MODELS OF RUMEN SULPHUR METABOLISM IN SHEEP

by

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This Thesis is submitted for the degree of
Doctor of Philosophy in the University of Tasmania

University of Tasmania
HOBART

January, 1981

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of the thesis.

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ACKNOWLEDGEMENTS

I wish to acknowledge most sincerely my supervisor, Dr A C Bray, Senior Lecturer in Animal Production of the Faculty of Agricultural Science at the University of Tasmania, for his continuous help, encouragement and constructive criticism given throughout the course of this project.

I express my thanks to the technical staff of the Faculty of Agricultural Science, in particular, the later Mr M Blacklow and Mr A Beswick for their assistance in the preparation and maintenance of the experimental sheep and help with the experimental work and to Mr W Peterson for his technical advice and assistance.

I also wish to thank Ann Rickards for the arduous task of typing the manuscript and Kieran Nixon for her assistance in drawing the diagrams.

Financial support was provided by the Australian Wool Research Committee, and a University Postgraduate Studentship Award and to them I am deeply grateful.

SUMMARY

A preliminary investigation (Part III) was carried out to investigate the losses of volatile sulphur compounds from sheep maintained on a high sulphur ration (total N = 1.980% of dry matter, total S = 0.395% of dry matter, N/S = 5/1), following intraruminal administration of sodium [^{35}S] sulphate.

Sheep were fed their rations at one hourly intervals with urea and inorganic sulphate being virtually the major sources of nitrogen and sulphur respectively in order to establish steady-state conditions in the rumen, that is constant rumen sulphide concentration and pH were maintained under these conditions.

The first experiment (Part IV) was conducted with a low sulphur intake (total N = 1.417% of dry matter, total S = 0.159% of dry matter, N/S = 8.9/1) in order to gain experience with modelling and test the proposed model. For the same purpose the sheep received a high sulphur ration (total N = 1.826% of dry matter, total S = 0.321% of dry matter, N/S = 5.7/1) in the second experiment (Part V).

Because of the anaerobic conditions predominant in the rumen, protein synthesis is mainly limited by available energy. In the third (Part VI) and fourth (Part VII) experiments the effect of energy intake on microbial protein synthesis and absorption rate from the rumen was studied. In the third experiment, the sheep were maintained on a high sulphur ration (total N = 1.753% of dry matter, total S = 0.312% of dry matter, N/S = 5.6/1) and 15% of the oat hulls were replaced by starch. In the fourth experiment, the sheep received a high sulphur ration as previously (total N = 1.681% of dry matter, total S = 0.302% of dry matter, N/S = 5.6/1) and 30% of the oat hulls were replaced by starch.

During the course of these experiments, recent marker techniques were used to study the flow of digesta and the sulphur flow rates from the rumen.

PART I

LITERATURE REVIEW

Section 1: INTRODUCTION

The rumen in its functional state is a complex continuous-flow system, therefore the control of ruminant digestion is multifactorial and the interactions of host, microbes, physical and chemical properties of feedstuffs and digestion end-products are very complex. In this part a review has been made of the models theory (Section 2); the simulation and tracer method (Section 3); models of nitrogen and sulphur metabolism in the rumen of sheep (Section 4); the metabolism of nitrogen and sulphur in the rumen (Section 5); sulphur toxicity (Section 6); the excretion and retention of sulphur (Section 7); and the proposed model of sulphur metabolism in the rumen of sheep (Section 8).

Section 2: MODELS THEORY

(a) The Concepts of a System

A *system* is a group of physical components connected or related in such a manner as to form and/or act as an entire unit (Patten, 1971). Systems can be considered as bounded sectors of reality.

The *state* of a system is the condition of its state variables.

The *state variables* of a system are its component parts or observable attributes, or arbitrary groupings of parts or attributes for particular purposes. State variables characterise and quantify all properties that describe the current state of the system.

Let

$$X(t) = [x_1(t), x_2(t), \ldots, x_n(t)]$$

represent the state of a system with n state variables

$x_i(t)$, $i = 1,2,\ldots, n$ where each state variable is a function of time t. If after a time interval Δt at least one of the n state variables has changed from $x_i(t)$ to $x_i(t+\Delta t)$, then the *amount of change* which has occurred can be expressed as $\Delta x_i = [x_i(t+\Delta t) - x_i(t)]$, and the *rate of change* by

$$\frac{\Delta x_i}{\Delta t} = \frac{x_i(t+\Delta t) - x_i(t)}{\Delta t}$$

Change, or lack of it, constitutes system behaviour. The *behaviour* of a system is its mode of acting, or more operationally, a sequence of state changes in time. The sequence may be *discrete* or *continuous*.

(b) Static and Dynamic Systems

Systems which do not change are *static* or *constant*. All other systems are *dynamic*.

(c) Models of Systems

All models are abstractions (Patten, 1971). The key to effective modelling is to strike a proper balance between realism and abstraction. Technically, a *model* is a "homomorphism" of some real system which it represents (Ashby, 1956). One of the definitions that the dictionary (Webster's New Collegiate Dictionary, 1953) gives for *model* is "a miniature representation of a thing; sometimes a facsimile". *Facsimile*,

according to Webster, means an exact duplicate. Baker (1969) states that,"a *model* is a representation of a hypothesis in which a formal structure for a system is proposed". It is usually a simplified representation of a physiological system. The model, like all hypotheses, is derived from experimental observations, previous knowledge, and a number of assumptions. Batschelet (1966) has pointed out that "models are of a transient nature"; therefore, some models are a better reflection of reality than others. With this in mind, there is no need for the scientist who has used a model to apologise in any way for having presented a model which may not be correct.

(d) Compartment Models

A *compartment model* is an abstraction of a system whose dependent variables can be thought of as describing the contents of various blocks or compartments or pools between which a flow of material or energy, represented by interconnecting arrows, takes place (Bledsoe and Van Dyne, 1971). In compartment models relations between the state variables are usually expressed as a system of differential equations. The system equations are based on an income-and-loss rationale dictated by the *conservation principle*: "All energy or substance transmitted must be accounted for". Once a system is made, what is part of the system and what is external to it becomes fixed. Input signals to the system which originate in energy or information sources outside the system will be termed *forcings* or *drivings*; they are not affected by processes within the system but characterise the influence from outside.

(e) Definition of Flows in Compartment Models

An elementary unit of a compartmentalised biological system is shown in Figure 1, where $F_{ij}(x,t)$ is the *flux* of energy or matter from compartment i *to* j (note emphasis on direction), and $x_i(x,t)$ and $x_j(x,t)$ are concentrations in the *donor* (or source) and *recipient* (or terminal) compartments, respectively. If the concentration units are, e.g. milligrams per litre, then corresponding flux units might be, e.g. milligrams per litre per day. The *flux* or *flow* over a branch in a compartment model is the amount of energy or material delivered to the terminal compartment in a unit interval of time. The *rate* of transfer, or *flow rate* (or rate constant), is the fractional quantity of some function of source or terminal materials delivered over a branch per

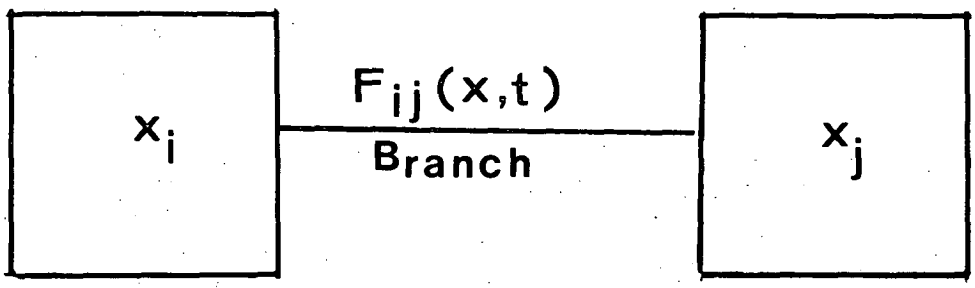


FIGURE 1. An elementary unit of a compartmentalised biological system.

unit time. The distinction between flux and rate is made best in terms of units. If a flux F_{ij} is in milligrams per litre per day, then the corresponding rate of flow ϕ_{ij} is expressed per day. Rate variables quantify the rate of change of the state variables. Their values are determined by the state variables and the driving variables. At any particular time, each rate depends only on the current values of the state variables and driving variables, and can therefore be calculated independently of all other rates.

(f) Model Hierarchy

A series of equations describing the fluxes associated with each compartment can be generated. These flux equations can be grouped on the basis on linearity or nonlinearity. Flux functions involving only one compartment (donor or recipient) are generally linear, while functions involving two or more compartments (e.g. donor and recipient jointly) are nonlinear. The mathematics of linear systems is well understood; the construction and analysis of linear models is relatively easy.

A hierarchy of useful mathematical expressions for flows in biological compartment models and their corresponding rationales is (Patten, 1971):

(1) $F_{ij} = \kappa$ (constant). Flow from compartment i *to* j does not change with time or system state.

(2) $F_{ij} = \phi_{ij} x_i$. Flow from compartment i *to* j is proportional to the content of i. The donor compartment only is controlling.

(3) $F_{ij} = \phi_{ij} x_j$. Flow is regulated by the receiving compartment only. These first three functions represent linear flows; those which follow denote nonlinear flows.

(4) $F_{ij} = \phi_{ij} x_i x_j$. Flow is regulated jointly by both source and terminal compartments.

(5) $F_{ij} = \phi_{ij} x_i (1-\alpha_{ij} x_j)$. The flow has two components: a positive, linear one regulated by the donor compartment and a negative, nonlinear one controlled by interaction of both compartments.

(6) $F_{ij} = \phi_{ij} x_j (1-\alpha_{ij} x_i)$. A positive linear compartment is controlled by the terminal compartment and a negative, nonlinear one by inter-compartmental interaction.

(7) $F_{ij} = \phi_{ij} x_j (1-\beta_{ij} x_j)$. The flow is regulated by a positive feedback, linear term and a negative feedback, nonlinear term.

(8) $F_{ij} = \phi_{ij} x_j (1-\alpha_{ij} x_i - \beta_{ij} x_j)$. This flow corresponds to a system with two negative feedback loops to represent both interactive ($-\alpha_{ij} x_i x_j$) and intrinsic ($-\beta_{ij} x_j^2$) flow control by the terminal compartment.

(g) Stages of Model Development

Model development is an orderly process (Baldwin *et al.*, 1977) and should advance through the achievement of the steps (Gordon, 1969) illustrated in Figure 2.

The need for attainment of each of these steps in sequence should be evident and has been discussed by many including Forrester (1971) and Riggs (1973). A most important step is stating the modelling objective. It provides the basis for selection of concepts and data for inclusion in the model, for determination of equation forms and mathematical techniques to be used, and for determination of the extent of aggregation of elements within the model.

Block diagrams represent, in diagrammatic form, the essential elements of complex systems and interaction among their elements. During the course of modelling, block diagrams are usually modified a number of times because of inadequacies identified during steps 3 to 5 as indicated by arrows in Figure 2. Conversion of the block diagram to mathematical statements is usually a straightforward process. The objective statement usually specifies or implies the type of equations required and the block diagram identifies the functions and transformations which must be represented.

