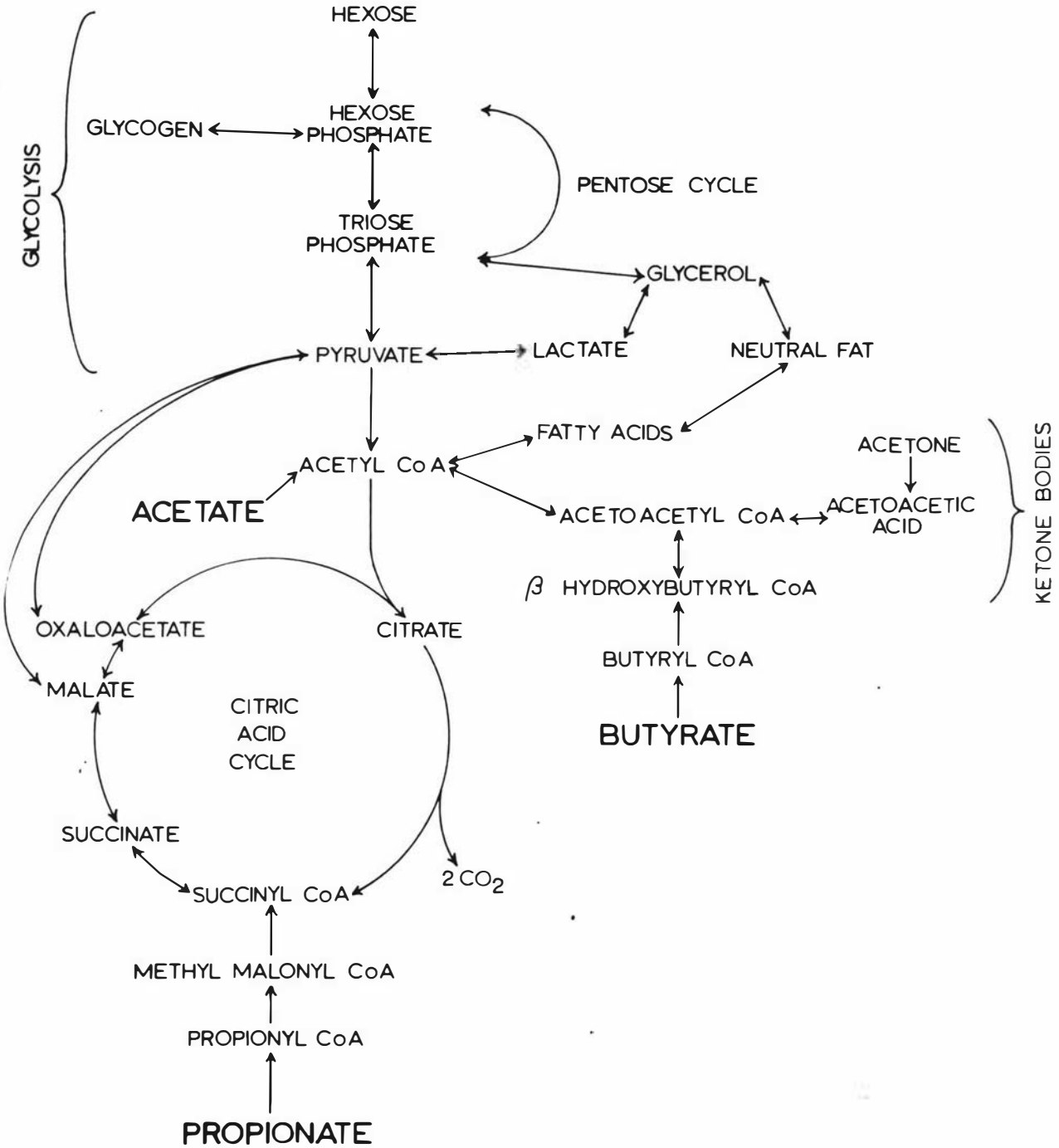

Table 32. Some Characteristics of the Metabolism of the Volatile Fatty Acids and Glucose in Sheep. (References are Given in Parentheses).

Characteristic	Acetic	Propionic	Butyric	Glucose
Amount Absorbed per Day:	100-150 g (347-527 kcal) (2, 3, 8, 15, 17, 21, 22, 37, 46)	20-78 g (100-389 kcal) (8, 17, 21, 22, 30, 37)	10-61 g (60-361 kcal) (17, 21, 22, 30, 37)	5-20 g (19-75 kcal) (8, 17, 43, 44)
Effect of Administration on Blood Ketones and Glucogenic Substances:	Ketogenic (9, 10, 18, 24, 25, 29)	Glucogenic (8, 9, 10, 18, 25, 48)	Ketogenic (10, 18, 25) ? Glucogenic (9, 14, 16, 18, 25, 28, 29, 32, 38, 45, 47)	Glucogenic (5, 6, 30)
Amount in Venous Blood:				
(a) Jugular Vein*	81-95% of VFA (1, 8, 41)	0-16% of VFA (1, 8, 41)	0-7% of VFA (8, 41)	18-85 mg/100 ml (5, 8, 41)
(b) Blood Draining Rumen	57-95% of VFA (8, 26)	6-33% of VFA (8, 26)	0-10% of VFA (8, 26)	48-78 mg/100 ml (8, 43, 44)
Main Site of Metabolism:	All tissues: brain and liver less active. (1, 3, 29, 33, 34, 35, 45)	Rumen epithelium and liver (4, 8, 9, 25, 26, 29, 35, 36, 39, 45)	Rumen epithelium and liver (9, 19, 26, 29, 31, 35, 39)	All tissues (30, 40)
Utilization Rate: (a) Fed	2.5-4.2 mg/min/kg (2, 3, 6) 6.6-10.8 mg/min/kg (20, 42)	2.8-3.7 mg/min/kg (4)	0.5-0.7 mg/min/kg (4)	1.8-3.2 mg/min/kg (5, 6, 27)
(b) Fasted	0.7-2.6 mg/min/kg (2, 3, 6)	0.7-0.9 mg/min/kg (4)	0 mg/min/kg (4)	1.2-1.5 mg/min/kg (5, 6, 27)
Pool Size:	0.9-2.0 g (20, 42)			5.6-8.1 g (27)
Contribution to Total Oxidative Metabolism: (a) Fed	35-45% (3, 42)			22-30% (5)
(b) Fasted	6% (3)			11-19% (5)
Calorimetric Efficiency of Utilization:				
(a) Maintenance (10)	59%	87%	84%	94%
(b) Lipogenesis (11)	33%	56%	62%	

Calorimetric Efficiency of Utilization of Mixtures of VFAs:	(a) Maintenance (12)	(b) Lipogenesis (13
Propionic:Butyric (60:40)	91%	
Acetic:Butyric (90:10)	65%	
Acetic:Propionic:Butyric (75:15:10)		32%
Acetic:Propionic:Butyric (25:45:30)		58%
Mixtures where acetic varied from 25-95% and the proportion of Propionic:Butyric was constant at 3:2	approx. 85%	

* Formic acid constitutes approximately 10% of jugular venous VFA (1, 8). Formate is mainly of endogenous origin and only contributes about 0.1% to total oxidative metabolism (1, 7).

References

1. Annison (1954)
2. Annison and Lindsay (1958)
3. " " " (1961)
4. " " " (1962)
5. Annison and White (1961)
6. " " " (1962a)
7. " " " (1962b)
8. Annison et al. (1957)
9. " " (1963)
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11. " " " (1957b)
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14. Ash et al. (1964)
15. Barcroft (1945)
16. Black et al. (1961)
17. Blaxter (1960)
18. Clark and Malan (1956)
19. Davis et al. (1958)
20. Essig et al. (1961)
21. Gray et al. (1960)
22. Halse and Veil (1956)
23. Heald (1952)
24. Jarrett and Potter (1950)
25. Jarrett et al. (1952)
26. Kiddle et al. (1951)
27. Kronfeld and Simesen (1961)
28. Kronfeld et al. (1959)
29. Leng and Annison (1963)
30. Lindsay (1959)
31. McCarthy et al. (1958a)
32. " " (1958b)
33. McClymont (1952)
34. McClymont and Setchell (1956)
35. Pennington (1952)
36. " (1954)
37. Pfander and Phillipson (1953)
38. Potter (1952)
39. Pritchard and Tove (1960)
40. Reid (1950a)
41. " (1950b)
42. Sabine and Johnson (1961)
43. Schambye (1951a)
44. " (1951b)
45. Shaw (1960)
46. Sheppard (1959)
47. Simesen et al. (1963)
48. Smith and Osborne-White (1961)

and Balch (1958) have estimated that up to 75% of the energy requirements of cattle can be provided as VFA.

Some of the metabolic characteristics of acetic, propionic, butyric acids and glucose are summarized in Table 32 and a simplified diagram of their metabolic relationships is presented in Fig. 10. Detailed reviews of the subject have been given by Annison and Lewis (1959), Lindsay (1959), Pearce (1960), Shaw (1960), Kronfeld (1961) and Annison and White (1961).

Of all the VFAs, acetic acid comes closest to fulfilling the theoretical requirements of an intake regulating metabolite because:

(a) It is the only VFA present in significant amounts in blood leaving the liver (Reid, 1950b; Annison, 1954; Annison, Hill and Lewis, 1957);

(b) It is the only VFA that shows appreciable arterio-venous differences after feeding (Reid, 1950b; Annison et al., 1957);

(c) It is the largest single energy source in ruminants: the sheep derives at least 35 to 45% of its energy from the oxidation of acetate (Annison and Lindsay, 1961; Sabine and Johnson, 1961); and

(d) Acetic acid is the main precursor of acetyl coenzyme A, a substance that occupies a key position in the metabolism of the VFAs (Fig. 10).

The position occupied by acetyl coenzyme A in the

intermediary metabolism of the VFAs makes it capable of being a metabolic pacemaker (Krebs and Kornberg, 1957). Under normal conditions ketone body formation does not seem significant and there appear to be three stages at which acetate metabolism can be limited:

(a) The formation of acetyl coenzyme A from acetic acid, which is dependent on a supply of ATP and coenzyme A.

(b) The combination of acetyl coenzyme A and oxaloacetate. This is the pathway by which acetyl coenzyme A enters the Citric Acid cycle, and the amount entering depends on a supply of oxaloacetate which must come from sources other than acetate.

(c) The synthesis of fat from acetyl units which requires reduced pyridine and flavine nucleotides as coenzymes as well as glycerophosphate.

All the above steps are dependent on some aspect of carbohydrate metabolism.

Further, acetate metabolism is closely related to plane of nutrition (Reid, 1958; Jarrett and Filsell, 1960; Annison and Lindsay, 1961) and to carbohydrate metabolism (Jarrett and Potter, 1950; Jarrett, Potter and Filsell, 1952; Reid, 1958; Jarrett and Filsell, 1961; Annison and Lindsay, 1961; Annison and White, 1962a; Annison, Leng, Lindsay and White, 1963).

There is thus a large volume of theoretical and experimental evidence to support a close relationship or

even dependence of acetate metabolism on carbohydrate metabolism. Manning et al. (1959) argued against acetic acid as regulatory metabolite on the grounds that it cannot supply the glucogenic substances that are essential to its own metabolism. This objection may be overcome by the evidence that acetate metabolism is rapid as long as an adequate supply of carbohydrate is available.

In the experiments to be described the effects of intraruminal infusions of various dose rates of acetic, propionic and to a lesser extent butyric acids on the food intake of sheep on high and low planes of nutrition were studied. It was hoped to put the reported regulatory effect of acetic acid on a firmer experimental basis.

Fig. 11. Disposition of the Sheep During the Infusion Experiments

(a) Before Feeding

(b) During Feeding

Table 33. A Chemical Analysis of the Diets (% DM)

	Carbohydrate				Lignin	Crude Protein	DM %
	Soluble Sugars	Soluble Polysaccharide*	Hemicellulose	Cellulose			
Concentrate 1	6.34	21.38	16.25	12.25	4.92	20.03	86.37
Concentrate 2	7.88	15.61	11.88	11.00	2.52	22.13	86.57
Chaff 1	6.75	9.69	10.25	27.00	15.44	12.69	84.34
Chaff 2	6.75	11.63	11.63	24.63	5.98	19.94	80.00

* Water soluble polysaccharide plus pectin.

EXPERIMENTAL METHODS

The sheep used in all experiments were adult Romney Marsh crossbred wethers prepared with permanent rumen fistulas by the technique of Jarrett (1948). The animals were housed individually in metabolism crates (Fig. 11) and had free access to a mineralized salt lick throughout the experiments.

Four diets were used in the experiments: two Concentrates and two Chaffs. Concentrates 1 and 2 were ground and pelleted mixtures of peameal, lucerne meal and barley meal, while both Chaffs were made from red clover hay. The chemical compositions of the diets are shown in Table 33. The main differences between the Concentrates and Chaffs were that the Concentrates contained more soluble polysaccharide while the Chaffs were higher in lignin and cellulose. Chaff 1 was low in crude protein but provided the sheep with protein in excess of maintenance requirements (Evans, 1960).