Provision of satisfactory data for solution of the model is a difficult step. It is unusual to find in the literature, the exact set(s) of rate constants, parameters and other numerical inputs required. Often, it is found during the course of modelling, that specific data are not available in any form in the literature. In this case, the modelling exercise has fulfilled one of its objectives - identification of key

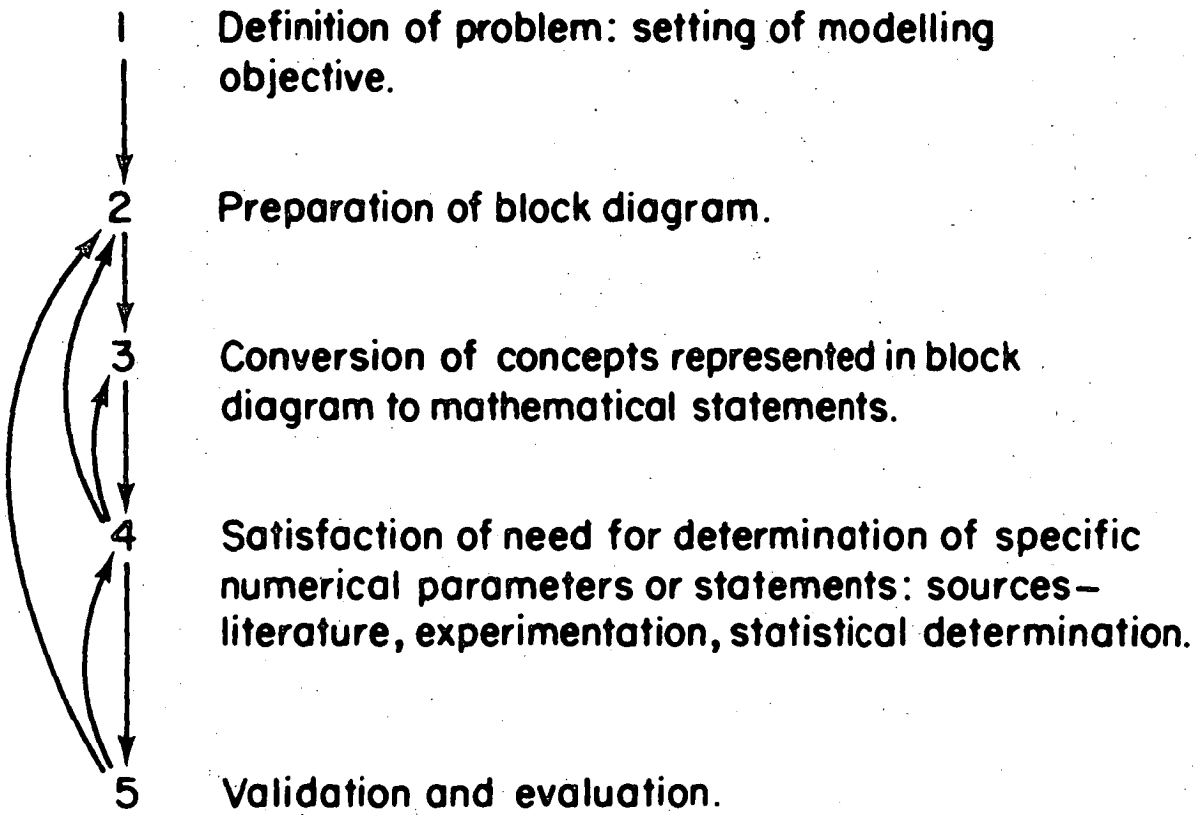


FIGURE 2. Stages of model development (Gordon, 1969)

experiments. Validation of models is essential to the modelling process, but is a difficult procedure (Fishman and Kiviat, 1967; Van Horn, 1971). A model must be constructed in such a way that it can be challenged experimentally by the authors of the model and by others (de Wit, 1969). As indicated by the arrows in Figure 2, the usual result of validation is a return to steps 2, 3 or 4 (i.e. the model requires additional work).

Section 3: THE SIMULATION AND TRACER METHOD

(a) The Simulation Method

Simulation is the use of a model to represent essential characteristics of a system (White, 1975). The construction of such a model helps to clarify ideas about the system. It identifies gaps in existing knowledge and may therefore indicate the relative importance of alternative avenues for further research. In general, simulation is considered a part of systems analysis. It normally involves use of a numerical model to study the behaviour of a system as it operates over time (Saila, 1972). An operational definition of *simulation* is the building of a dynamic model and the study of its behaviour (de Wit and Goudriaan, 1978). The use of simulation is likely to contribute to an understanding of the dynamics of the system. A simulation model imitates (simulates) the behaviour of a complex system in some different medium - most commonly a computer - so that the behaviour of the model is conceptually similar to that of the real system. A simulation model is based on a mathematical representation of the dynamics of a real-life system (Frenkiel and Goodall, 1978). Although simulation modelling existed earlier, it could not be developed to any significant extent until reasonably advanced computers were available, and these were not manufactured until the 1950s. This is not to suggest that simulation modelling cannot be performed without the use of computers; in fact, some simple models not relying on computers have had appreciable relevance in the biological field (Frenkiel and Goodall, 1978). However, complex simulation models could not be developed and applied successfully - and would probably not have even been conceived - without the availability of computers. The main advantages of using computers for modelling are two (Frenkiel and Goodall, 1978):

Firstly, a computer can store enormous amounts of data and retrieve them almost instantaneously. *Secondly*, computers can operate much faster than the human brain, carrying out millions of calculations per minute without making mistakes. Use of the computer thus increases our computational ability by approximately six orders of magnitude. As Biswas (1975) remarks, "The computer is probably the most patient and obedient servant that man has ever found to carry out instructions without asking any embarrassing questions"; but it should be noted that the computer will obey instructions even if the instructions are nonsensical.

In the early days of the development of computers, programmes were necessarily written in what were called 'machine-languages', and these languages were specific to a particular type of machine, and sometimes to an individual machine. Fairly quickly, however, it became possible to write programmes in simpler ways, at first in what were called 'autocodes' and then in several high-level languages. The best known of these languages are undoubtedly FORTRAN (standing for FORmula TRANslation) and ALGOL (standing for ALGOrithmic Language), and a very large number of all computer programmes for scientific computations is written in either of these two languages. More recently, the languages of BASIC (Beginner's All-purpose Symbolic Instructional Code) has been added to the list of commonly used languages, its main advantages being that it is easy to learn and that it is particularly appropriate for interactive use of computers through terminals.

(b) Types of Models

Many types of models have been used in system studies and have been classified in a number of ways. The classification is sometimes made in terms of the nature of the system they model, such as continuous versus discrete or deterministic versus stochastic. A classification of models is illustrated in Figure 3 (Gordon, 1978).

(1) Physical Models

The state variables of the system are represented by physical measures. The best known examples of physical models are scale models used in wind tunnels and water tanks to study the design of aircraft and ships.

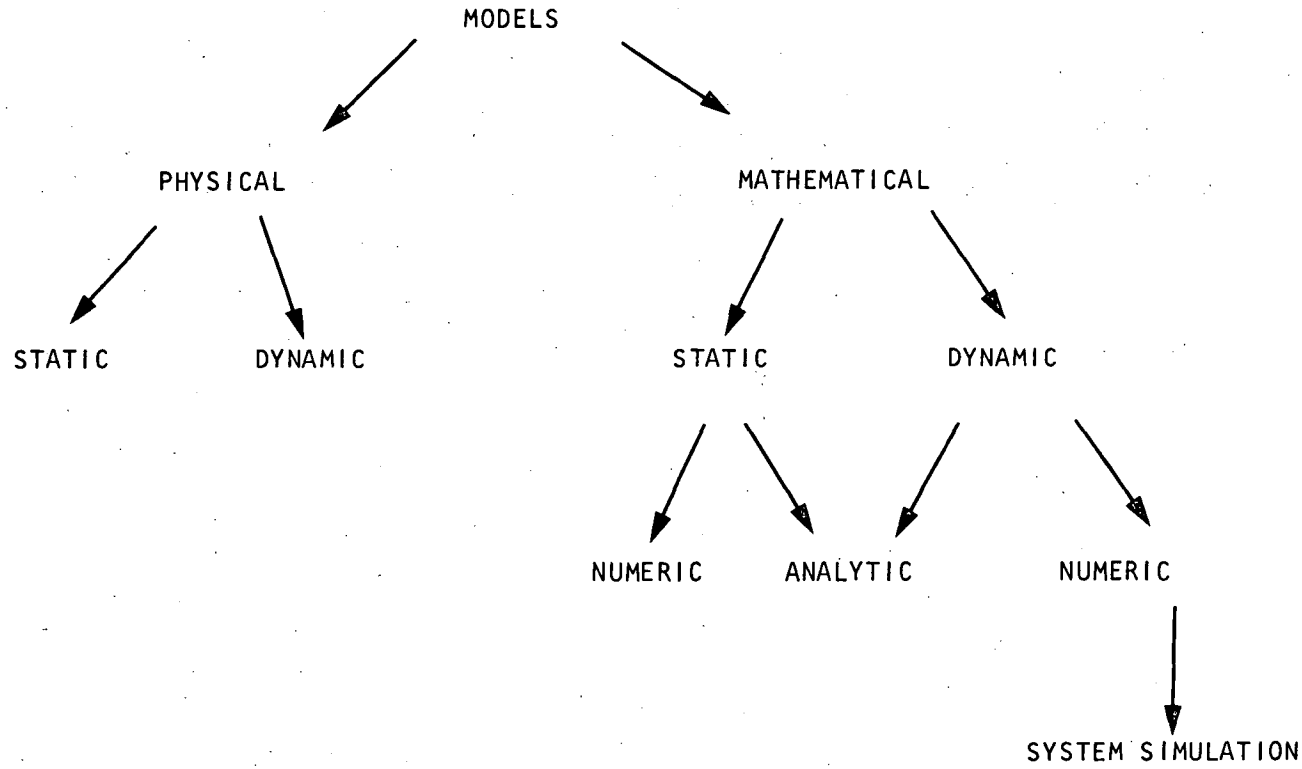


FIGURE 3. Classification of models (Gordon, 1978)

Other types of physical models have been described as *iconic models*, that is, models which "look like" the system they model; for example, the models of molecular structures that are made from spheres representing atoms with rods representing atomic bonds. Both scale models and iconic models are examples of *static physical models*.

Dynamic physical models rely upon an analogy between the system being studied and some other system of a different nature, the analogy usually depending upon an underlying similarity in the forces governing the behaviour of the systems.

(2) Mathematical Models

In a mathematical model, the state variables and their relationships of a system are expressed by mathematical symbols. Mathematical models are considered to be either *static* or *dynamic*.

A *static* model displays the relationships between the system attributes (state variables) when the system is in equilibrium. The model may be solved analytically or it may be necessary to solve it numerically, depending upon the nature of the model.

A *dynamic mathematical model* allows the changes of system attributes (state variables) to be derived as a function of time. The derivation may be made with an analytical solution or with a numerical computation, depending upon the complexity of the model.

Simulation is considered to be a numeric method for solving the dynamic mathematical model.

Baldwin *et al.* (1976); Baldwin (1977), have used the rumen digestive system to illustrate various aspects of modelling philosophy and techniques. According to the above authors, models may be considered as balance models, based on simultaneous algebraic equations or as dynamic models, based on ordinary differential equations.

(i) Balance Models

Whenever a series of metabolic equations describing pathways for conversion of nutrients to products is written and solved to obtain a net

balance, a balance model has been constructed and used. Many such models have been worked out by hand and published (Wolin, 1960; Hungate, 1966; Milligan, 1971; Krebs, 1964; Blaxter, 1962; Ball, 1965; Baldwin, 1968; 1970 a,b; Baldwin *et al.*, 1970; Baldwin and Smith, 1971 a,b; Smith, 1970). Several advantages can be obtained if such balance models are programmed for solution on computers. These include a reduction in labour required for solution, improved accuracy and speed, elimination of simplifying assumptions usually made to facilitate hand solution, and, as a result of the latter, the development of more general models. A very powerful application of balance models is their use along with statistical procedures to estimate parameter values not directly measurable.

(ii) Dynamic Models

Several dynamic models representing various aspects of rumen digestion have been published. Blaxter *et al.* (1956) mathematically analysed the excretion of marker in the faeces of sheep. They suggested that passage of feed residues through the gastrointestinal tract could be represented as a simple two-compartment model (tentatively assigned as rumen and abomasum) with a time delay in the intestines.

An approach, favoured by many, to the development of dynamic models is to start with a very simple model and to add equations representing additional concepts as one is required to do so in order to simulate better an increasing range of conditions or to make the model more generally applicable. Baldwin *et al.* (1976) state that this approach can be illustrated by starting with the model of Waldo *et al.* (1972, Figure 4) which can be represented by two differential equations:

$$dA/dt = -K_1A - K_2A$$
$$dB/dt = -K_2B$$

These workers demonstrated that good agreement between experimental results and computed estimates of rates of cellulose passage from and digestion in the rumen can be obtained using this model on a single chart. However, the rate constants K_1 and K_2 must be changed to accommodate changes of dietary composition. Thus, the numerical values in the model are not generally applicable. A general causal model of rumen digestion must contain provisions for variation in rates of fermentation and passage with diet. A more complex model by Baldwin *et al.* (1977) incorporating

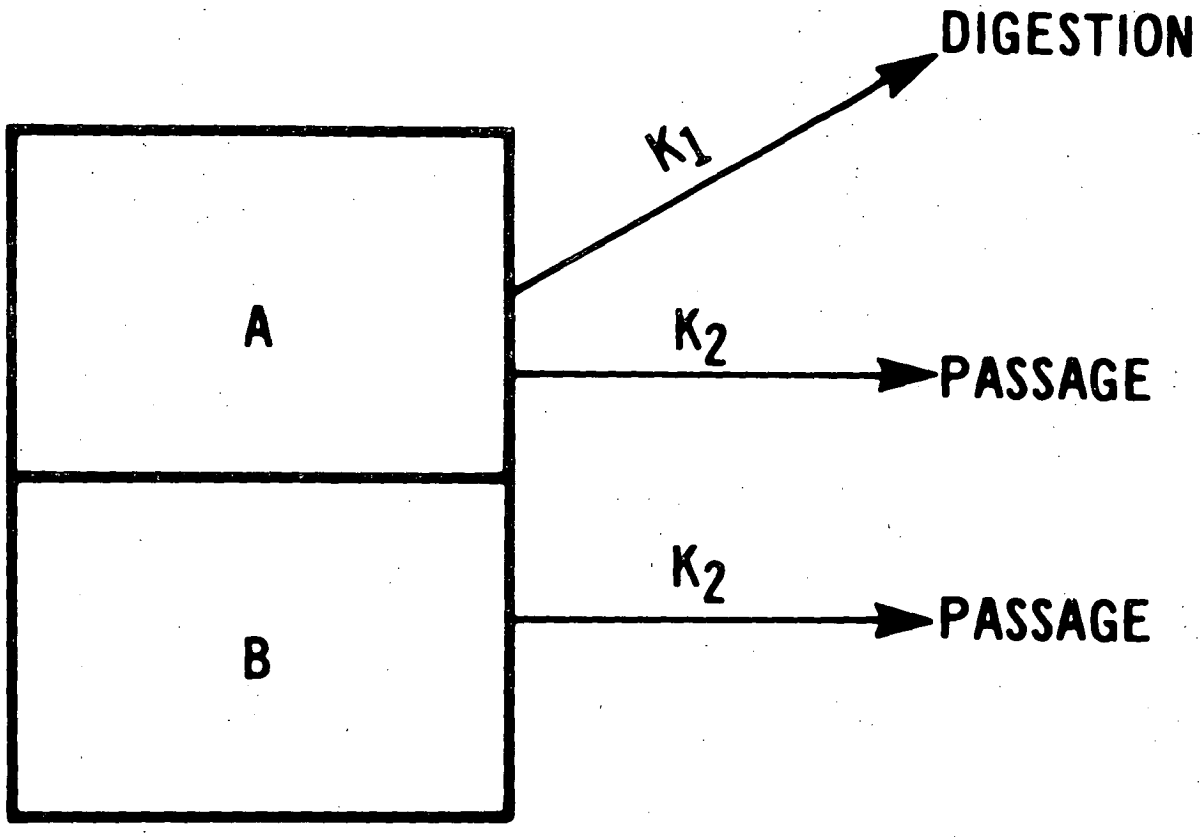


FIGURE 4. Diagrammatic representation of the model of Waldo *et al.* (1972). A = potentially digestible cellulose; B = indigestible cellulose.

the concepts represented in the Waldo model and, in addition, the concept of large and small particle pools in the rumen (Hungate *et al.*, 1971) and provision for cellulose fermentation in the lower gut, proved to be more general in that computed digestibilities of a wider range of feedstuffs compared favourably with experimental results. Smith (1970) constructed, based on an extensive analysis of the literature, a comprehensive model of ruminant animal metabolism. According to Baldwin *et al.* (1976), this represented the first attempt made toward dynamic analyses of ruminant metabolism and of the question posed earlier regarding causal relationships determinant of patterns and varying efficiencies of nutrient utilisation (Baldwin and Smith, 1971b). Evaluation of the behaviour of this model emphasised the inadequacy of our knowledge, at that time, of ruminant adipose function. This finding indicated that experimental studies of ruminant adipose function should receive high priority.

(c) The Tracer Method

(1) General Considerations

The tracer method is a technique to observe certain characteristics of a population of specific things such as molecules, organisms or other entities by observing the behaviour of the tracer (see Technical Reports Series No. 60, 1972). The substance to be traced is generally termed the *tracee*. Nolan *et al.* (1972) have used the following terminology in studies of tracer kinetics.

Tracee: the defined substance whose movement and behaviour in the system is under study.

Tracer: defined substance labelled (e.g. by use of isotopes) so that its movement and behaviour in the system can be studied. The criteria for an ideal tracer is that it be indistinguishable from the tracee at the unit level and that the introduction of the tracer does not disturb the system. Both of these criteria are nearly perfectly met by radioactive isotope tracers. Isotopes have identical chemical properties (only slight mass differences) and they can be obtained in very high specific activity. Therefore, the introduction of an isotopic tracer generally adds negligible mass to the system and does not disturb its kinetics. Tracer can be delivered to a pool system as a single, abruptly

administered dose, or delivered over an extended period as by continuous infusion at a constant rate, with or no priming dose (see Shipley and Clark, 1972).

(2) Tracer Dilution

The tracer dilution technique has been very useful in determination of the exchangeable mass of a substance in a system. The principle is: that for a given constant amount of tracer radioactivity, the final specific activity is inversely proportional to the exchangeable mass of tracee in the system. The technique is particularly useful when quantitative separations are not possible or are too tedious for the systems under study. In addition, it is the principal technique used to measure the exchangeable mass *in vivo*. Consider a system that contains an unknown amount, S grams, of a substance. To this system is added a known amount of a radioactive tracer of initial specific activity a_i, so that

$$a_i = \frac{R}{\sigma} \qquad (1)$$

where R = activity of the tracer in μCi (or counts/min)

σ = mass of tracee associated with tracer

If the tracer is allowed to mix in the system then, according to the dilution principle, the final specific activity, a_f, will be

$$a_f = \frac{R}{S+\sigma} \qquad (2)$$

Substituting (1) into (2) and solving for S

$$S = \sigma \frac{a_i}{a_f} - 1 \qquad (3)$$

Therefore, to determine S, only the final specific activity need be measured, as a_i (and therefore σ) is known. Quantitative separation of the tracee in the sample from the system is not necessary because the specific activity is independent of sample size, recovery etc. However, it is a necessary condition that the tracer be *completely mixed* with the tracee in the system. This condition becomes very important in tracer dilution studies *in vivo*.

Very commonly, σ is negligible compared with S. This is the case with

"carrier-free" or high specific activity tracers. By inspection of Eq. (2), if σ is negligible with respect to S, then

$$S = \frac{R}{a_f} \qquad (4)$$

and only the initial tracer activity and the final specific activity need be known.

(3) Tracer Kinetics

The principal difficulty in the tracer dilution technique is to ensure uniformity of mixing of the tracer. To determine the mixing time it is necessary to take repetitive samples as a function of time from the system. Such data contain valuable information on the kinetics of the mixing processes. It is such analysis as well as the response of the system at "tracer equilibrium" that is treated by the field of *tracer kinetics*.

Most biological systems are open, that is, there is exchange with their environment. Consider an open compartment as shown in Figure 5 in the steady state. A compartment is a subdivision of a system in which the tracer specific activity is constant within its boundaries at any given time. Thus, the tracer specific activity defines the boundaries of a compartment and they may or may not coincide with any chemical, physical or physiological boundaries. Mixing within a compartment is rapid compared with the rate that tracer leaves the compartment. If the compartment is in the steady state then the input rate is equal to the output rate or

$$I = \kappa S \qquad (5)$$

Therefore, S is constant but the tracer activity R and the specific activity *a* vary with time (see caption to Figure 5 for the symbol definitions).

Consider now a compartment in which a tracer has been injected and allowed to mix. Most compartments in nature are observed to follow first-order kinetics. That is, the specific activity of the tracer is observed to decline exponentially, as