In an attempt to characterize the four feeds in terms of their metabolism in the rumen, digestibility and VFA production were measured in two sheep on each feed. A conventional digestibility trial with 5 days of faecal collection was conducted following the procedures outlined in Chapter 2. Samples of rumen liquor for VFA determinations were collected 0, 1.5, 3, 6, 12 and 24 hr after feeding. The proportions of individual

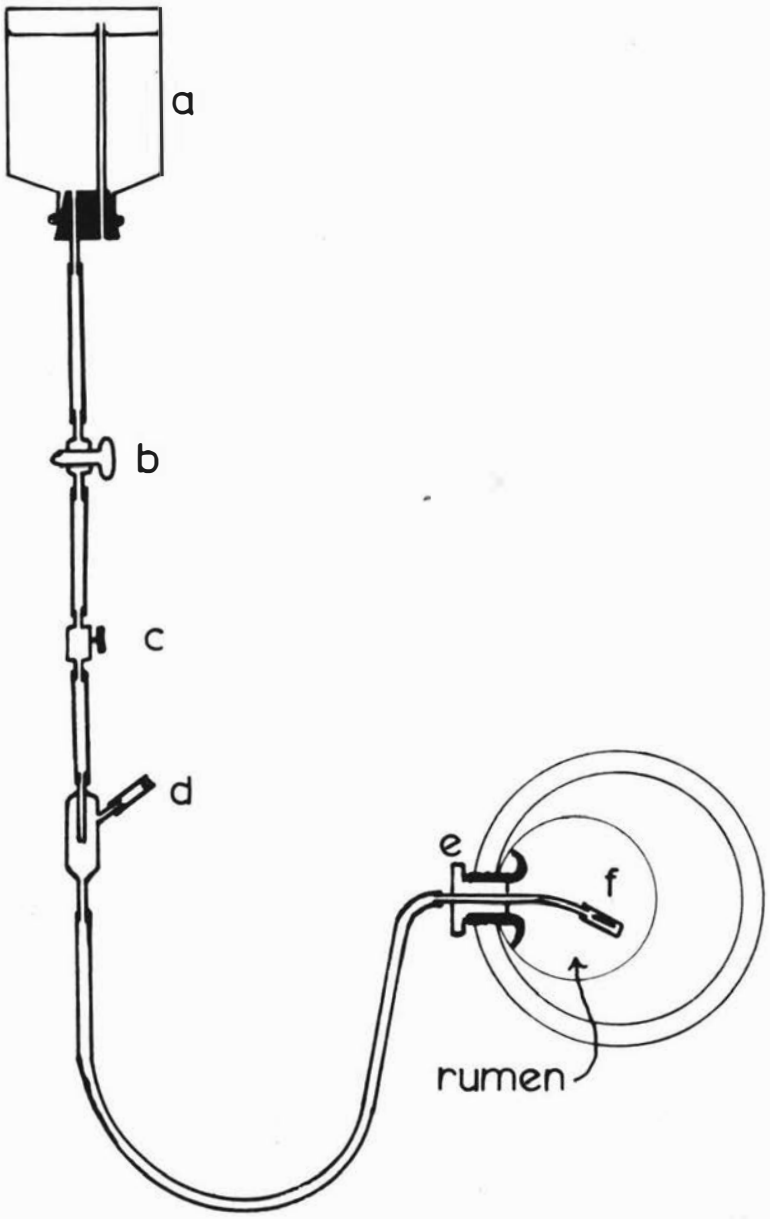


Fig. 12 Diagram Showing Intraruminal Infusion Apparatus

VFAs in the 6 hr samples were analyzed by the method given in Chapter 2.

Infusion Apparatus

All intraruminal infusions were carried out with the gravity feed system shown diagrammatically in Fig. 12. A 6 litre polythene reservoir (a) graduated with 500 ml divisions was position 10 ft above the animal to minimize differences in flow rate due to changes in the head of liquid when the sheep were standing or lying. Polythene tubing passed from the reservoir to a simple glass on-off tap (b) and then to a flow regulator (c). The latter was either a polythene needle valve or an adjustable metal clamp. Below the regulator was a glass drop counter (d) fitted with a side-arm which terminated in a simple ball valve. From the drop-counter the polythene tubing passed through the cannula (e) and ended with a rubber bunsen valve (f), which eliminated back pressure from the rumen. At the beginning of each infusion the apparatus was connected to the animal and then completely filled from the reservoir. The ball valve on the drop-counter was then used to partially empty the counter so that the flow could be seen and regulated. With this apparatus it was possible to maintain a steady flow into the rumen over the 6 hr infusion period.

Daily Routine

The sheep were trained to consume their daily food over a period of 6 hr starting at 10.00 AM. The amount of food offered to each sheep was calculated as its pre-experimental intake plus 15%.

Infusions of VFAs were started 1 hr prior to feeding in order to raise the rumen VFA concentration at feeding to approximately that normally found 1 hr after food was offered. Thus it was hoped that if the infusion had any effect on food intake, it would slow down the rate of eating in the first hr. Food intake was therefore measured after 1 and 6 hr. The daily routine in all experiments was as follows:

9.00 AM	Start infusion
10.00 AM	Start feeding
11.00 AM	Weigh feed
3.00 PM	Stop infusion
4.00 PM	Stop feeding and weigh refusal

Psychic factors can have a marked influence on food intake so the animals were disturbed as little as possible during the experiments.

Design of Individual Experiments

A series of preliminary trials showed that:

1. The sheep drank approximately 3 litres of water during the day when on the experimental diets.
2. Infusions of 3 litres of water into the rumen had

no effect on food intake. Thus all doses were made up to 3 litres with water. Drinking water was removed for the infusion period but replaced over night.

3. Intraruminal infusions of 400 kcal of acetic, propionic and butyric acids all caused depressions in daily intake. Below this dose rate effects were variable.
4. During such infusions rumen pH was not grossly abnormal.

Using this preliminary work as a guide, four experiments were carried out.

Experiment 1

Two sheep, GB and C, were fed on Concentrate 1 and subjected to intraruminal infusions as outlined below:

	Infusion Period (3 days)					
Sheep	1	2	3	4	5	6
GB	Water	Propionic	Water	Butyric	Water	Acetic
C	Water	Acetic	Water	Propionic	Water	Butyric

400 kcal of each acid (1.91 moles acetic, 1.09 moles propionic, 0.76 moles butyric) were infused each treatment day in three litres of water. Armstrong and Blaxter (1957a) and Rook et al. (1963) found that infusions of this magnitude caused no apparent abnormalities in sheep.

Blood ketone and carbon dioxide concentrations and rumen pHs were determined each day to check the physiological normality of the animals. Samples of blood were collected from the jugular vein at 11.00 AM. Rumen liquor samples were collected at 9.00 AM, 11.00 AM, 1.00 PM and 4.00 PM.

Experiment 2

Propionic acid was chosen for Experiment 2 because of its known gluconeogenic characteristics. The experiment was designed to study the amount of propionic acid required to produce a significant depression of intake and to ascertain the repeatability of any such effect. Four sheep were used; L and GB on Concentrate 1 and BR and C on Chaff 1. The daily routine was the same as for the first experiment except that blood and rumen sampling were omitted because the procedures involved upset the animals. The sheep received intra-ruminal infusions of water and acid on alternate days: the basic plan was to start with three infusions of 100 kcal and to increase this by steps of 100 kcal every 6 days until a depression in intake was obtained. There were some minor alterations to this plan as will be seen in the results.

Throughout Experiment 2 time was lost by animals losing their cannulae. This was minimized by lining the left side of each crate with a plate of galvanized

iron. The sheep was removed from the experiment, when a cannula was lost, and was not returned until it had regained its previous intake level and had maintained this for several days.

Experiment 3

This experiment was similar in design to Experiment 2 but acetic acid was used instead of propionic acid. Dose rates were randomized and a recovery period allowed between each dose rate to avoid cumulative effects such as experienced in Experiment 2. Four sheep were used in this experiment: BR and BB on Concentrate 2 and C and BW on Chaff 1.

After the acetic acid treatments had finished 300 kcal of each of the VFAs was infused in the order shown in Fig. 16. Water was infused every alternate day. Blood samples were collected from the jugular veins each day at 4.00 PM for ketone and carbon dioxide determinations.

Experiment 4

The first aim of Experiment 4 was to test whether intravenous infusions of glucose would cause depressions of food intake similar to those caused by propionic acid infusion in animals on a low plane of nutrition. Unfortunately Chaff 2 was of considerably higher nutritive value than Chaff 1 (Tables 34 and 35) and the proposed comparison was not possible. It was

decided to carry on however to see if intravenous glucose infusions would influence the intake of Concentrate 2 and Chaff 2, and to examine the effects of intravenous glucose, or intraruminal propionic acid infusions, on blood glucose concentrations of animals on the two diets.

Three sheep were used in Experiment 4: BR and BB on Concentrate 2 and C on Chaff 2. At the start BW was included on the Chaff ration but subsequently had to be removed because of an infection.

Experiment 4 was divided into three periods:

1. Intraruminal infusions of 300 kcal of propionic acid and water were alternated for 4 days.
2. Intravenous infusions of glucose were alternated with intravenous infusions of physiological saline for 6 days.
3. Period 1 was repeated with 1 day of propionic acid and 1 day of water control.

Intravenous cannulae for the glucose infusions were inserted as follows. The sheep were immobilized with an intravenous injection of 5% sodium thiopentone. A 15 cm length of 1 mm diameter polythene cannula (Sterivac, Size 2. Allen and Hanburys Ltd., London) was introduced into one jugular vein through a bleeding needle and the 2 cm left protruding from the vein was sutured to the animals' skin. These cannulae were

filled with heparinized saline (1000 IU/ml) and closed with polythene stoppers. Two days were allowed for post-operative recovery. The intravenous infusion apparatus was essentially the same as for intraruminal infusions except that the polythene reservoir was replaced by a 1 litre Abbot bottle (Abbot Laboratories, N.Z., Ltd.). All intravenous infusions were of 6 hr duration and coincided with the feeding period. The dose of glucose was 1 g per kg of body weight made up to 1 litre with sterile physiological saline and this was infused on 3 days with 1 litre of sterile saline on alternate days as a control. At the beginning of each infusion the jugular cannulae were washed out with saline and the infusion apparatus connected. When the infusion was ended the cannulae were re-filled with heparinized saline and stoppered. Blood was sampled for glucose determinations from the non-cannulated jugular vein at 9.00 AM (before feeding), 12.00 noon and 4.00 PM during each of the treatment periods.

Analytical Methods

1. Feed Analysis

The Concentrates and Chaffs were analyzed for carbohydrates and lignin by the method of Bailey (1964). Details of this method have been given in Chapter 2. Water soluble polysaccharide and pectin fractions were combined and named soluble polysaccharide as the former

was incompletely extracted, as indicated by the detection of starch with iodine in the pectin fraction.

Nitrogen analyses were carried out by the Kjeldahl method (Humphries, 1956) using a selenium-potassium sulphate catalyst. Crude protein was obtained by multiplying the nitrogen percentage by 6.25.

2. Volatile Fatty Acids

These were determined by the procedure outlined in Chapter 2.

3. Blood analyses

Blood for ketone and carbon dioxide determinations was collected from the jugular vein and stored under liquid paraffin in oxalated sample tubes. All blood determinations were made within 24 hr of sampling.

Blood carbon dioxide content (volume %) was determined by the Van Slyke manometric method (Milton and Waters, 1949).

Blood ketones were estimated as total acetone bodies after oxidation with acid dichromate by the method of Thin and Robertson (1952) as amended by Armstrong and Blaxter (1957a). This method underestimates β-hydroxybutyric acid, which is an important component of total blood ketones (Leng and Annison, 1963), by approximately 40% (Thin and Robertson, 1952). For this reason data on blood ketones presented in this chapter are intended for comparative purposes only.

Blood glucose was determined by the method of Somogyi (1945, 1952) using the colour reagent of Nelson (1944). The blood was deproteinized immediately after sampling and the protein-free supernatant frozen until analyzed.