$$a = a_o e^{-\kappa t} \qquad (6)$$

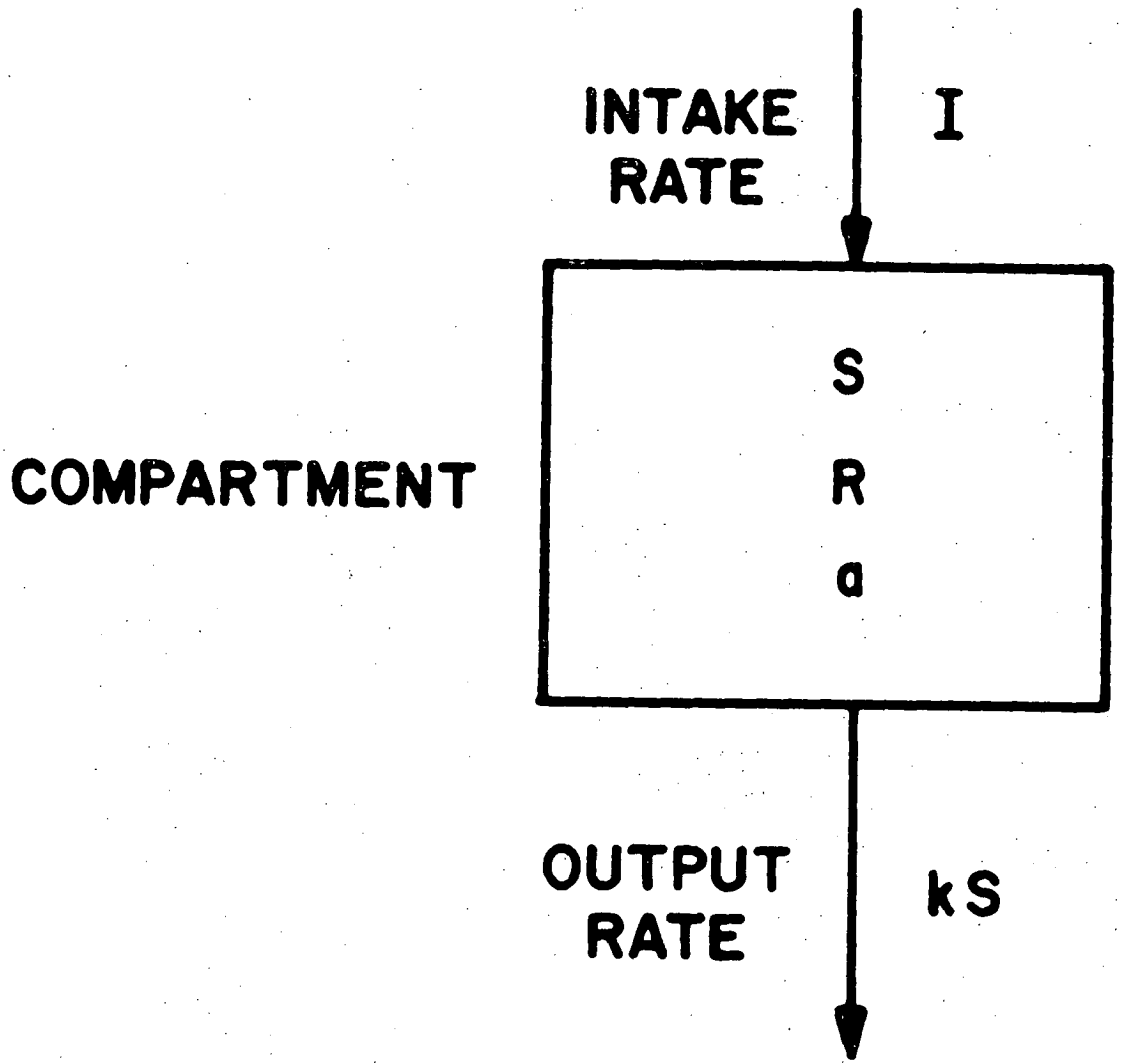


FIGURE 5. Schematic model of single open compartment.

S = Tracee in grams
R = Radioactivity of tracer in μCi
a = Specific activity

$$= \frac{R}{S} \quad \frac{\mu Ci}{gram}$$

I = Intake rate in grams of S per unit time

where κ is the first-order rate constant.

A plot of specific activity (after mixing) versus time would appear as in Figure 6. The slope of the line allows calculation of κ. Exactly analogous to radioactive decay, a half-life may be determined graphically or by

$$T_{\frac{1}{2}} = \frac{0.693}{\kappa} \tag{7}$$

$T_{\frac{1}{2}}$ is commonly termed the *biological half-life*. Since it was assumed that the tracer behaved exactly as the tracee it is now possible to calculate the output rate κS. S can be determined by tracer dilution.

Consider a closed two-compartment model ("closed system" means there is no communication with the external environment) as shown in Figure 7.

If compartment 1 is initially labelled, the following differential equations may be written

$$\frac{dR_1}{dt} = \frac{S_1 da_1}{dt} = \rho\ (a_2 - a_1) \tag{8}$$

$$\frac{dR_2}{dt} = \frac{S_2 da_2}{dt} = \rho\ (a_1 - a_2) \tag{9}$$

Let $\Delta_{1,2}$ equal the difference in specific activities at any time.

$$\Delta_{1,2} = a_1 - a_2 \tag{10}$$

$$da_1 - da_2 = d\Delta_{1,2} = -\rho\ (\frac{1}{S_1} + \frac{1}{S_2})\ \Delta_{1,2} \tag{11}$$

Equation (11) is observed to be a first-order differential equation and may be integrated directly to give

$$\Delta_{1,2} = a_1(0)e^{-\rho(\frac{1}{S_1} + \frac{1}{S_2})t} \tag{12}$$

Where $a_1(0)$ is the initial condition that at $t = 0$ all the activity is in compartment 1. Therefore, $\Delta_{1,2}(0) = a_1(0)$.
Now since the system is closed, the total activity is constant. This can be expressed as

$$S_1 a_1 + S_2 a_2 = S_1 a_1(0) \tag{13}$$

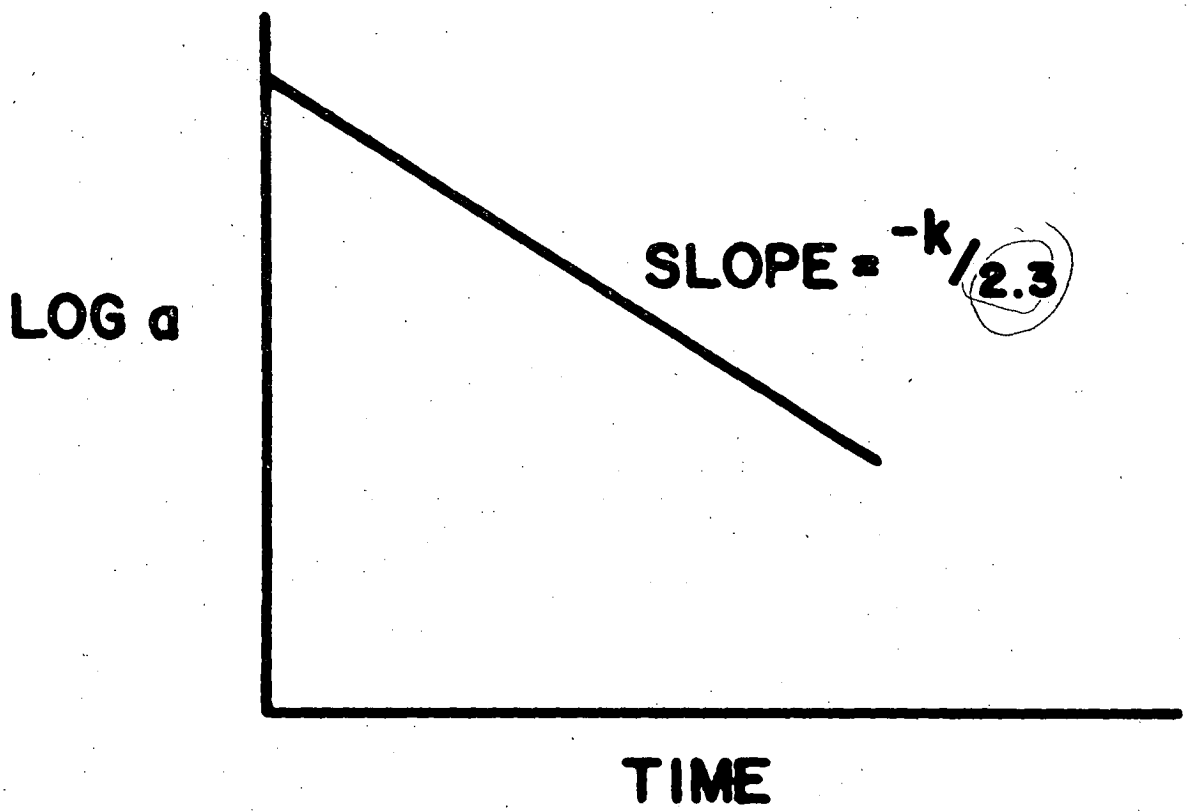


FIGURE 6. Specific activity versus time in a single compartment after mixing of the tracer

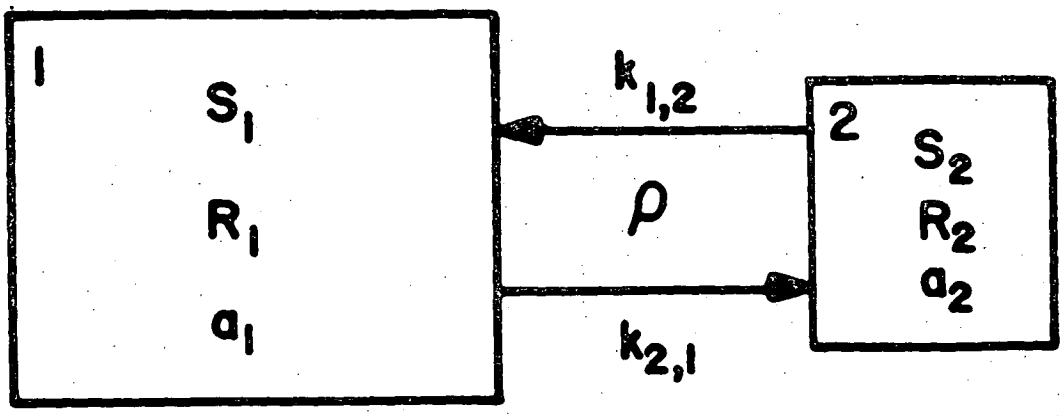


FIGURE 7. Closed two-compartment model in steady state

$K_{1,2}$ = first-order rate constant describing exchange of tracee or tracer from compartment 2 to compartment 1

$K_{2,1}$ = first-order rate constant describing exchange of tracee or tracer from compartment 1 to compartment 2

ρ = rate at which tracee is exchanged, i.e. grams/unit time.

If Eqs (12) and (13) are solved simultaneously, the following solutions are obtained

$$a_1 = \frac{a_1(0)}{S}\,(S_1 + S_2 e^{-\rho St/S_1S_2}) \qquad (14)$$

$$a_2 = \frac{a_1(0)S_1}{S}\,(1 - e^{-\rho St/S_1S_2}) \qquad (15)$$

where $S = S_1 + S_2$

Plots of a_1 and a_2 versus time are shown in Figure 8.

Total S may be determined by the dilution technique. Now by inspection of Eq. (14) at long times, i.e. when the tracer is completely mixed, a_1 equals the equilibrium value

$$a_1 = \frac{a_1(0)S_1}{S} \qquad (16)$$

Therefore, one can solve for S_1 and hence S_2 by difference. Now a plot of Eq. (12) will allow calculation of the slope

$$\rho\left(\frac{1}{S_1} + \frac{1}{S_2}\right)/2.3 \quad \text{(see Figure 9)}$$

Therefore, from the slope, the *transfer rate* ρ may be determined. To obtain transfer rates between compartments is the objective of most tracer kinetic experimentation. It should be noted that

$$\rho = k_{1,2}S_2 = k_{2,1}S_1 \qquad (17)$$

which expresses the steady-state condition.

(4) Analysis of Isotope Dilution Curves

When a tracer is injected into any (primary) compartment of a system in steady-state, and the change in concentration of tracer with time is determined in the compartment, it is possible to estimate several parameters : the size of that compartment, the total rate of flux of the traced substance through that compartment, and the rate of entry and irreversible loss of substance, the difference between the last two parameters being the rate of recycling of traced substance back to that

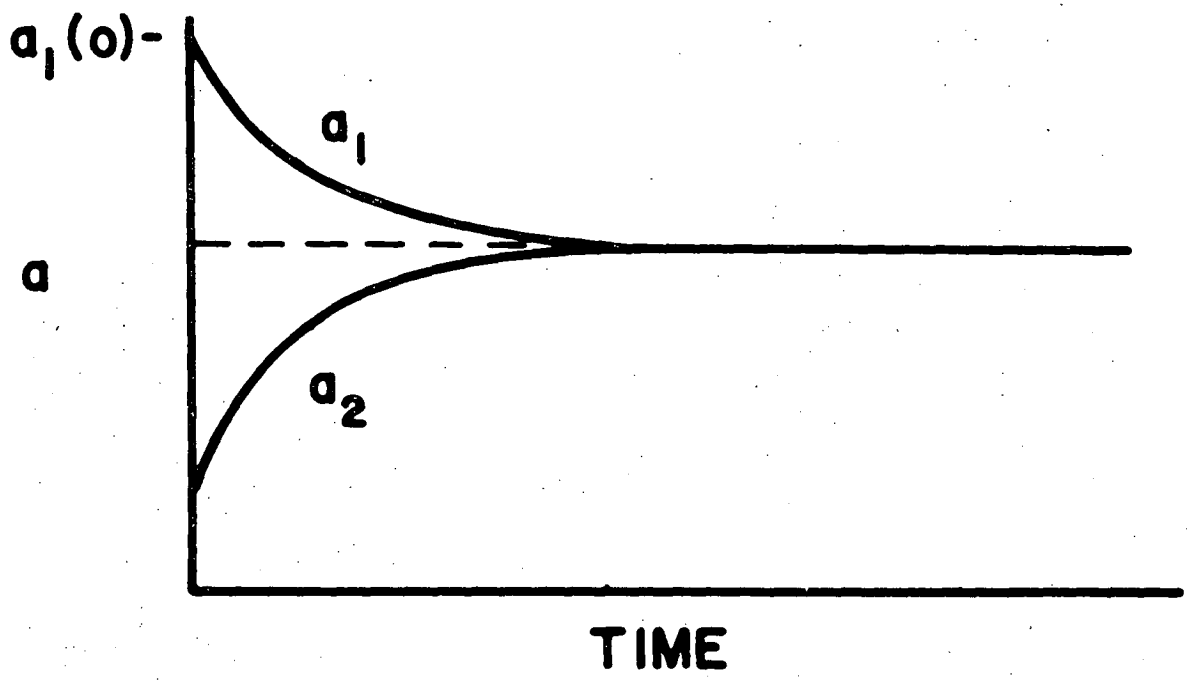


FIGURE 8. Specific activity in a closed two-compartment system with compartment 1 initially labelled

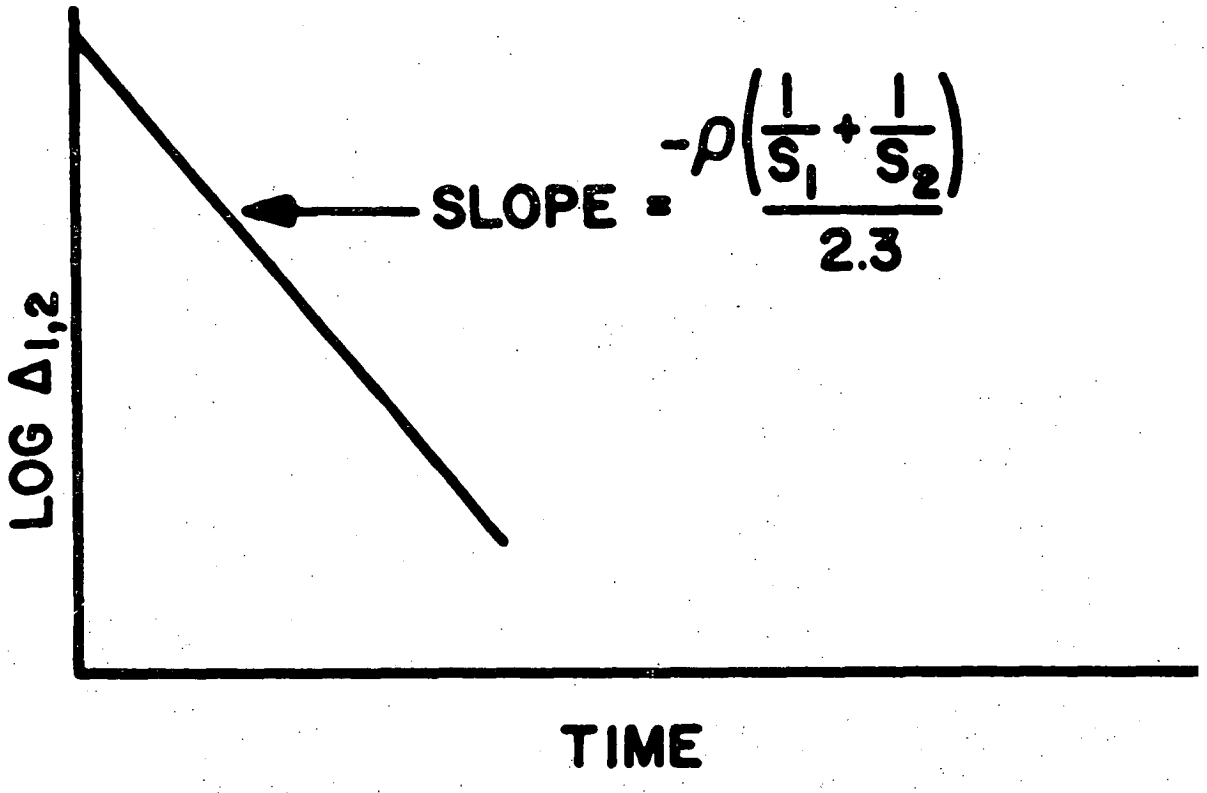


FIGURE 9. Plot of Eq. (12) for closed two-compartment model

compartment during the experiment (Nolan and Leng, 1974; Nolan and Rowe, 1976). If another (secondary) compartment is sampled concurrently, and its size is independently determined, it is theoretically possible to obtain a complete solution to a general two-compartment model by a suitable algebraic analysis in which a mathematical description of the time-course of concentration of tracer in both compartments is used, i.e. the multi-exponential equations to the curves. Once the coefficients and exponents of the equations are obtained, these are used algebraically to define the differential equations for which the experimental curves are the model solution under that set of conditions. Even two-compartment models are often quite difficult to solve by this procedure since, in tracer dilution experiments with animals, the curves of tracer concentration with time in sampled compartments, are usually composed of more than two exponential functions. Nevertheless, this type of analysis requires that the curves be fitted by only two such functions, and forcing such a fit to experimental data (which are derived from a much more complex multi-compartmental system and are also subject to considerable errors) can pose difficulties (Nolan and Rowe, 1976). Notwithstanding, the procedures have been used in numerous studies, and these studies and methods of analysis have been reviewed, for example, by Atkins (1969) and Shipley and Clark (1972). Alternatively, a similar solution (but, in addition, the size of the second compartment) is obtainable if two different tracer infusions or injections are made separately, one into each of the two compartments. In this procedure, the information used is either the "plateau" tracer concentrations from continuous infusions experiments or the area under the curves describing tracer concentration with time for single injection experiments. During analysis of single injection experiments, the areas under the curves can be obtained relatively simply (e.g. by planimetry), and the difficulties associated with curve fitting can be avoided (Katz *et al.*, 1974 ; Nolan and Leng, 1974). The information available from the time course of the tracer curves is not required, time is removed as a variable, and solution is by means of linear simultaneous equations. This alternative procedure which is also mathematically simpler, seems to have received little attention recently, although the theory of this analysis has been described in a number of publications that are summarised by Mann and Gurpide (1966). (See also Gurpide *et al.*, 1963; Perl,

1960; Steele, 1964). This analysis is easily extended to large multi-compartment models and becomes more appropriate than the algebraic procedure for more complex models.

Section 4: MODELS OF NITROGEN AND SULPHUR METABOLISM IN THE RUMEN OF SHEEP

(a) Nitrogen Models

There have been several reports in the literature of models of nitrogen metabolism in sheep (Pilgrim *et al.*, 1970; Nolan *et al.*, 1972; Nolan and Leng, 1972; Nolan, 1975; Morris *et al.*, 1975; Mazanov and Nolan, 1976; Nolan *et al.* 1976; Nolan and Rowe, 1976; Nolan and Stachiw, 1979; Kempton *et al.*, 1979; Egan and Ulyatt, 1980).

A model describing the flows of nitrogen through pools in the rumen of sheep given a low-quality-roughage diet was proposed by Nolan and Stachiw (1979) and is given in Figure 10 (the values given in the model are mean values for four sheep). The total flux of ammonia through the rumen NH_3 pool, estimated by $^{15}NH_3$ dilution methods, was 8.2 g N/d of which 3.5 g N/d was irreversibly lost; thus, 4.7 g N/d was recycled, partly within the rumen (approximately 3.8 g N/d) and partly via endogenous secretions (approximately 0.9 g N/d). There are several important points that arise from the model results : (1) the total incorporation of nitrogen into microbial cells in the rumen is almost twice the outflow of microbial nitrogen, emphasising the extensive turnover of microbial nitrogen that occurred in the rumen; (2) 34% of the microbial nitrogen was derived from nitrogenous compounds more complex than ammonia, presumably mainly peptites and amino acids; and (3) the amount of ammonia absorbed was considerable despite quite low concentrations of ammonia in rumen fluid (63 mg N/ℓ).

Nolan and Leng (1972), Nolan *et al.*, (1972), Nolan (1975) and Mazanov and Nolan (1976) constructed quantitative whole-animal models of nitrogen metabolism in sheep by integrating and superimposing sub-models representing parts of the body which were derived from individual isotope tracer experiments made in sheep in steady state.

Figure 11 shows the structure of the final, mean, seven-pool model (Mazanov and Nolan, 1976) that was assumed to be characteristic of the

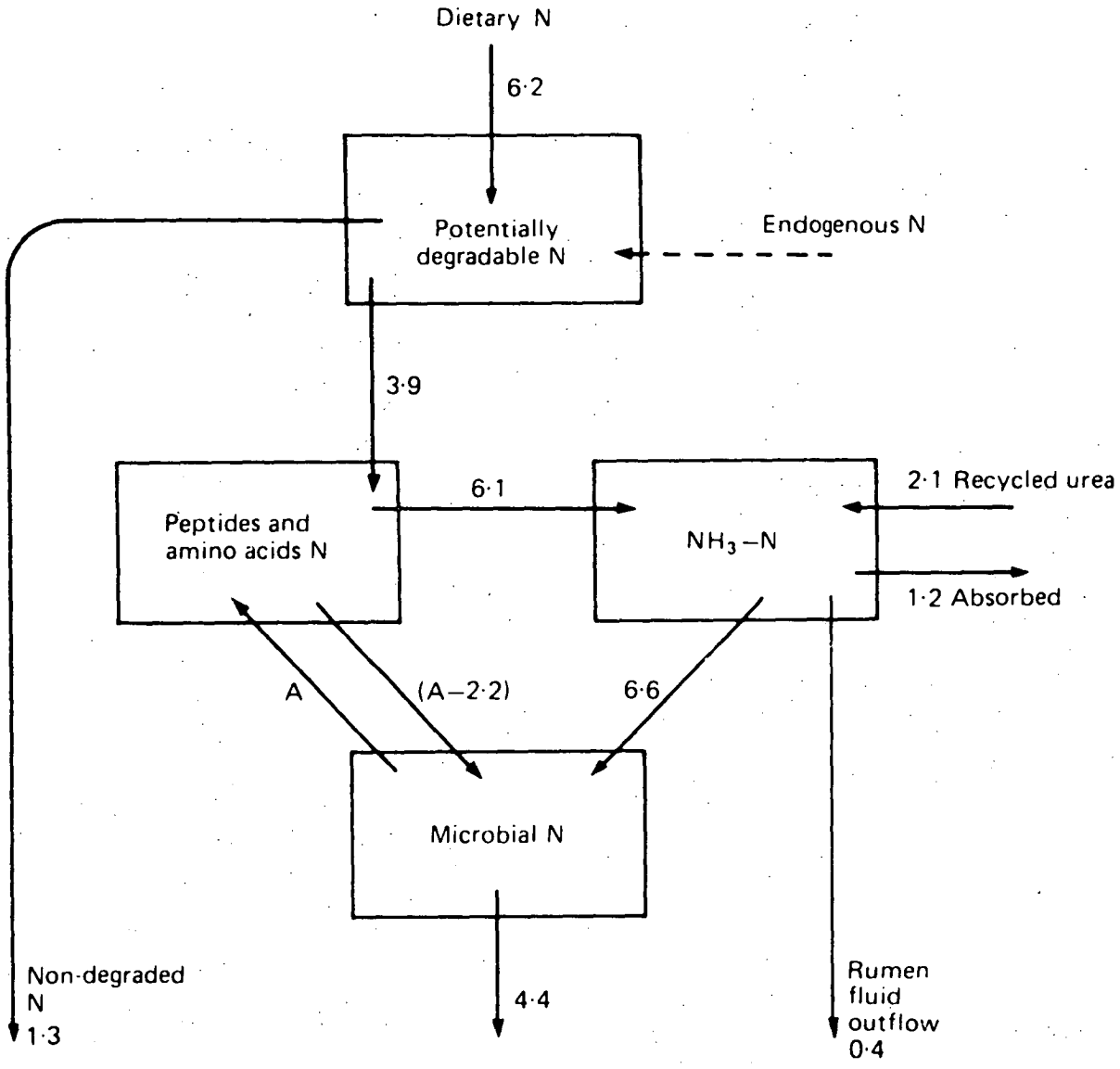


FIGURE 10. A model of nitrogen flows in the rumen (g N/d) of Merino sheep given a low-quality-roughage diet (Nolan and Stachiw, 1979)
A = 3.8
(A-2.2) = 1.6