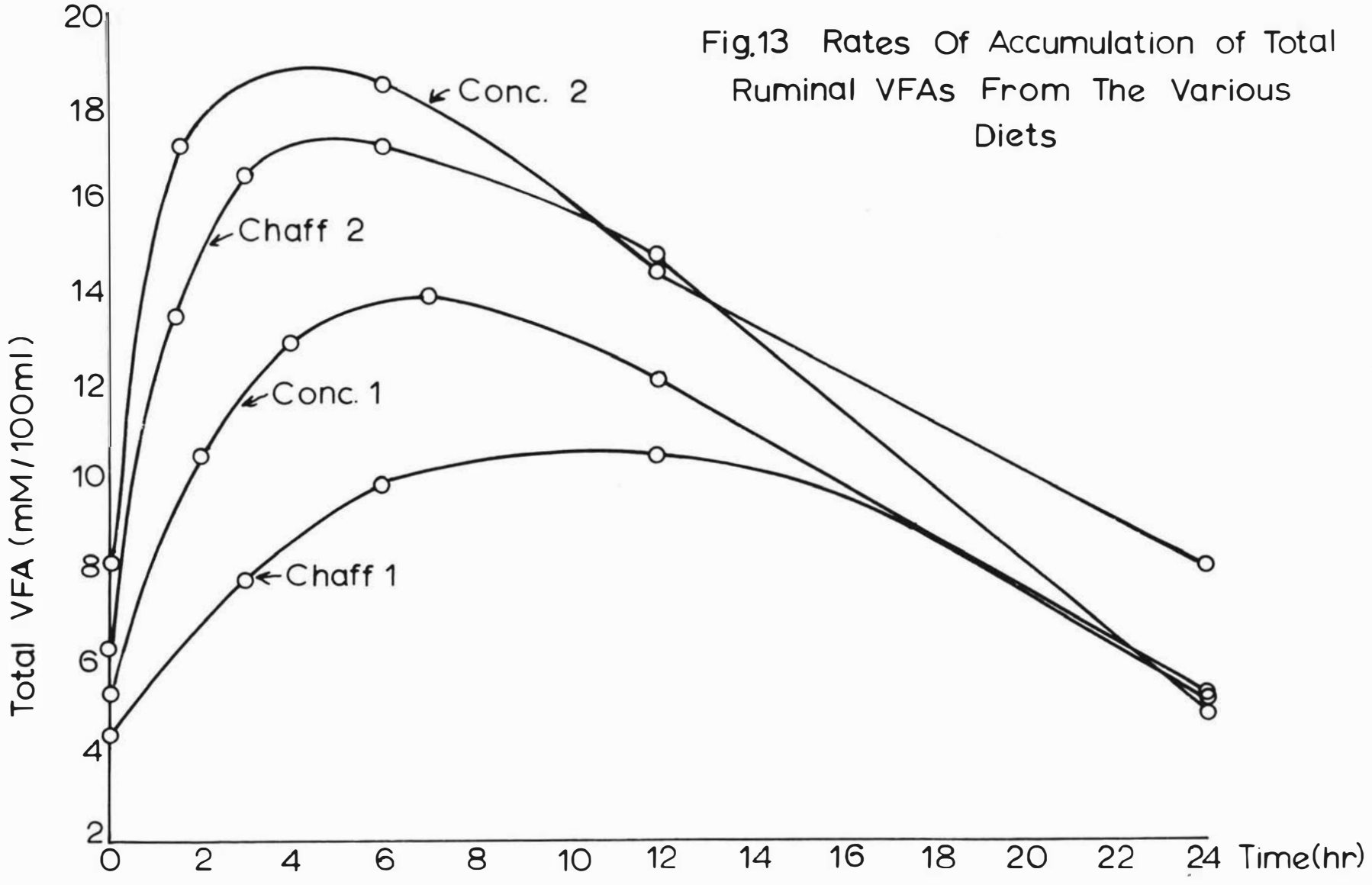


Fig.13 Rates Of Accumulation of Total Ruminal VFAs From The Various Diets

Table 34. A Comparison of the Nutritive Values of the Diets (All Data are Means from Two Sheep).

Diet	Digestibility (% of DM)	Total VFA (mM/100 ml)	VFA Proportions (%)				Individual VFA Concentrations (mM/100 ml)			
			Acetic	Propionic	Butyric	Higher Acids	Acetic	Propionic	Butyric	Higher Acids
Concentrate 1	71.4	13.80	50.2	22.2	19.3	8.3	6.928	3.064	2.663	1.145
Concentrate 2	81.6	18.42	47.3	25.1	25.0	2.6	8.713	4.623	4.605	0.479
Chaff 1	29.4	9.96	76.2	17.7	6.1	-	7.590	1.763	0.608	-
Chaff 2	67.9	17.11	70.8	17.2	12.0	-	12.114	2.943	2.053	-

Table 35. Live Weight Changes of Sheep During the Experiments

Expt No.	Diet	Live Weight Gain (kg/week)			
		Sheep	Gain	Sheep	Gain
1	Concentrate 1	C	0.796	GB	1.307
2	Concentrate 1	L	0.796	GB	0.455
	Chaff 1	C	-0.568	BR	-0.606
3	Concentrate 2	BR	1.121	BB	0.212
	Chaff 1	C	-0.607	BW	-0.545
4	Concentrate 2	BR	0.950	BB	1.322
	Chaff 2	C	1.075		

RESULTS

Characterization of the Diets

Chaff 1 was a relatively poor quality diet as is indicated by its fermentation pattern in the rumen (Fig. 13, Table 34) and its low digestibility. The fermentation of both Concentrates produced high proportions and concentrations of propionic and butyric acids in the rumen, and low proportions of acetic acid compared to the Chaff diets. Very low concentrations of propionic and butyric acids accumulated in the rumens of sheep fed Chaff 1.

Live weight gains of sheep in the various [redacted] are presented in Table 35. Losses always occurred on the Chaff 1 diet while gains were produced on the other three rations. This result complements those on digestibility and VFA production presented above, and emphasizes that both Concentrates and Chaff 2 were productive diets while Chaff 1 was a sub maintenance ration.

[redacted] 1: The Effects of Intraruminal Infusions of 400 kcal of Acetic, Propionic and Butyric Acids on the Intake of the Concentrate 1 Diet

Food intakes during the experiment are presented in Table 36 and Fig. 14. Effects on first hour intakes were variable but daily intakes were depressed by all treatments. The order of depression appeared to be:

Fig.14 Effects Of Intraruminal Infusions Of 400 kcal Of Acetic, Propionic And Butyric Acids On The Intake Of Concentrate 1 (Shaded Areas Indicate First Hour Intakes)

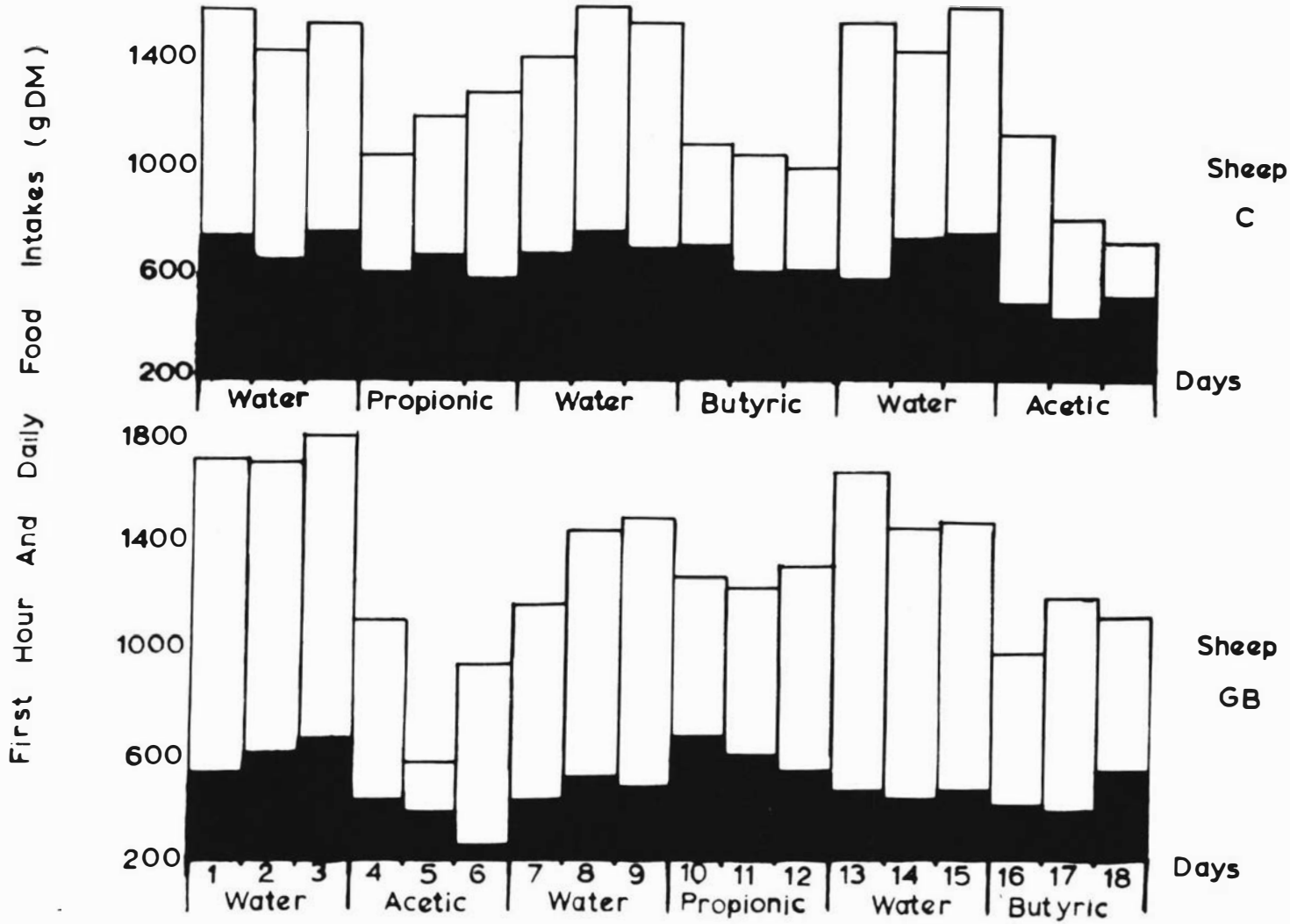


Table 36. Effect of Intraruminal Infusions of 400 kcal of Acetic, Propionic and Butyric Acids on Food Intake in Experiment 1.

Sheep	Infusion	Mean Food Intake (g DM) (n = 3)	
		First Hour	Daily
GB	Water	590	1725
	Acetic	350	870
	Water	470	1355
	Propionic	590	1260
	Water	450	1530
	Butyric	440	1090
C	Water	715	1510
	Propionic	615	1170
	Water	710	1520
	Butyric	650	1045
	Water	695	1530
	Acetic	485	890

Table 37. Effect of Intraruminal Infusions of 400 kcal of Acetic, Propionic and Butyric Acids on Blood Ketones, Blood Carbon Dioxide, and the pH and VFA Concentration of Rumen Liquor in Experiment 1 (n = 3)

Sheep	Infusion	Blood Ketones (mg Acetone/ 100 ml Blood)	Blood Carbon Dioxide (Volume %)	pH of Rumen Liquor				Concentration of VFAs in Rumen Liquor (mM/100 ml)			
				9.00 AM	11.00 AM	1.00 PM	4.00 PM	9.00 AM	11.00 AM	1.00 PM	4.00 PM
GB	Water	4.03	47.76	7.00	6.12	5.63	5.63	4.37	6.62	7.80	12.28
	Acetic	5.91	44.16	7.12	5.77	5.62	5.70	3.90	11.12	10.68	10.27
	Water	3.96	50.73	6.98	6.02	5.83	5.42	3.53	9.97	11.06	13.37
	Propionic	4.37	48.45	7.00	5.47	5.43	5.42	4.58	11.64	12.42	11.74
	Water	4.03	47.33	6.74	5.80	5.67	5.20	5.42	10.33	12.45	15.02
	Butyric	6.07	43.77	7.00	5.48	5.43	5.18	4.83	11.50	12.23	14.39
C	Water	4.92	41.26	6.68	5.57	5.30	5.33	5.32	12.23	13.65	14.65
	Propionic	4.83	42.29	6.77	5.45	5.47	5.47	5.35	12.24	13.59	13.12
	Water	4.71	42.59	6.72	5.53	5.40	5.30	4.95	12.14	13.28	14.63
	Butyric	6.01	39.09	6.75	5.43	5.43	5.53	5.95	12.69	12.99	13.14
	Water	4.64	44.03	6.77	5.57	5.48	5.26	5.53	11.54	13.02	14.40
	Acetic	4.38	47.33	6.60	5.28	5.15	4.93	6.21	14.55	14.20	19.64

Table 38. Effect of Intraruminal Infusions of 400 mol of Acetic, Propionic and Butyric Acids on the Proportions of VFAs in Rumen Liquor in Experiment 1 (Samples Analysed From Second Day of Each Infusion Period at 4 PM)

Sheep	Infusion	Proportions of VFAs in Rumen Liquor (%)		
		Acetic	Propionic	Butyric
GB	Water	53.2	29.7	17.1
	Acetic	81.8	9.8	8.4
	Water	55.0	22.8	22.2
	Propionic	45.5	38.2	16.3
	Water	47.0	25.5	27.5
	Butyric	34.8	21.1	44.1
C	Water	47.2	25.0	27.8
	Propionic	35.8	49.4	14.8
	Water	47.5	27.3	25.3
	Butyric	33.0	21.6	45.4
	Water	41.3	24.6	34.1
	Acetic	61.0	15.4	23.6

acetic > butyric > propionic.

Blood ketone and carbon dioxide concentrations together with mean daily rumen liquor pHs and VFA concentrations are presented in Table 37. Infusions of butyric acid caused a rise in blood ketones and a depression in blood carbon dioxide content. The other acids had variable effects. Rumen pHs were slightly lower and total rumen VFAs higher at 11.00 AM on treatment days. Thus no marked abnormalities were detected after the addition of 400 kcal of VFAs to the rumen using this infusion procedure.