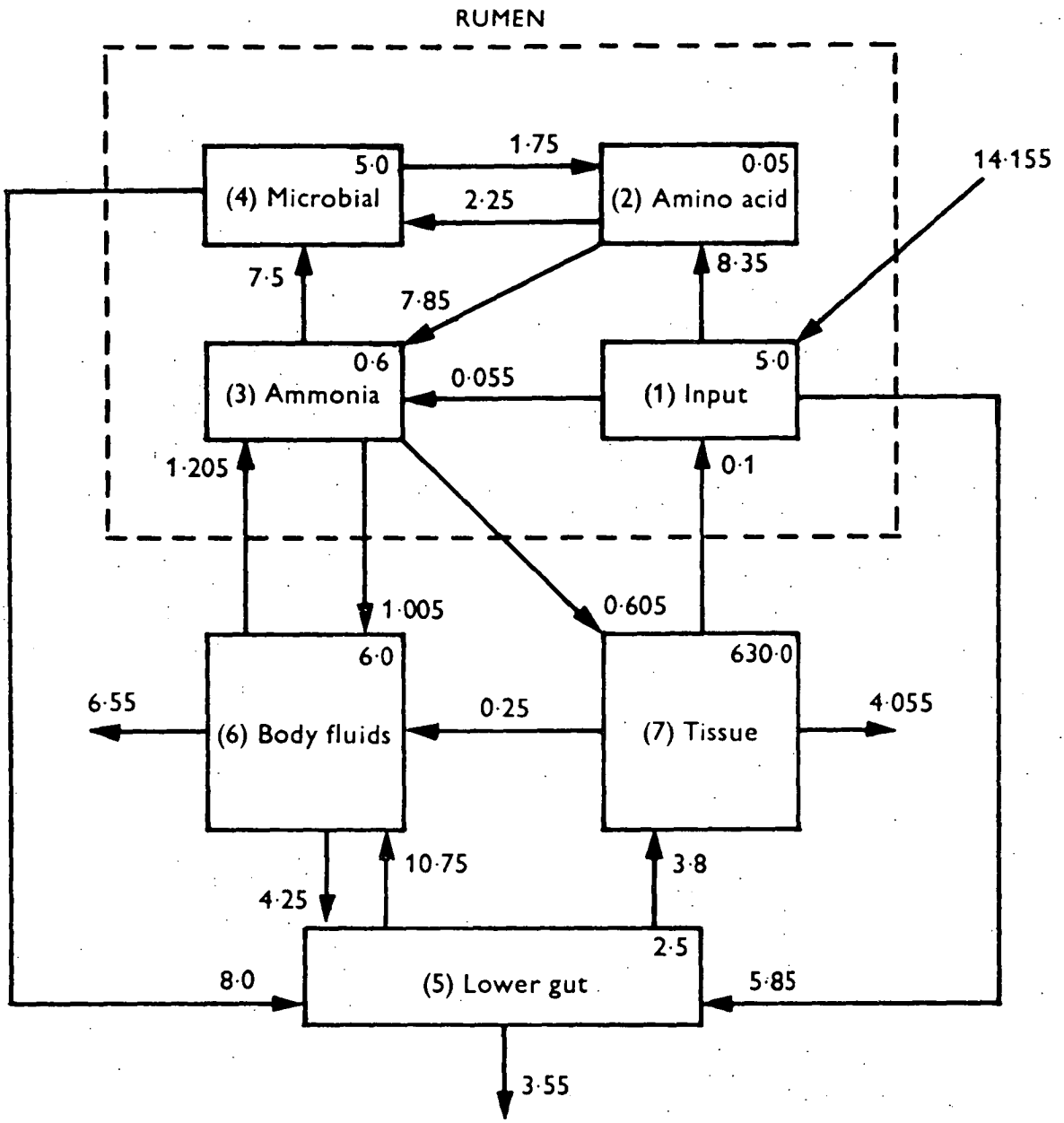


FIGURE 11. Seven-pool model of the dynamics of nitrogen metabolism in sheep consuming forage diets, with values for N transfers (g N/d) shown for a dietary N intake of 14.155 g N/d. The value (g N) in the top right-hand corner of each box represents the steady-state pool size for the average dietary N intake corresponding to the N flows given. (Mazanov and Nolan, 1976)

dynamics of nitrogen metabolism in sheep consuming forage diets containing between 4.9 and 23.4 g N/d. The 'lower gut' represents all the gut pools shown below the rumen microbial nitrogen pool in the twenty-five-pool flow model of Nolan and Leng (1972). The flows shown in Figure 11 are simply the average of the 'high-N' and 'low-N' balanced model flows. The average model was in steady-state with the input and the sum of the losses of nitrogen exactly equal to 14.155 g N/d. Even in this simplified model, some of the nitrogen flows were not known for sheep given a 'high-N' diet of 800 g lucerne chaff (containing 23.4 g N/d). Therefore, estimated flows of nitrogen which made the seven-pool model conform to the assumption of steady-state were used. That is, the sum of the amounts of nitrogen flowing daily into any pool was made exactly equal to the total nitrogen flowing out of that pool; this was also done for the model as a whole. Therefore, when both 'cell sloughings' and the result of 'nitrogenous base' flows from 'tissue components' (Nolan and Leng, 1972) were each assumed to be 0.1 g N/d, the transfer of nitrogen from the lower gut to body fluids became 5.6 g N/d while the flow of nitrogen from rumen fluid ammonia to the tissue pool became 1.2 g N/d to 'balance' each pool in this seven-pool model.

A diagram is given in Figure 12 to illustrate the progress of nitrogen through the sheep's rumen (Pilgrim *et al.*, 1970). Values in parentheses are based on values from the trial in which a lucerne-hay diet was used and the nitrogen intake was 22.9 g N/d. The assumption of 5.0 g recycled N does not affect other values quoted except that if it were less, then a correspondingly greater value would be given to NH_3-N formed from plant N to make up the measured total of 17.5 g formed. The above work by Pilgrim *et al.* (1970) was considered to be of a preliminary and exploratory nature. For this reason, only one sheep was used throughout the trial; the animal was fed equal parts of its ration at hourly intervals. Pilgrim *et al.* (1970) have found that, for two diets of different nitrogen content fed to the same sheep the amount of microbial - N shown to be derived from NH_3-N was between 62% and 78% of the total. It was concluded that synthesis of microbial protein was more dependent on ammonia as a starting point with the low-N diet than with the higher-N diet. Additional calculations, based on measured entry rates of NH_3-N into the rumen showed that

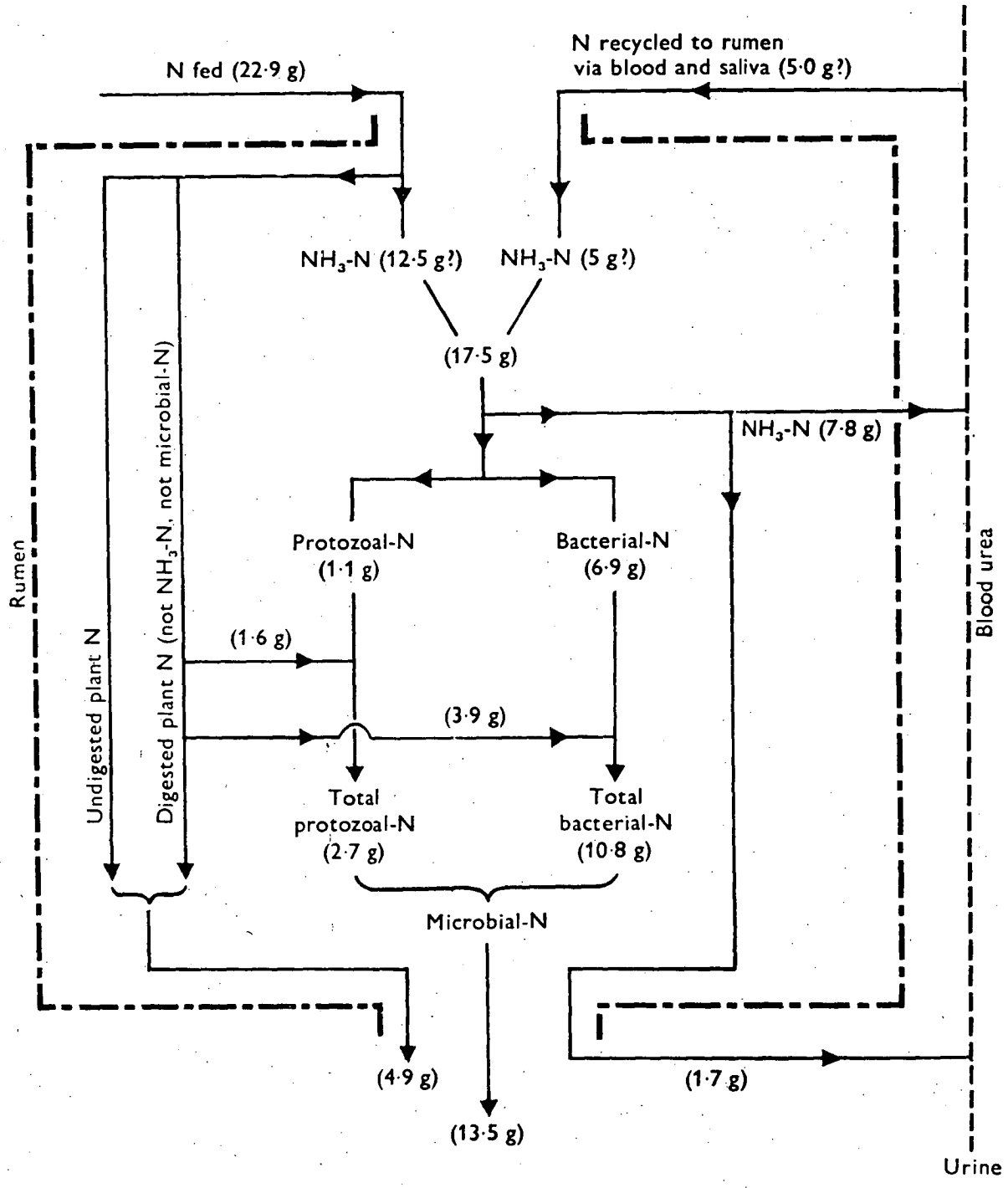


FIGURE 12. Diagram of progress of nitrogen through the sheep's rumen (Pilgrim *et al.*, 1970)

minimal values for conversion of dietary - N into microbial - N ranged from 53% to 68%.