The effect of intraruminal infusions of each acid on the proportions of VFAs found in the rumen are set out in Table 38. The infusions changed VFA proportions in favour of the acid infused. There appeared to be a carry-over after the infusion of butyric acid to sheep C.

Experiment 2: The Effects of Intraruminal Infusions of Propionic Acid at Different Dose Rates on the Intakes of Two Contrasting Diets

Two different types of effect were produced in this experiment and it is necessary to define them. A statistical comparison was made within each dose rate of the results of the three treatment days against those of the three water infusion days preceding each of them. This was considered a measure of short-term intake regulation. On the Concentrate 1 diet there

Fig.15 Effects Of Intraruminal Infusions Of Propionic Acid(P) On Food Intake (Shaded Areas Indicate First Hour Intakes : Dose Rates In kcal). 1962.

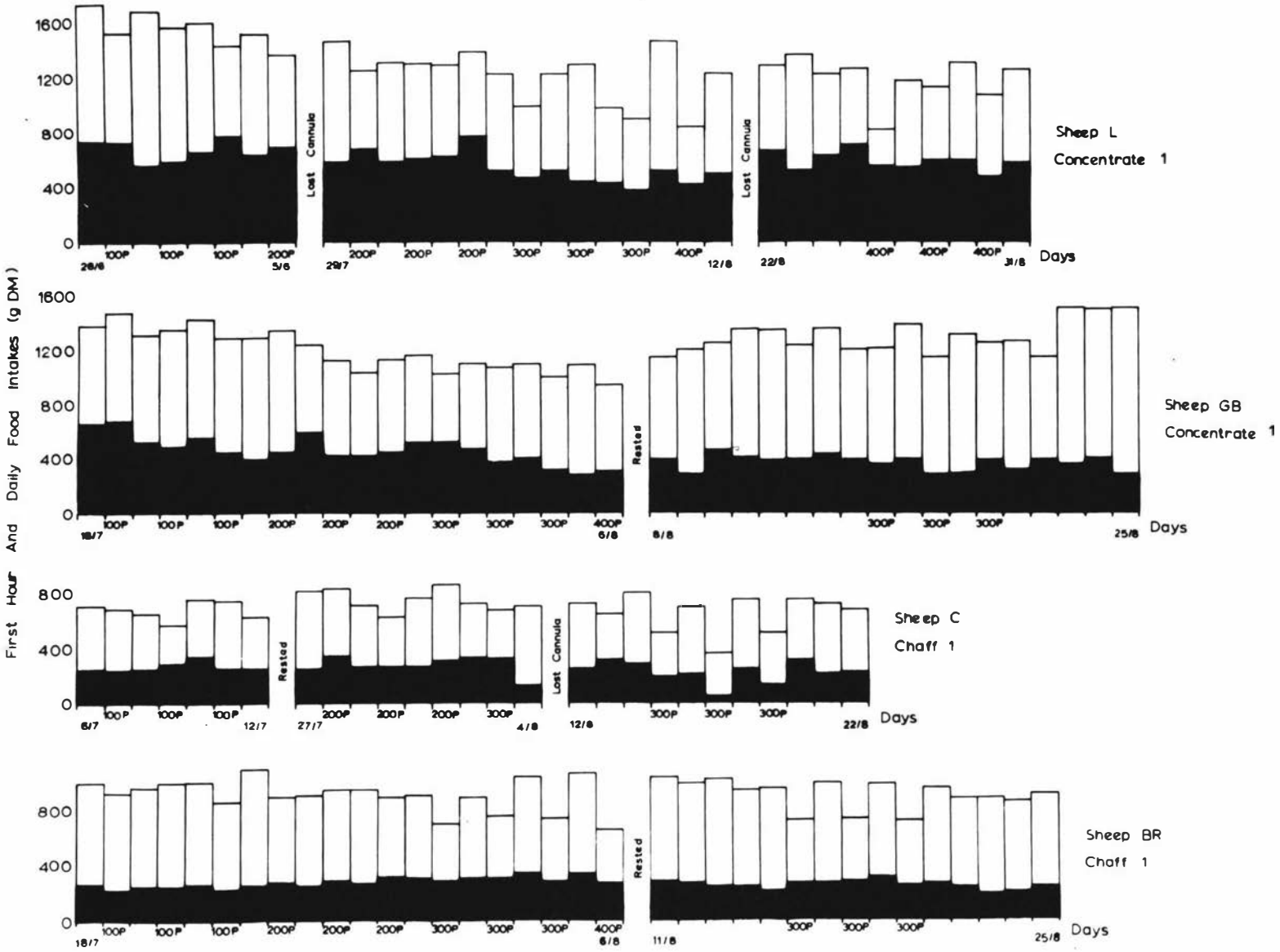


Table 39. Effect of Intraruminal Infusions of Propionic Acid (HPr) on the Food Intake of Sheep in Experiment 2 (n = 2)

Diet	Infusion	Intake (Means and S.E.s) (g DM)	
		First Hour	Daily
Concentrate 1	Water	612 ± 40	1512 ± 30
	100 kcal HPr	615 ± 40	1440 ± 30
	Water	533 ± 30	1272 ± 45
	200 kcal HPr	564 ± 30	1257 ± 45
	Water	490 ± 30	1130 ± 52
	300 kcal HPr	427 ± 30	1047 ± 52
	Water (1)	623 ± 43	1263 ± 73
	400 kcal HPr	547 ± 43	1020 ± 73
Chaff 1	Water	269 ± 12	847 ± 25
	100 kcal HPr	248 ± 12	797 ± 25
	Water	255 ± 9	874 ± 35
	200 kcal HPr	295 ± 9 *	894 ± 35
	Water	280 ± 17	850 ± 28
	300 kcal HPr	205 ± 17 *	597 ± 28***

* $P < 0.05$

*** $P < 0.001$

(1) These results are from sheep L only

were no significant short-term depressions of either the first hour or daily intakes even though 300 and 400 kcal depressed intake (Table 39, Fig. 15). However, as the dose rate of propionic acid was increased there was a progressive decrease of both control and treatment daily intakes ($P < .01$). This trend was followed by the first hour intake although it was not statistically significant. This cumulative effect of propionic acid on the Concentrate 1 diet can be seen clearly in Fig. 15. To check whether this effect was really cumulative, or just a random effect, propionic acid infusions to sheep GB were ceased on 7 August 1962 and water alone was continued for 9 days. Daily intake increased stepwise for 4 days and then remained constant at a level similar to that at the beginning of the experiment (Fig. 15).

On the Chaff 1 diet 300 kcal of propionic acid caused a significant short-term reduction in both first hour and daily intakes (Table 39). Two hundred kcal of propionic acid caused an increase in both daily ($P > .05$) and first hour ($P < .05$) intakes.

Experiment 3: The Effects of Intraruminal Infusions of Acetic Acid at Different Dose Rates on the Intakes of Two Contrasting Diets

The results of this experiment are presented in Fig. 16 and Table 40. Significant short-term

Fig. 16 Effects Of Intraruminal Infusions Of Acetic Acid On Food Intake (Shaded Areas Indicate First Hour Food Intakes: Dose Rates In kcal Of Acetic (A), Propionic (P) And Butyric (B) Acids). 1963.

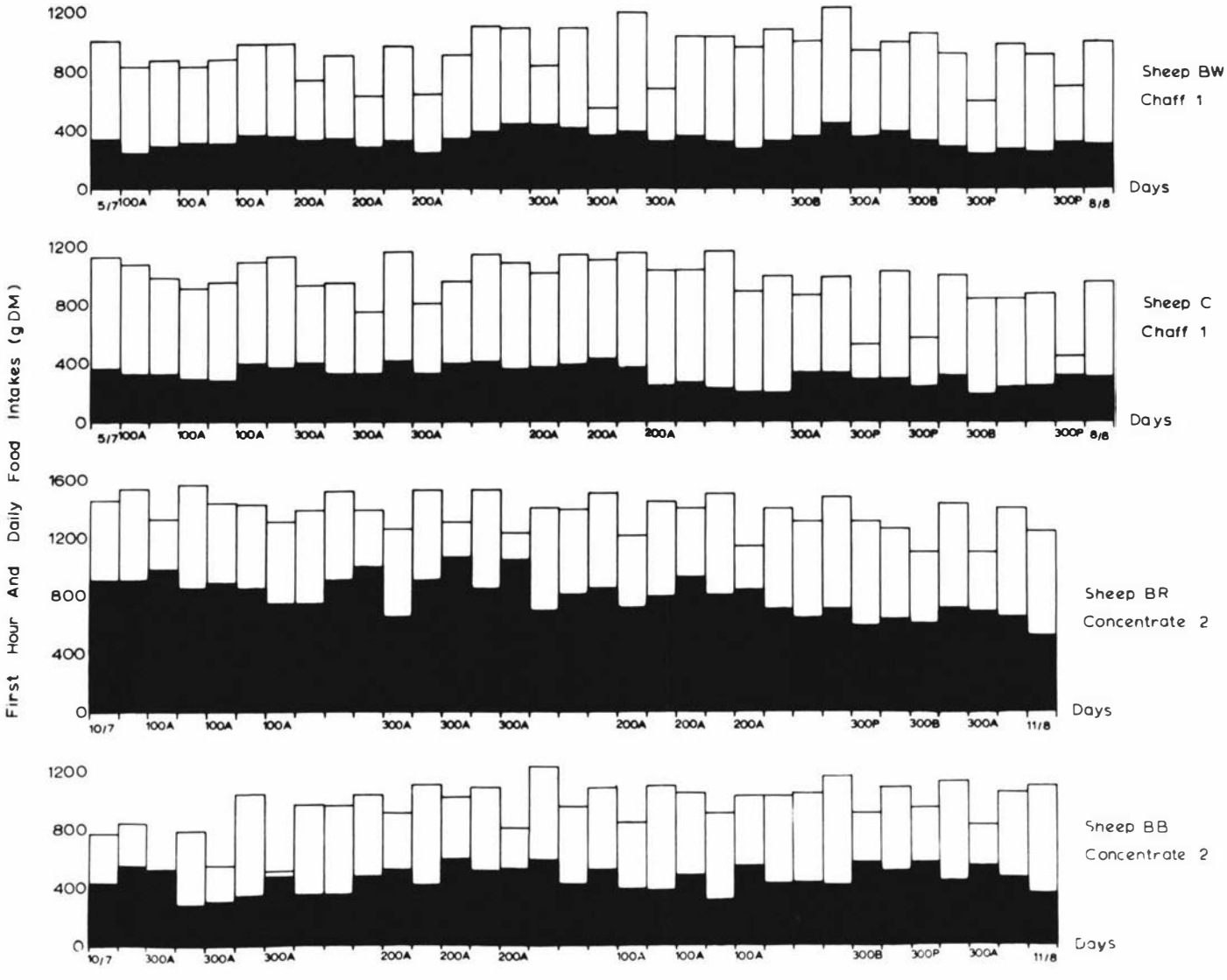


Table 40. Effect of Intraruminal Infusions of Acetic Acid (HAc) on the Food Intake of Sheep in Experiment 3 (n = 2)

Diet	Infusion	Intake (Means and S.E.s) (g DM)	
		First Hour	Daily
Concentrate 2	Water	636 ± 37	1271 ± 37
	100 kcal HAc	672 ± 37	1165 ± 37
	Water	657 ± 27	1276 ± 35
	200 kcal HAc	688 ± 27	1080 ± 35 *
	Water	653 ± 62	1186 ± 33
	300 kcal HAc	678 ± 62	896 ± 33***
Chaff 1	Water	320 ± 20	971 ± 37
	100 kcal HAc	321 ± 20	950 ± 37
	Water	353 ± 22	1039 ± 20
	200 kcal HAc	311 ± 22	858 ± 20***
	Water	391 ± 18	1100 ± 44
	300 kcal HAc	358 ± 18	757 ± 44 **

* $P < 0.05$

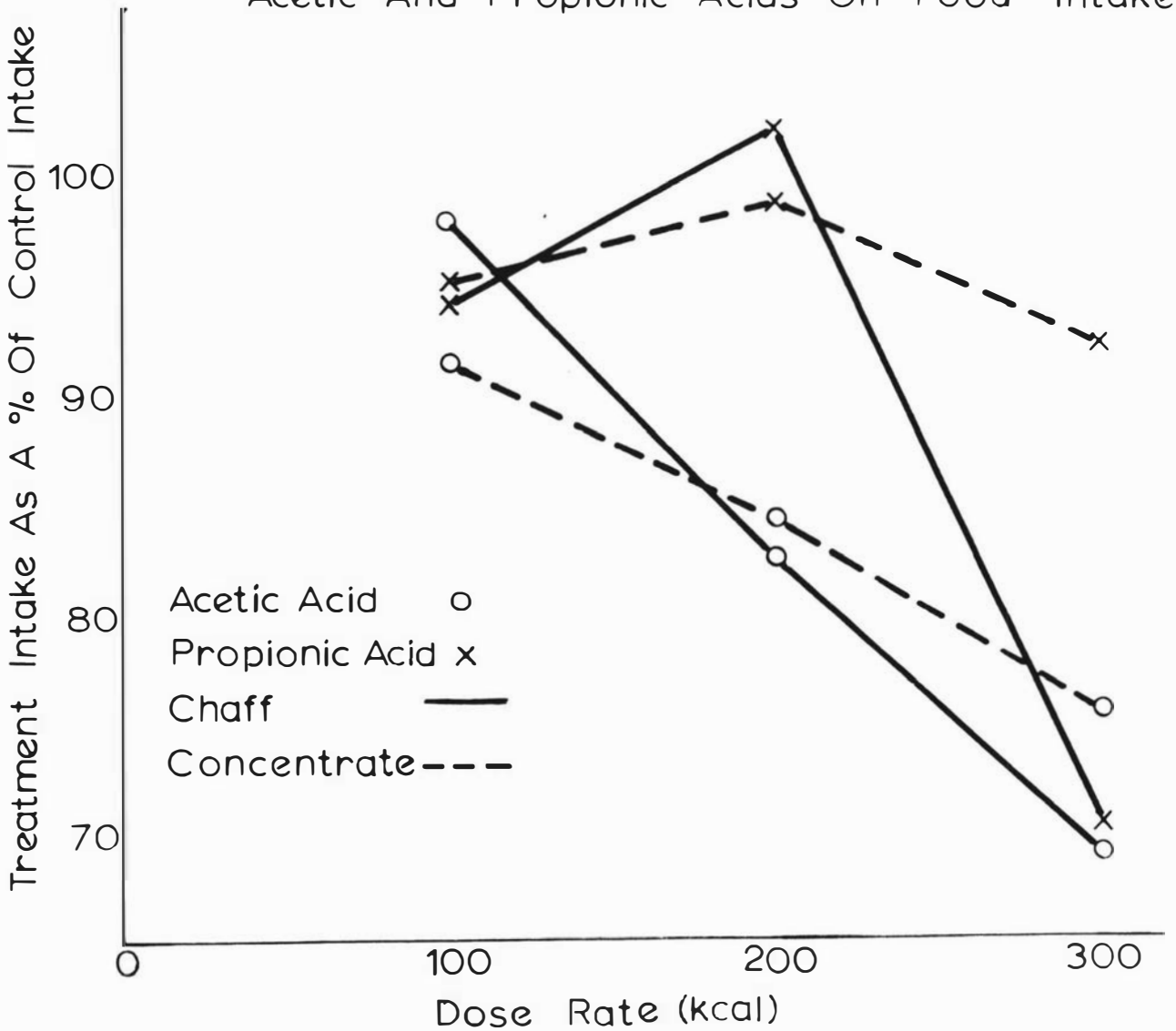
** $P < 0.01$

*** $P < 0.001$

Table 41. Effect of Intraruminal Infusions of VFAs on Blood Ketones and Carbon Dioxide in Experiment 3

Diet	Sheep	Blood Carbon Dioxide (Volume %)				Blood Ketones (mg acetone/100 ml blood)			
		Mean Water (n = 3)	Acetic	Propionic	Butyric	Mean Water (n = 3)	Acetic	Propionic	Butyric
Chaff 1	C	48.29	47.10	49.03	48.71	2.55	4.76	1.15	6.31
	EW	49.02	48.77	46.84	44.60	2.16	2.46	2.21	5.08
Concentrate 2	EB	46.58	46.22	47.97	46.93	5.96	8.04	4.76	8.94
	ER	46.48	45.05	46.98	45.62	5.47	6.56	4.51	11.81

Fig.17 A Comparison Of The Effects Of Infusions Of Acetic And Propionic Acids On Food Intake



depressions of food intake were caused on the Concentrate 2 diet by 200 kcal ($P < .05$) and 300 kcal ($P < .001$) of acetic acid. Significant short-term depressions of Chaff 1 intake were caused by infusions of acetic acid at the 200 kcal ($P < .001$) and 300 kcal ($P < .01$) dose rates. No cumulative effects on food intake were produced.

The effects of 300 kcal of acetic, propionic and butyric acids on blood ketone and blood carbon dioxide concentrations are shown in Table 41. The infusions had no consistent effect on blood carbon dioxide content. The effects on the concentrations of blood ketones were in line with those reported in the literature: acetic and butyric acids were ketogenic while propionic acid was antiketogenic. Blood ketone concentrations were higher on the Concentrate 2 than Chaff 1 diet after water infusions.

Several infusions of 300 kcal of propionic acid were carried out on the Chaff 1 diet (Fig. 16) and these confirmed the results of Experiment 2.

The results of Experiments 2 and 3 have been expressed in a different manner in Fig. 17. Daily treatment food intakes were calculated as a percentage of control intake for each dose rate. Two distinct effects were seen: an acid effect and a diet or plane of nutrition effect. There were straight line relationships

Table 42. Effect of Intravenous Infusions of Glucose on the Food Intake of Sheep in Experiment 4

Sheep	Diet	Infusion*	Intake (Means and (S.E.s) (g DM)	
			First Hour	Daily
BR	Concentrate 2	Saline	530 ± 48	1230 ± 67
		Glucose	566 ± 48	1279 ± 67
BB	Concentrate 2	Saline	344 ± 48	857 ± 67
		Glucose	425 ± 48	1012 ± 67
C	Chaff 2	Saline	442 ± 24	1195 ± 67
		Glucose	486 ± 24	1278 ± 67

* Dose rate of glucose was 1 g per kg body weight

Table 43. Effect of Intraruminal Infusions of Propionic Acid and Intravenous Infusions of Glucose on Blood Glucose Concentration (mg/100 ml Blood) and Food Intake in Experiment 4

Sheep	Diet	Infusion*	Sampling Time			Daily Food Intake (g DM)
			9.00 AM	12.00 Noon	4.00 PM	
BR	Concentrate 2	Propionic ir	34.5	35.0	32.5	1138
		Water ir	35.1	34.3	35.3	1461
		Glucose iv	34.2	58.5	45.4	1203
		Saline iv	35.3	35.0	38.5	1119
		Water ir	33.1	37.7	37.3	1272
		Propionic ir	30.0	51.3	48.0	1087
EB	Concentrate 2	Propionic ir	39.1	48.0	43.9	924
		Water ir	38.0	47.8	39.5	1171
		Glucose iv	40.2	70.0	52.0	904
		Saline iv	39.5	43.5	46.0	736
		Water ir	40.0	43.8	42.0	1259
		Propionic ir	48.5	59.0	43.7	977
C	Chaff 2	Propionic ir	45.7	59.0	43.0	1070
		Water ir	49.0	45.5	48.7	1201
		Propionic ir	49.0	46.5	54.5	856
		Water ir	46.3	47.5	46.5	1135
		Glucose iv	42.0	80.0	34.9	1150
		Saline iv	42.1	40.4	41.4	1150
		Water ir	40.0	51.3	47.6	1456
		Propionic ir	40.4	44.0	41.0	1286

* ir = intraruminal
iv = intravenous

All glucose doses were of 1 g per kg body weight.

All propionic acid doses were of 300 mmol.

with acetic acid infusions on both diets: as the dose rate was raised the intake level was depressed. Intake was depressed more on the Chaff 1 than Concentrate 2 diet. Propionic acid had a different effect on food intake: on both diets 200 kcal caused a small stimulation while 300 kcal caused a depression which was dramatic on Chaff 1. This suggested a threshold for propionic acid around 200 kcal. Animals on the low plane Chaff 1 diet were more sensitive to infusions of acetic and propionic acids than those on the Concentrate rations.

Experiment 4: The Effects of Intravenous Infusions of Glucose and Intraruminal Infusions of Propionic Acid on Intake and Blood Glucose Levels

The effects of intravenous infusions of glucose on the intake of Concentrate 2 and Chaff 2 are presented in Table 42. There were no statistically significant effects, yet glucose infusions stimulated both the first hour and daily food intakes on both diets.

Blood glucose concentrations during propionic acid and glucose infusions are presented in Table 43. The effects of intraruminal infusions of 300 kcal of propionic acid were variable. There were rises in blood glucose concentration in sheep on both diets during intravenous infusions of glucose. Blood glucose

concentrations did not seem to correlate with food intakes (Table 43).

DISCUSSION

The possibility that absorbed VFAs can act chemostatically to signal satiety in sheep has been investigated in this chapter. The results presented support the published reports of intake depressions caused by acetic acid (Dowden and Jacobson, 1960; Rook et al., 1960, 1963; Blaxter, 1962; Montgomery et al., 1963) but do not agree with Holder (1963). Decreased intakes were also caused by infusions of propionic acid, a finding that supports Dowden and Jacobson (1960) but not Rook et al. (1960, 1963) or Montgomery et al. (1963). The results also agree with the depressions of intake found with butyric acid by Montgomery et al. (1963).

The hypothesis that satiety in sheep can be influenced by the amount of acetate absorbed from the rumen will be considered. The rate of acetate metabolism in sheep appears to be related to two things:

1. Plane of Nutrition

Acetate metabolism is more rapid in sheep on a high plane of nutrition than in those which are on a low plane or have been starved (Jarrett and Potter, 1950; Jarrett et al., 1952; Reid, 1958; Jarrett and Filsell, 1960, 1961; Annison and Lindsay, 1961).

2. Carbohydrate Metabolism

Jarrett and Potter (1950), Jarrett et al. (1952),

Reid (1958) and Jarrett and Filsell (1960, 1961) all suggested that there is a close relationship between the metabolism of carbohydrate and that of acetate in sheep. Recently Annison and Lindsay (1961) found that acetate utilization rate could be decreased by intravenous infusions of insulin or increased by intravenous infusions of moderate amounts of glucose. Annison et al. (1963) infused C^{14}-labelled VFAs into the portal veins of sheep that had been starved on the experimental day. They found that blood acetate concentrations were depressed by infusions of propionic acid and suggested that propionate either inhibits endogenous acetate production or increases the rate of removal of acetate from the blood. There is thus a large volume of evidence to support a relationship between the rates of carbohydrate and acetate metabolism. Jarrett and Filsell (1961) suggested that a supply of available carbohydrate aids the utilization of acetate possibly by supplying oxaloacetate for the Citric Acid cycle. If an acetate load were imposed, those animals on a high plane of nutritition or with a large amount of available carbohydrate should be able to cope more efficiently with the extra acetate. In the present work ruminal fermentation of the Concentrate diets produced large amounts of the glucogenic propionic acid (Table 34), and presumably resulted in a high glucose turnover

in the animal. Conversely, fermentation of Chaff 1 produced large amounts of acetic acid and little propionic acid, which would indicate a low glucose turnover in the Chaff-fed animals. Therefore, animals on the Concentrate rations should have been able to cope with an acetate load more efficiently than those on the Chaff 1 diet. This is apparently what happened: the effect of acetic acid infusions on food intake was more pronounced on the Chaff 1 than on the Concentrate 2 ration (Fig. 17). Thus it appears that satiety can be related to the amount of acetate circulating in the blood.

Explanation of the propionic acid effects on food intake in terms of an interaction of carbohydrate and acetate metabolism does not satisfy the results. Animals on the Chaff 1 diet had available from rumen fermentation adequate acetate but small amounts of propionate and butyrate. If oxaloacetate was in short supply for condensation with acetyl coenzyme A for subsequent entry into the Citric Acid cycle, then acetate should accumulate as ketone bodies, particularly as the animals on Chaff 1 were losing weight. But blood ketones were not high in animals on the Chaff 1 diet. Annison et al. (1963) showed that intravenous infusions of propionic acid can depress blood acetate and Armstrong et al. (1957) found that the addition of

propionic acid to the rumens of fasted sheep increased the efficiency of utilization of acetate by the animals. These observations suggest that the propionic acid infusions to sheep on Chaff 1 should have reduced the amount of acetate circulating in the blood and thus increased food intake. In this respect three observations from the present experiments are of interest:

1. Two hundred kcal of propionic acid caused a significant increase ($P < .05$) in the first hour intakes of sheep on Chaff 1 in Experiment 2 (Table 39).
2. Infusions of 200 kcal of propionic acid caused small increases in the daily food intake of sheep on both Chaff 1 and Concentrate 1 in Experiment 2 (Fig. 17).
3. Intravenous infusions of glucose caused small increases of food intake in all cases in Experiment 4 (Table 42).

There would appear to be a threshold level for propionic acid, particularly on the Chaff 1 diet, between 200 and 300 kcal (Fig. 15, Fig. 17). Beyond 200 kcal, infusions of propionic acid caused marked depressions in the intake of Chaff 1. It could be postulated that abnormally high concentrations of propionate in peripheral blood depress food intake. In a starved animal, such as found on Chaff 1, the ability of the liver to metabolize VFAs, particularly propionic acid,

is depressed (Leng and Annison, 1963) and under these conditions infusions of propionic acid could have produced abnormally high concentrations of propionate in blood leaving the liver. If the depression of intake caused by 300 kcal of propionic acid on Chaff 1 was due to increased concentrations of blood propionate, then the depression was probably similar to that found by Dowden and Jacobson (1960) with intravenous infusion of propionic acid.