A quantitative sub-model of nitrogen transactions in the stomach of a sheep is given in Figure 13 (Nolan, 1975). Values (g N/d) were obtained from results derived from one particular animal; values within brackets refer to flows of organic matter (g/d). Nolan (1975) states that, a rumen sub-model which adequately described processes of nitrogen metabolism in the reticulo-rumen, omasum and abomasum might include quantitative assessment of the following : (1) the quantities of soluble and insoluble protein and non-protein N from the diet and of endogenous urea and other endogenous N compounds, and their contribution to the total pool of N compounds available for fermentation in the rumen; (2) the extent to which these N compounds are degraded to simpler compounds (i.e. peptides, amino acids and ammonia) or pass undegraded from the rumen; (3) the extent to which peptides, amino acids, ammonia and nucleic acids are assimilated by bacteria and protozoa, and the net rate of efflux of microbial N to the small intestine; (4) the quantities of soluble N compounds absorbed through the walls of the forestomachs, or their efflux in water; and (5) the quantities of N recycled through pools within the rumen itself (e.g. as a result of ingestion of other micro-organisms by protozoa, and lysis of bacteria). One approach to the problem of analysing kinetic data from isotope tracer experiments is to use compartmental analysis as a means of obtaining explicit solutions for relatively simple, assumed models (Baker *et al.*, 1959). However, as Nolan (1975) states, models representing the multiplicity of nitrogen exchanges occurring in the animal are too complex for it to be practicable to obtain sufficient information from a single experiment to allow explicit solutions of such a complex system. Furthermore, because nitrogenous compounds take time to move throughout the body, the body system cannot realistically be considered to be instantly in equilibrium after injection of tracers such as ^{15}N, even though this assumption is a necessary pre-requisite of conventional compartmental model analysis. Nolan (1975) used an alternative approach to the development of a whole-animal model, that is, several sub-models representing parts of the body were developed from individual isotope dilution experiments made under identical conditions and these were used to develop a

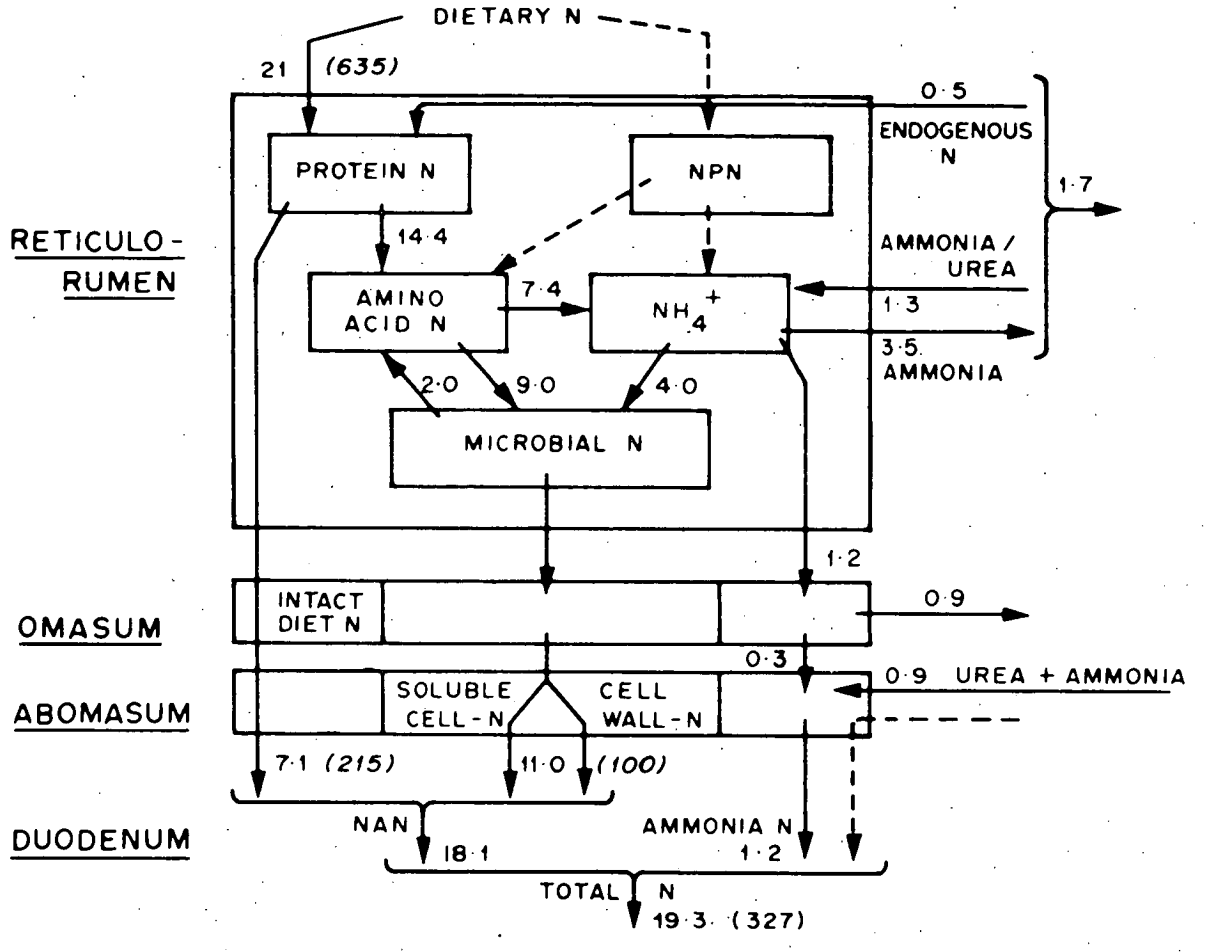


FIGURE 13. A quantitative sub-model of nitrogen transactions in the stomach of a sheep (Nolan, 1975)

quantitative whole-animal model of nitrogen metabolism, which is shown in Figure 14. Values (g N/d) were derived from various sources and, in that time, were considered to be 'best-available' estimates for sheep given 800 g/d of lucerne chaff. A' represents the total uptake of ammonia from the gut made up of individual pathways each labelled A. B' represents the total urea degradation in the gut made up of individual pathways each labelled B. (———) pathways that appear to be quantitatively well understood, (------) more tentative pathways. The steps in developing the above mathematical model were as follows: (1) the relatively complex flow diagram (Figure 14) was modified, initially by amalgamation of pools to produce the simplest model that would be capable, when represented in mathematical form, of producing outputs that could be validated. It was reasoned that a model can be added to as required, but each added step must improve the model's ability to mimic the system it represents. When improvement does not occur, the last step added can be studied further; (2) the results for sheep on the high-N and low-N diets were averaged. Inputs and outputs for each pool and for the whole system were balanced by small adjustments of the data in order to satisfy the assumptions (a) that the animals were in steady state and (b) that the model was linear within the dietary range. Time delays (lags) were included in the whole-animal model to account for the time taken for digesta to move between sampling sites in the digestive tract. In a similar report of studies of the dynamics of N metabolism in sheep, a model of nitrogen metabolism in sheep (given a ration of 800 g lucerne) showing the movement of nitrogen (g N/d) between the various nitrogenous pools in the body is shown in Figure 15 (Nolan and Leng, 1972; Nolan *et al.*, 1972). For convenience, the digestive tract has been divided into three areas: (1) the anterior area of microbial colonisation (i.e. the reticulo-rumen), (2) the abomasum, duodenum, jejunum and anterior ileum, and (3) the major areas of microbial colonisation in the lower digestive tract (i.e. lower ileum, caecum and large bowel). The pools of nitrogen in the digestive tract are considered separately from the pools of ammonia, urea and amino acids in the body, which are considered separately from the larger pools with slower turnover rates such as the muscle proteins and structural proteins. Several interesting points became immediately apparent from an appraisal of this model of nitrogen metabolism in sheep. Approximately 60% of the dietary N was digested in the

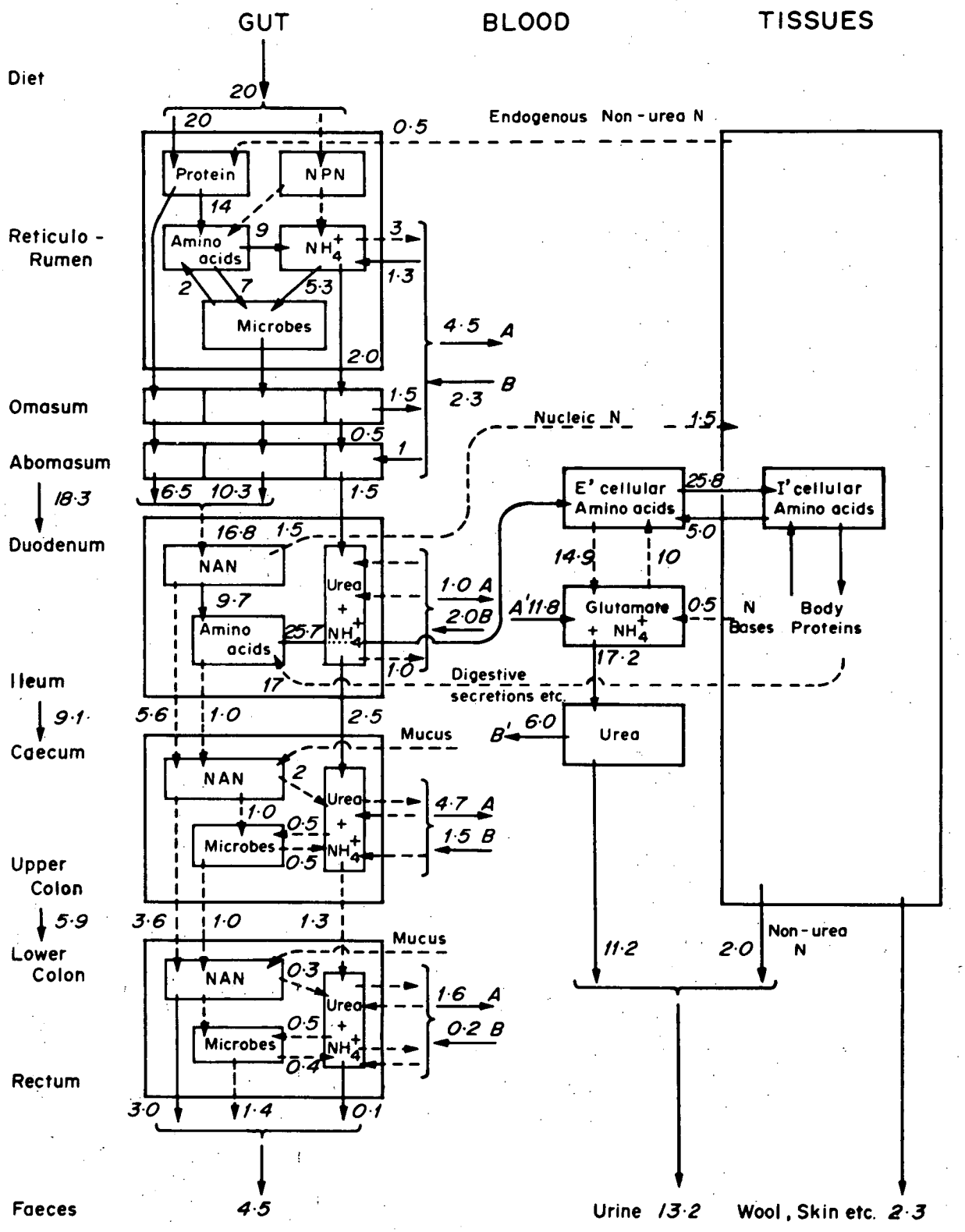


FIGURE 14. A quantitative, whole-animal model of nitrogen transactions in the sheep (Nolan, 1975)