Propionic acid infusions to the rumens of sheep on the Concentrate 1 diet apparently had a cumulative effect on feed intake (Fig. 15, Table 39). This effect could also be explained by postulating that elevated concentrations of propionate in peripheral blood caused an intake depression. Large amounts of propionic acid were produced from the fermentation of Concentrate 1 and intraruminal infusions of additional propionic acid could have placed a strain on the ability of the liver to metabolize propionate. Leng and Annison (1963) showed *in vitro* that the oxidation of propionate and its incorporation into glucose by liver slices from fed sheep increased with propionate concentration up to a maximum of about 6 mM. Above this concentration there was a plateau in oxidation rate and additional propionate had no effect. This evidence suggests that the liver of fed sheep can only

cope with a certain amount of propionate and above this amount additional propionate could escape to peripheral circulation.

Butyric acid was not studied in detail because the pathways of its metabolism are not fully understood and because the intraruminal infusion of butyric acid results in high blood ketone concentrations. However, depressions of food intake were caused by infusions of butyric acid in Experiments 1 and 3. It is not known if blood ketones per se depress food intake.

While the results of the present work provide some evidence for a relationship between satiety and the level of acetate circulating in the blood, there is little to explain the depressions of intake that resulted from propionic acid infusions. It is thought that acetate is a more likely regulatory metabolite than propionate because concentrations of the latter are normally low in circulating blood. The following working hypothesis is therefore suggested: that one of the factors regulating satiety in the normal sheep could be the amount of acetate circulating in the blood.

It is obvious from the above discussion that further work is required to test the hypothesis offered. Investigation of the following topics could be profitable:

1. The effects of VFAs on food intake need to be tested under carefully controlled environmental condit-

ions, for long periods of time, and at several planes of nutrition. Under these conditions the acids should be infused intraruminally and intravenously, and blood concentrations and rates of utilization of acetate, propionate, butyrate and glucose determined. The most reliable way of measuring such variables would be with radio-isotope techniques.

2. More information is required on the metabolism of butyric acid and blood ketones. Until this is available little progress can be made in assessing the contribution of butyric acid to food intake regulation. Butyric acid is quantitatively important on high quality diets and it is the most efficiently utilized VFA for lipogenesis (Baxter, 1960).

3. More work is required on the physiology of the stimuli that evoke a satiety response from the nervous system in ruminants.

Reports in the literature that acetic and possibly propionic acids can have effects on ruminant food intake have been confirmed, i.e., chemostatic regulation of intake is possible. This can now be considered in relation to the well established rumen load reduction theory of ruminant food intake regulation. It is suggested here that the two theories are complementary and that with roughages, intake is largely governed by the physical repletion of the rumen and the

time taken to reduce rumen load to the point where hunger recurs. When rapidly fermented diets such as concentrates or certain high quality pastures are being fed, the concentration of metabolites reaching peripheral circulation may cause satiety before physical repletion of the rumen is attained. The point at which chemostatic satiety is reached may be dependent on the physiological state of the animal and might be affected by factors such as pregnancy, lactation, plane of nutrition and environmental temperature.

It seems likely that chemostatic regulation of food intake in ruminants is possible. No more than this can be said at the moment: the results provided cannot be regarded as unequivocal proof. If the VFAs are involved in the regulation of food intake it appears that their effect is not a major one, the maximum depression obtained in the present work was about 30%. Further, it seems unlikely that any single mechanism would be responsible for food intake regulation in ruminants: an interaction of many factors as suggested for monogastric animals by Janowitz and Grossman (1947b) and Brobeck (1955) would seem more reasonable.

SUMMARY

1. The effects of intraruminal infusions of acetic, propionic and to a lesser extent butyric acids on the food intake of sheep fed contrasting diets were studied. Concentrate diets provided a high plane of nutrition while a poor quality chaff diet provided a submaintenance plane of nutrition.

2. Acetic and propionic acids were infused at varying dose rates and two effects were observed: an acid effect and a plane of nutrition effect:

(a) As the dose rate of acetic acid was increased intake decreased and was significantly different from the control intake at 200 kcal on both the high and low planes of nutrition. However the depression of intake was more pronounced on the low than the high plane of nutrition.

(b) Propionic acid had a different effect: 200 kcal caused a small increase in the food intake of both diets while 300 kcal caused a significant depression of only the Chaff intake ($P < .001$). Propionic acid infusions had a cumulative effect on the high plane of nutrition where both treatment and control intakes decreased as the dose rate of acid was increased.

3. Butyric acid was not studied in detail but infusions of it were observed to depress food intake.

4. The results are discussed in relation to the intermediary metabolism of the VFAs by sheep.

CHAPTER 4

THE ABSORPTION AND EXCRETION OF LITHIUM BY SHEEP IN RELATION TO ITS USE AS A MARKER FOR DETERMINING RUMEN WATER VOLUME

INTRODUCTION

The principles involved in the use of markers to measure the volume of water in the reticulo-rumen and the flow of water on to the omasum have been elaborated in Chapter 1. In brief, the method involves the introduction of a known quantity of marker into the reticulo-rumen and the determination of the dilution of marker concentration over a period of time. The volume of water is then calculated by extrapolating the dilution curve back to zero time, and the rate of flow from the slope of the curve.

Not many substances are suitable for use as markers, and ideally, a marker should fulfil the criteria elaborated in Chapter 1. These are: it must not be absorbed from, or metabolized in the rumen; it must not be adsorbed on to the rumen epithelium; it must be non-toxic; it must be completely water soluble; and it must be adaptable for routine chemical analysis.

It has been difficult to find a substance that will fulfil all these criteria and in many cases a compromise has been accepted.

The element lithium, because of its low occurrence in herbage (Scharrer, 1941) and its ease of analysis has been suggested as a marker for estimating rumen water volume in pasture-fed animals (Mangan, unpublished). Against these admitted advantages, other

properties of the lithium ion seem likely to reduce its value for this purpose. Little work has been done on determining the fate of lithium in ruminants but it is rapidly absorbed from the gut of non-ruminants (Good, 1903; Berger, 1906; Kent and McCance, 1941). The absorbed lithium is evenly distributed through the animal body and its distribution volume is equal to or greater than the body water (Radomski, Fuyat, Nelson and Smith, 1950; Talso and Clarke, 1951; Foulks, Mudge and Gilman, 1952; Trautner, Morris, Noack and Gershon, 1955). In non-ruminants the largest part of any lithium dose appears to be excreted in the urine over the first few days (Good, 1903; Kent and McCance, 1941; Noack and Trautner, 1951; Foulks et al., 1952; Trautner et al., 1955). A smaller amount of lithium is excreted via the faeces (Good, 1903; Kent and McCance, 1941; Radomski et al., 1950; Talso and Clarke, 1951) while small amounts have been detected in saliva (Good, 1903), and in sweat (Noack and Trautner, 1951).

Much of the work on lithium toxicity stems from medical and pharmacological studies. Schou (1957) has reviewed the symptoms of lithium toxicity in great detail. The gastro-intestinal tract is affected and symptoms such as nausea, salivation, vomitting, diarrhoea and enteritis have been observed. The nervous system

is very sensitive to lithium intoxication and early symptoms are tremors of the limbs and jaws. More severe signs are drowsiness, muscular hyperirritability, coma and finally death.

The toxic and morphogenetic effects of the lithium ion on lower organisms have been reviewed by Needham (1942) and Schou (1957).

Lithium was considered when a marker was being chosen for measuring rumen water volume in the rye-grass strain comparison (Chapter 2). Because of the potential disadvantages mentioned above, a preliminary investigation was carried out to study the absorption and excretion of lithium by the sheep, and its toxicity to rumen micro-organisms. The results of these experiments, which led to the discarding of lithium as a likely marker, are described in the present chapter.

EXPERIMENTAL METHODS

1. Animals

The animals used were Romney Marsh crossbred wethers containing permanent rumen fistulas prepared by the method of Jarrett (1948). They were housed indoors in separate pens and fed on a mixture of chaffed red clover hay and pelleted lucerne concentrate. When required urine and faeces were collected by means of a harness incorporating a faecal bag and urine tank. All doses of lithium were administered through the fistula at a dose rate of 0.3 to 0.5 g of elemental lithium per sheep. This gave an initial rumen liquor concentration of approximately 100 µg lithium per ml.

2. Analytical

Monohydrous lithium sulphate was found to be the most suitable lithium salt for use as a marker because it was not deliquescent and was readily soluble in water.

All lithium determinations were made by flame photometry using the flame attachment of a Beckman model DU spectrophotometer and measuring the emission at a wavelength of 671 mµ. Calibration curves with standard solutions were determined before, during and after each run to correct for any drift. The atomizer was cleaned after each reading by flushing with distilled water.

Table 44. The Recovery of Lithium Added to Rumen Liquor

Sample	Amount of Lithium Added (µg)	Amount of Lithium Recovered (µg)	% Recovered
1	2000	1941	97.05
2	2000	2015	100.80
3	2000	1971	99.72
4	2000	1986	99.87
			Mean 99.36

Polyethylene glycol (PEG) was determined turbidimetrically by the method described in Chapter 1.

3. Collection and Preparation of Samples

(a) Rumen Liquor

Samples of ingesta were collected through the rumen fistula and strained through muslin to remove any coarse suspended plant material. For lithium determination, duplicate 20 ml samples of rumen liquor were added to crucibles, evaporated on a water bath, and then ashed in a muffle furnace at 600°C for 4 hr to remove organic matter. By this means all lithium in the samples, including that absorbed by micro-organisms and plant debris was accounted for. The ashed samples were dissolved in 10 ml 2N HCl, transferred quantitatively to 50 ml volumetric flasks and made to volume with distilled water. The flasks were allowed to stand overnight then samples for analysis were carefully decanted off.

The accuracy of the method of lithium analysis was measured with a recovery test as shown in Table 44. It was considered that lithium could be recovered quantitatively from rumen liquor by the method described.

(b) Blood

Blood was collected from the jugular vein by syringe, allowed to clot, and 1 ml of the serum diluted to 50 ml with distilled water. Samples of this

solution were taken for analysis.

(c) Urine

Urine was collected thrice daily and its volume measured. One ml was diluted to 50 ml with distilled water and a sample of this solution taken for analysis.

(d) Faeces

Faeces were collected three times daily. An aliquot from each collection was homogenized in a Waring blender with a measured amount of distilled water. Duplicate 20 g samples of homogenate were evaporated to dryness, ashed, dissolved in 2N HCl and transferred to 50 ml volumetric flasks following the procedure used for rumen liquor.

(e) Saliva

One parotid salivary gland was cannulated in each of three sheep following the method of Ash and Kay (1959). The animals were dosed with lithium through the fistula and saliva was collected at intervals for 24 hr. At this stage the salivary cannulae were removed in order to avoid degenerative changes in the glands. Duplicate 5 ml samples of saliva were made up to 50 ml for analysis. Any saliva remaining after a collection period was returned to the animal through the rumen fistula to avoid upsetting acid-base metabolism.

4. Manometric Technique

The effect of lithium upon the gas production of rumen micro-organisms was determined manometrically. The apparatus consisted of double side-arm 150 ml Warburg-type flasks connected by pressure tubing to 200 ml constant pressure manometers which measured to 0.5 ml gas evolved (Clarke, 1964). The flasks were agitated in a water bath at 40°C. Because of the large quantities required, rumen liquor was obtained from a fistulated cow following overnight fasting.

The following typical experimental design was used. Forty ml of rumen liquor was added to the main body of each flask and various mixtures were added to one of the side-arms as follows:

Flask 1 - 10 ml $\frac{M}{15}$ phosphate buffer, pH 5.7.

Flask 2 - 10 ml red clover juice, pH 5.7.

Flask 3 - 10 ml red clover juice plus lithium sulphate (to give a flask concentration of 100 µg Li per ml when mixed).

Flask 4 - 10 ml red clover juice plus lithium sulphate (to give a flask concentration of 250 µg Li per ml when mixed).

Flask 5 - 10 ml red clover juice plus sodium sulphate (to give a sulphate concentration equimolar to flask 4).

All flasks were placed in the water bath for

10 min for temperature equilibration and then gassed for 10 min with a mixture of 5% carbon dioxide and 95% nitrogen. The flasks were then tipped, mixing the side-arm contents with the rumen liquor, and gas production was measured in the ensuing hr.

This type of experiment was conducted five times.

5. Comparison of Lithium with PEG as a Rumen Water Volume Marker

Two experiments were conducted, each with three sheep fed a ration of pelleted lucerne. The animals were starved overnight and fed their daily ration at 8.00 AM. A solution containing 0.25 g lithium and 7.5 g PEG was introduced through the fistula immediately before feeding. Rumen samples were collected at 2, 4, 6 and 8 hr after dosing. Rumen water volume (V) was calculated from the ratio:

$$V = \frac{p}{C'' - C'}$$

where p is the amount of marker added and C' and C'' are the marker concentrations immediately before and after dosing. The flow of water from the rumen was expressed two ways:

(a) as the percentage of rumen water volume being removed per hr (Vol %), which is equivalent to 100 k; where k is the slope of the marker dilution curve; and

(b) as the volume of water leaving the rumen in unit time (u), expressed by the equation $u = kV$.

Table 45. The Concentration of Lithium in Rumen Liquor and Jugular Blood After Dosing a Sheep with 0.5 g of Lithium

Sample Time (hr after dosing)	Concentration in Rumen Liquor (µg Li/ml)	Blood Concentration (µg Li/ml serum)
2	177.5	
3		4.3
4	57.5	
6	48.8	
7		2.8
8	46.3	
12	30.0	8.8
24	21.3	4.0
27		5.3
31	13.8	6.0
48	16.3	6.3
72	12.5	2.3
96	8.8	2.3

Table 46. Urinary and Faecal Excretion of a Lithium Dose of 0.5 g by Sheep 3 (DM intake 400 g/day)

	Sampling Period (hr after dosing)						
	0-12	12-24	24-48	48-72	72-96	96-121	121-151
Urine: Volume Excreted (ml)	518	215	364	717	1040	890	
Concentration of Li in Urine (µg/ml)	13.40	20.97	162.65	77.69	29.88	15.66	
Total Urinary Li (mg)	6.86	45.09	59.21	55.70	31.08	13.94	
Faeces: Wet Wt. (g)	198	117	473	400	543	783	1195
Concentration of Li in Wet Faeces (µg/g)	2.39	6.00	1.56	3.57	5.90	12.49	6.65
Total Faecal Li (mg)	0.51	0.70	0.74	1.43	3.20	9.78	7.95

Table 47. Urinary and Faecal Excretion of a Lithium Dose of 0.5 g by sheep 33 (DM intake 800 g/day)

	Sampling Period (hr after dosing)				
	0-12	12-24	24-48	48-72	72-96
Urine: Volume Excreted (ml)	410	675	765	870	1170
Concentration of Li in Urine (µg/ml)	98.03	111.48	85.20	22.13	1.97
Total Urinary Li (mg)	40.19	75.25	65.18	19.25	2.30
Faeces: Wet Wt. (g)	282	398	1009	975	1114
Concentration of Li in Wet Faeces (µg/g)	41.85	26.61	29.01	23.73	26.63
Total Faecal Li (mg)	11.80	10.59	29.35	23.14	29.66

RESULTS

1. Absorption and Excretion of Lithium

The concentration of lithium found in the rumen liquor and venous blood of sheep 3 after dosing with 0.5 g lithium is shown in Table 45. The concentration in rumen liquor declined exponentially with time: only small amounts of the element were present after 4 days. Note the rapid reduction in concentration over the first 4 hr compared to the second 4 hr after dosing. Lithium was detected in venous blood within 3 hr of dosing. The concentrations in blood were always low and decreased to a trace after 2 days.

There was some variation between animals in their urinary and faecal excretion of lithium. Data for sheep 3 are given in Table 46. Urinary excretion of the element reached a peak about 48 hr after dosing and then fell away to a low concentration. An appreciable amount was detected within 12 hr of dosing. Faecal excretion of lithium was comparatively low and irregular, and seemed to reach a maximum about 5 days after dosing. Similar data for sheep 33 are presented in Table 47. This second animal had twice the food intake of sheep 3 and a correspondingly greater faecal output. Sheep 33 had a larger faecal excretion of lithium. This can also be seen from the cumulative urinary and faecal excretion curves which are presented

Fig.18 Cumulative Urinary And Faecal Excretion Of Lithium

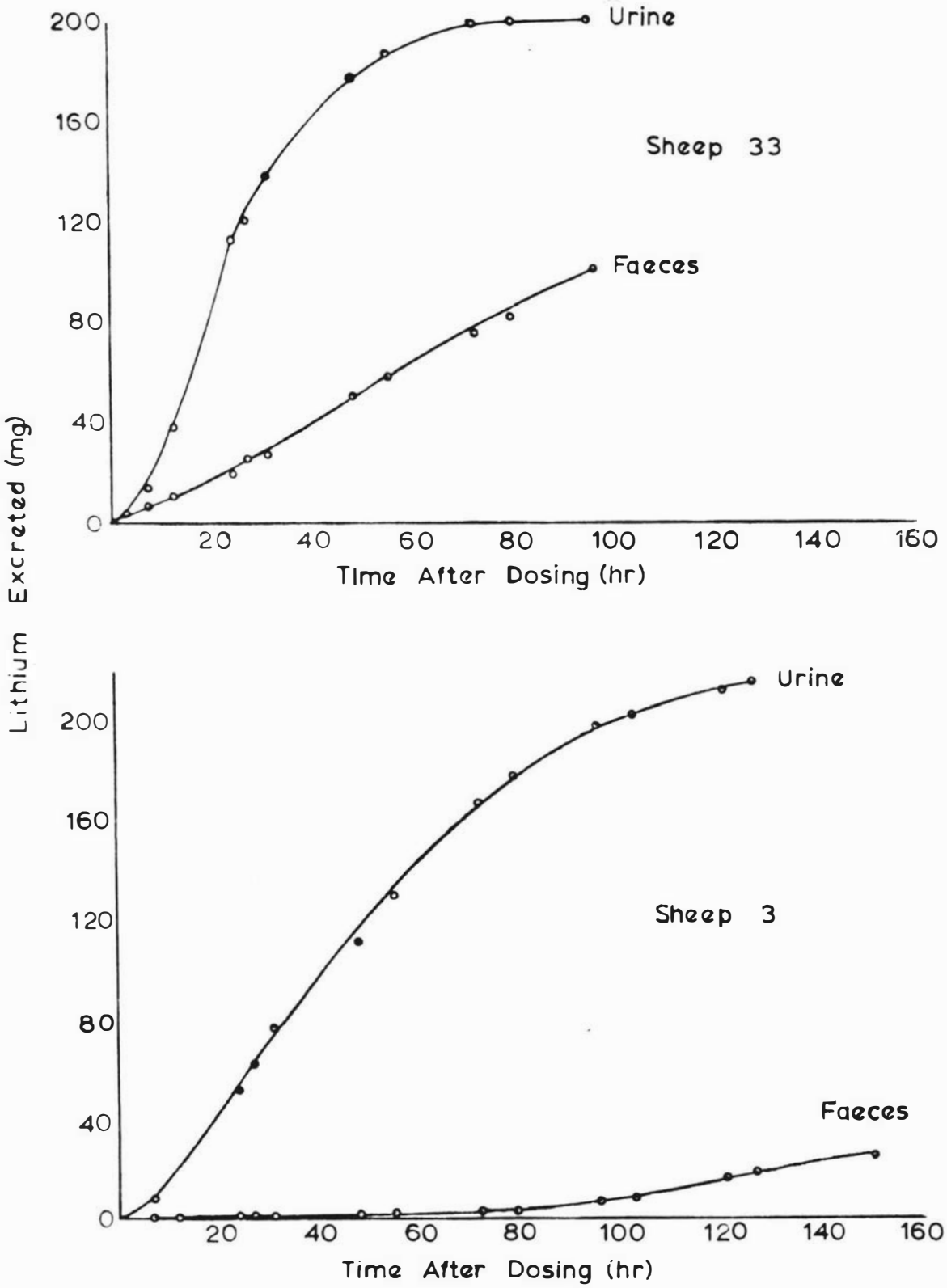
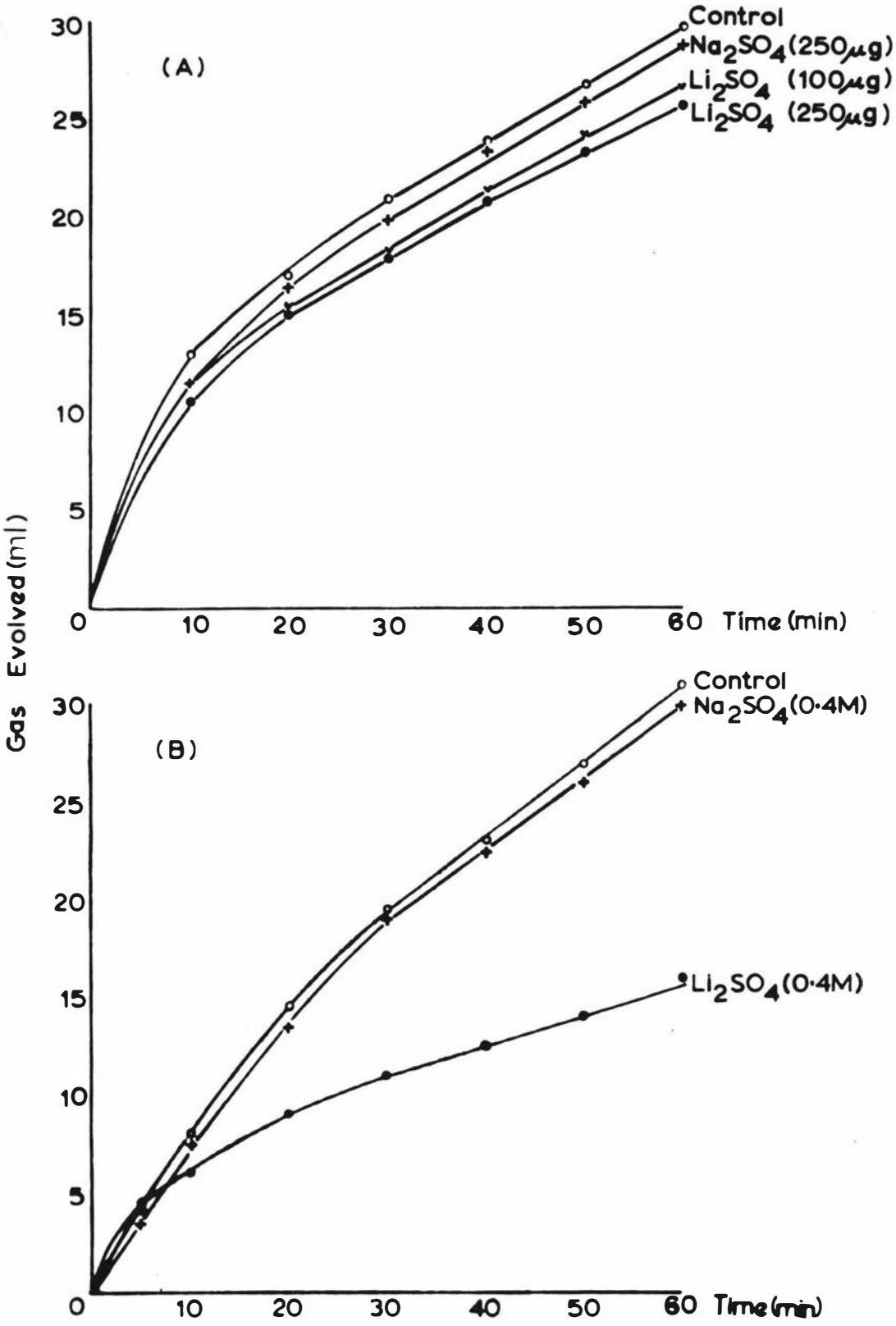


Table 48. Parotid Salivary Secretion of Lithium by Sheep 4

Sample Time (hr after dosing)	Amount of Saliva Secreted		Concentration of Lithium in Saliva (µg/ml)
	ml	ml/hr	
1	95	95	0
2	92	92	1.75
3	84	84	3.75
4	76	76	6.25
5	52	52	8.25
6	46	46	8.75
7	63	63	9.50
11	166	42	10.25
22	490	45	10.75
23	58	58	10.75

Fig. 19 Effect Of Lithium On The Gas Production Of Rumen Micro-organisms



in Fig. 18. From these curves it can be seen that, (a) the urinary excretion of lithium was faster in sheep 33 (40% of the administered dose was excreted in 72 hr as compared with 96 hr for sheep 3), and (b) sheep 33 excreted a much higher proportion of the dose in the faeces.

The concentration of lithium in the parotid saliva of sheep was measured after dosing with 0.5 g of lithium. The results from sheep 4 are presented in Table 48. Lithium was detected in the saliva during the second hour after dosing and the concentration steadily increased for 10 hr.

2. Toxicity of Lithium to Rumen Micro-organisms

The results of two of a series of experiments where the effect of lithium on the gas production of rumen micro-organisms was studied, are presented in Fig. 19. In Fig. 19A it can be seen that both 100 and 250 µg lithium per ml caused a reduction in gas production. Fig. 19 also shows that the reduction was not associated with the sulphate radical of lithium sulphate. A severe depression in gas production was obtained with a 0.4 M solution of lithium sulphate (Fig. 19B).

3. Comparison of Lithium with PEG as a Rumen Water Volume Marker

The results of two experiments in which lithium

Table 49. Comparison of PEG and Lithium as Markers for Estimating Rumen Water Volume and Rate of Flow of Water from the Rumen

Date	Sheep	Rumen Water Volume (ml)		Flow Rate (vol %)		Flow Rate (ml/hr)	
		PEG	Lithium	PEG	Lithium	PEG	Lithium
6 September 1961	1	1933	2818	22.5	21.8	435	612
	5	2577	3920	13.8	12.9	356	504
	6	2107	3497	16.0	12.2	338	425
27 September 1961	1	2492	3369	19.9	19.9	496	670
	5	2239	3401	15.6	12.3	349	418
	6	935	1812	30.9	24.0	289	435
	Mean	2047	3136	19.8	17.2	377	511

and PEG were compared as rumen markers are shown in Table 49. Rumen water volumes estimated with lithium were considerably higher than those with PEG. Flow rate estimated as a percentage of the rumen water volume changing per hr gave a higher figure for PEG. Water flow rates in ml per hr were largely a reflection of rumen water volumes.

DISCUSSION

The experiments described here on the absorption and excretion of lithium were intended to be qualitative only. The results obtained confirm those of other workers on lithium metabolism (Good, 1903; Berger, 1906; Kent and McCance, 1941; Radomski et al., 1950; Talso and Clarke, 1951). Lithium added to the rumen of sheep was rapidly absorbed, as was demonstrated by its appearance in venous blood and saliva within 3 hr of dosing, and because 40% of a lithium dose was excreted in the urine within 4 days. Faecal excretion of lithium was more variable and was much slower in the sheep having a lower food intake (Fig. 18). Because lithium has such a large distribution volume in the animal (Radomski et al., 1950; Talso and Clarke, 1951; Foulks et al., 1952) it is likely that lithium would continue to be excreted in small amounts for a long period.

In a note published since the present work was completed, Harrison, Hill and Mangan (1963) also presented results of studies on the absorption and excretion of lithium by sheep. Following the administration of 2 g of lithium sulphate to the rumen they found that lithium ions appeared in urine within 2 hr, reached a peak between 4 and 5 hr, and that 50% of the added lithium was excreted in the urine within 3

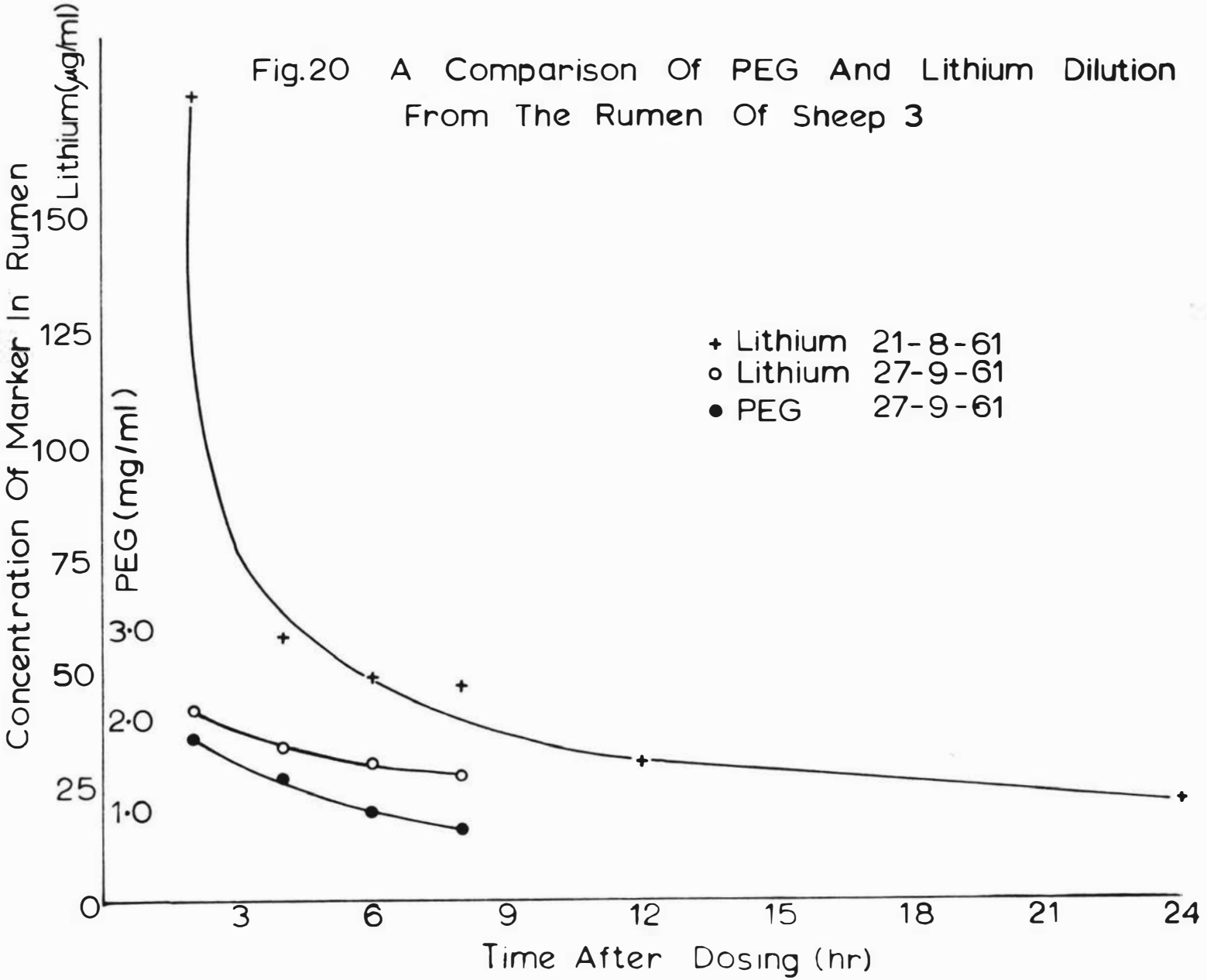
days. Further, these authors inferred that lithium was absorbed rapidly by the anterior part of the digestive tract and that a small amount was recirculated into the rumen in saliva. These results of Harrison et al. (1963) agree well with the present work.

Results of the in vitro fermentation experiments (Fig. 19) showed that lithium is toxic to rumen micro-organisms particularly in higher concentrations. However, for the purpose of a rumen marker, an initial rumen concentration of approximately 100 µg per ml would be adequate, and this level should not cause a large depression in microbial activity.

None of the symptoms of lithium intoxication in mammals described by Schou (1957) were observed in the present work. This is not surprising because the dose rates were kept low. If the dose rates were increased, or if frequent doses were administered, toxic effects on animals might become evident.

Lithium gave a 51% higher estimate of rumen water volume than PEG (Table 49). Hydén (1961) calculated that the distribution volume of PEG in rumen contents was approximately 95% of total rumen water. Using this figure it was calculated that lithium had an apparent distribution volume of 146% of the rumen water in the present experiments. There are two possible explanations for this high value with lithium: either

Fig.20 A Comparison Of PEG And Lithium Dilution From The Rumen Of Sheep 3



PEG gave a low estimate of water volume, or the lithium estimate was high. PEG has been dealt with in detail in Chapter 1: it is not absorbed or metabolised and appears to be adequate as a marker for determining rumen water volume. A more likely explanation would be that the estimates of initial lithium concentration were low, thus leading to a high estimate of rumen water volume. The reason for a low initial lithium concentration is not obvious. Apparently there was no analytical error as lithium could be recovered quantitatively from rumen liquor (Table 44). If lithium was being absorbed rapidly from the rumen the slope of its dilution curve should have been steeper than that of PEG; but this was not the case (Table 49). A possible explanation is that there was a rapid initial absorption of lithium in the 2 hr after dosing in the lithium-PEG comparison, and that by the first sampling time the rate had decreased. In addition, an increasing salivary influx of lithium into the rumen could have stabilized the concentration of lithium in the rumen. The work of Harrison et al. (1963) supports this view. When the rumen concentrations of lithium shown in Table 45 were plotted against time (Fig. 20), there appeared to be a very rapid reduction over the first 4 hr. The dilution curves obtained with this same sheep during the lithium-PEG comparison on 27 September are also

shown in Fig. 20. When the two lithium curves in Fig. 20 are compared it is conceivable that there may have been a rapid absorption of lithium before the first sampling at 2 hr on 27 September 1961.

From the results presented it was considered that lithium is unsuitable as a marker for estimating rumen water volume, mainly because of its rapid absorption.

SUMMARY

1. Lithium was tested as a marker for measuring rumen water volume.

2. A dose of lithium added to the rumen of a sheep was rapidly absorbed, as was indicated by its appearance in venous blood and parotid saliva within 3 hr. Forty percent of a lithium dose was excreted in urine within 4 days but faecal excretion was slower and more variable.

3. *In vitro* fermentations showed that lithium was toxic to rumen micro-organisms, particularly at concentrations of 250 µg/ml and higher.

4. Lithium was found to give a 51% higher estimate of rumen water volume than did PEG. Possible reasons for this are discussed.

5. It was considered that lithium is unsuitable as a marker for determining rumen water volume.

GENERAL DISCUSSION

In the work described in this Thesis two aspects of food intake regulation by sheep were encountered. Firstly, some of the factors influencing the intake of a palatable feed were considered. Secondly, a situation was described where an apparent lack of palatability restricted food intake.

1. The Regulation of Intake of a Palatable Ration

It appears that the basic urge in the feeding behaviour of mammals is hunger (Anand, 1961). When an animal eats to satisfy this urge a point is reached where the mechanism of satiety is stimulated to inhibit feeding behaviour. After a period of time the inhibitory effects of satiety diminish, the urge of hunger increases, and a point is reached where the animal starts eating again. Thus it appears that satiety and not hunger is regulated.

In this Thesis two theoretical mechanisms for regulating food intake in sheep were considered: the reduction of rumen load and chemostasis.

(a) Reduction of Rumen Load Theory

With ruminants on a poor quality feed it seems that physical repletion of the rumen can provide a stimulus for satiety. The time required for the rumen load to be reduced to the point where hunger recurs will be dependent on the rate of break down of the food in the rumen and the rate of flow of digesta

through the alimentary tract. The higher the food quality, the quicker the food is broken down and passed along the digestive tract, and the more frequently can the animal eat.

The present work confirmed that the rumens of P-fed sheep contained larger amounts of ingesta than those from S-fed sheep. Evans (1964) has shown that P is physically stronger than S. Consequently, it could be implied that P accumulates in the rumen because it is more resistant to break down than S. This hypothesis has not been tested experimentally. However, it is possible that S is broken down and passed from the rumen more rapidly than P, and that a higher food intake is a major factor contributing to the superior live weight gains normally found in S-fed sheep.

(b) Chemostatic Theory

When high quality foods are fermented in the rumen large amounts of metabolites are rapidly produced and absorbed and could cause satiety by chemostatic means. An example of such a chemostatic mechanism was provided in the experiments where VFAs were infused into the rumen and produced significant depressions in food intake. Another possible example can be drawn from the ryegrass comparison. The hypothesis that S is broken down and passed through the rumen more rapidly than P does not explain fully why the rumens of the

rumens of the S-fed sheep should contain less digesta. In other words, what stops the S-fed animals eating to repletion of their rumens? A feasible explanation is that the fermentation of S produces metabolites which are absorbed and cause a chemostatic limitation of intake. A similar situation was described by Freer and Campling (1963) where cows ate less and had smaller rumen "fills" on a concentrate than on a hay diet.

It is considered that in the normal ruminant, satiety can be regulated by a balance of the physical repletion of the rumen and chemostatic means. There may be thresholds at which either of these factors are limiting. The physical bulk of low quality feeds may limit intake because repletion of the rumen is attained before the regulating metabolites reach a threshold level in the blood, while on high quality diets the chemostatic threshold may be reached before repletion of the rumen is attained. The point at which chemostatic satiety is reached may be dependent on the physiological state of the animal and might be affected by factors such as pregnancy, lactation, plane of nutrition and environmental conditions.

2. <u>The Situation where an Apparent Lack of Palatability Limited Food Intake</u>

The present experiments provided an example

of how the mechanisms governing food intake in the normal animal may be counteracted by factors which cause an unpalatability of the food. In this case S was rendered unpalatable in the spring of 1962 and intakes of it were low. This effect, which was described as unpalatability, may have been due to either of two causes:

(a) The sheep might have found the grass repugnant to taste or smell. This is palatability in the accepted sense: a subjective assessment of the food by the animal.

(b) Toxic factors might have been present in the grass or produced during digestion in the rumen. Examples of this type of situation are: lactic acid poisoning, where the fermentation of high starch diets can lead to excess lactic acid production and normal buffering mechanisms in the rumen are overwhelmed; and nitrate poisoning where nitrite formed by the reduction of high concentrations of nitrate in the feed is absorbed and results in methaemoglobinaemia.

In practice it is often impossible to distinguish between (a) and (b), particularly when a toxic factor is operating subclinically.

The work presented here is the result of a preliminary examination of some factors implicated in the productive performance of sheep on pasture. It

has confirmed the complexity of the problem, particularly in regard to animal and plant variation. This variation is such that greater animal numbers are required than can be handled by one investigator. The volume of work involved in maintaining the pastures, handling experimental animals, processing the many samples for analysis and carrying out the chemical determinations calls for a team effort. The writer is fully conscious of the limited conclusions that can be drawn from the restricted number of experiments that he was able to carry out.

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