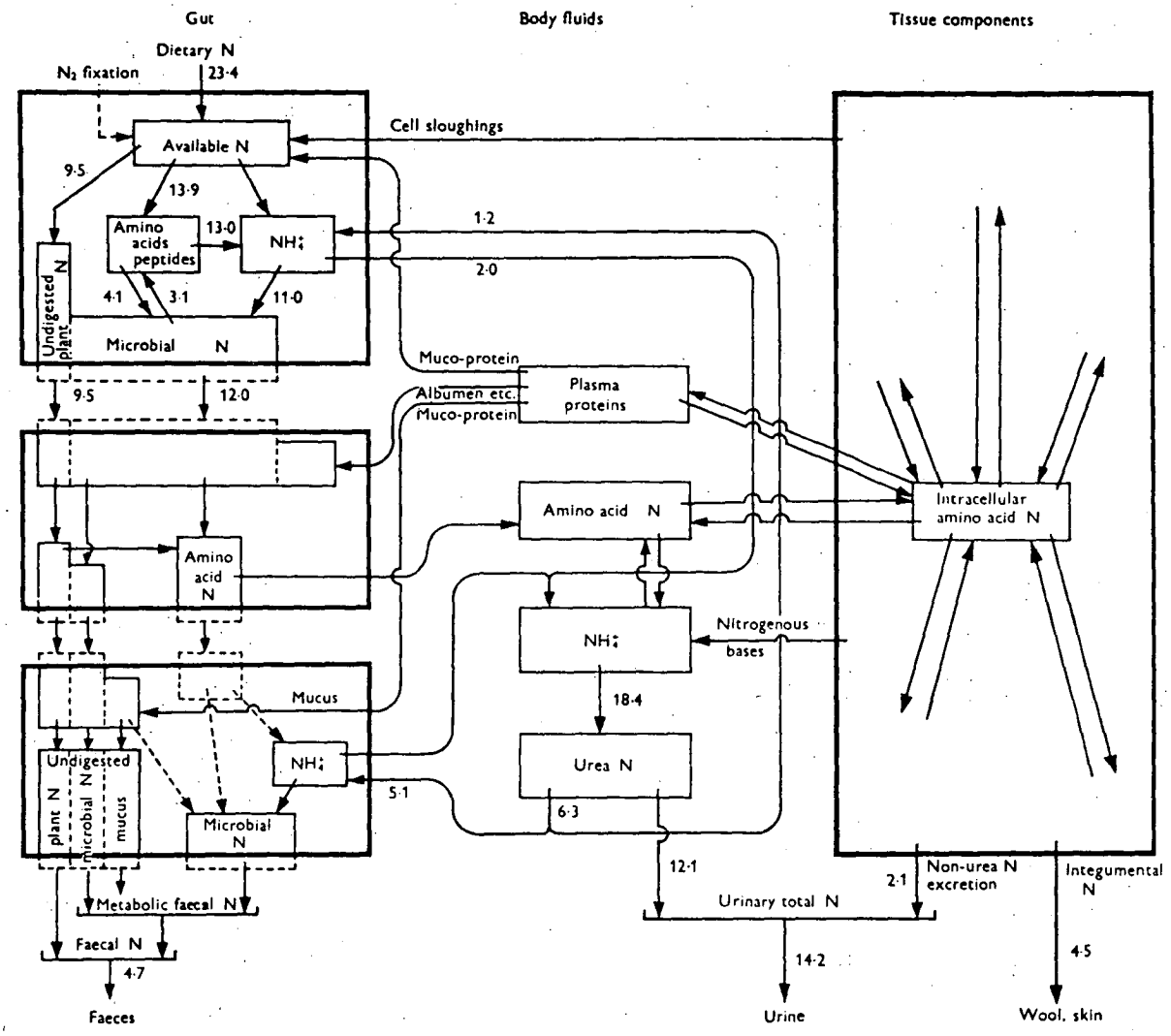


FIGURE 15. A model of nitrogen metabolism in sheep (Nolan and Leng, 1972; Nolan *et al.*, 1972)

rumen. A maximum of 80% of N of bacteria came from the ammonia pool. Only a small amount of ammonia was absorbed from the rumen and only a relatively small amount of the degraded urea was degraded in the rumen. This means that a measured recycling rate of ammonia N in the rumen was apparently through a cycle restricted to the rumen (through the pathways: ruminal ammonia ⟶ microbial protein ⟶ amino acids ⟶ ammonia). It was suggested that around 30% of the bacteria were degraded *in situ* in the rumen. All the urea degraded in the rumen could have been added in saliva rather than by passage across the rumen wall.

In the models discussed so far, the primary objective of the modelling process was to provide a way of analysing experimental data from isotope dilution experiments and of summarising those data in a generally applicable form. In this form the models can be used as a basis for comparison with other results and also for development of hypotheses about the nature of the much more complex whole-animal system. Nolan and Rowe (1976) suggest that a dynamic model of nitrogen metabolism in sheep, though capable of simulation of short-term experiments, will not necessarily be able to generate longer-term production responses. Many factors other than dietary N intake can limit animal production. It is, therefore, somewhat surprising that a nine-compartment model (Mazanov and Nolan, 1976), which includes only N transactions and takes no account of energy flows or other variables, appears to predict satisfactorily responses of animals on a range of forage diets. The reason for the apparently satisfactory simulations by the N model probably lies in the fact that digestible nitrogen and metabolisable energy are highly correlated in dried, forage diets. It seems obvious that this model will be inadequate when other types of diet are considered; in fact, Egan and Ulyatt (1980) state that, the seven-pool and nine-pool dynamic models proposed by Mazanov and Nolan (1976) do not predict well the quantitative movements of nitrogen for animals fed fresh diet. To have general applicability, and to be suitable for inclusion in larger ecological or animal production system models, many other factors such as the environment, physiological state, disease must also eventually be taken into account.

(b) Sulphur Models

Bray and Till (1975) suggest that in terms of sulphur (S) metabolism, the gut can be divided into two main systems: (1) the reticulo-rumen, in which S in feed and secretions is largely reduced to H_2S and converted into microbial proteins or absorbed directly as H_2S; (2) the post-ruminal section, in which the overall processes are the digestion of protein and other S-containing materials, and the absorption of amino acids, peptides and inorganic and organic sulphates. The fate of S in the animal may be illustrated by a 'box and arrow' diagram in which the boxes represent pools, and the arrows flows of S (Figure 16, Bray and Till, 1975). Such a diagram (Figure 16) can form the basis for a model which may then be used to estimate the effects of metabolism in other body components on the digestive processes. In ruminants there are many similarities between the metabolism of S and nitrogen (N) and at times it is advantageous to consider the relationships between them. The major metabolic pathways of sulphur metabolism in the rumen are presented in Figure 17 (Bray and Till, 1975). Sulphide is the key intermediate between the breakdown of ingested and recycled S and its subsequent utilisation and/or loss from the system. There has been only a small number of models of sulphur metabolism in sheep reported in the literature (Kennedy and Siebert, 1975; Gawthorne and Nader, 1976; Kennedy and Milligan, 1978; Doyle and Moir, 1979 a,b).

A schematic representation of the apparent turnover (g sulphur/d) of sulphate, sulphide, and microbial-protein-S in the rumen of sheep, intraruminally infused with 10 g sodium sulphate (values in small type)/d or with 10 g Na_2SO_4 plus 126 mg sodium molybdate/d (values in large type) is shown in Figure 18 (Gawthorne and Nader, 1976). In this model, Gawthorne and Nader (1976) found that only 52% - 57% of the S in microbial protein originated from the sulphide pool. They suggest that approximately half the S-amino acid content of microbial protein in ruminal digesta was synthesised *de novo* from sulphide, and the remainder resulted from the direct incorporation of amino acids from digested plant and salivary proteins. Another suggestion made by Gawthorne and Nader (1976) is that there may be some sulphate absorption from the rumen, but their evidence is inconclusive. In fact Bray (1969a) has found that sulphate is not absorbed from buffer solutions placed in the rumen of sheep.

A schematic diagram of the apparent movements of organic S, microbial S,

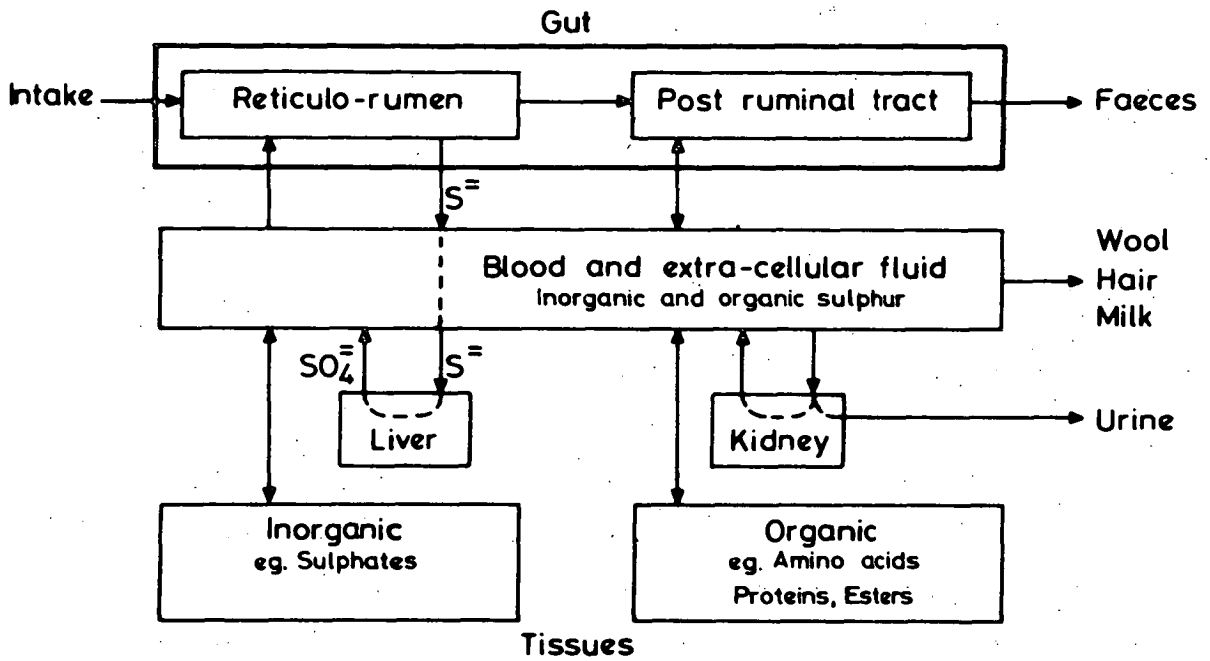


FIGURE 16. The fate of sulphur in ruminants (Bray and Till, 1975)

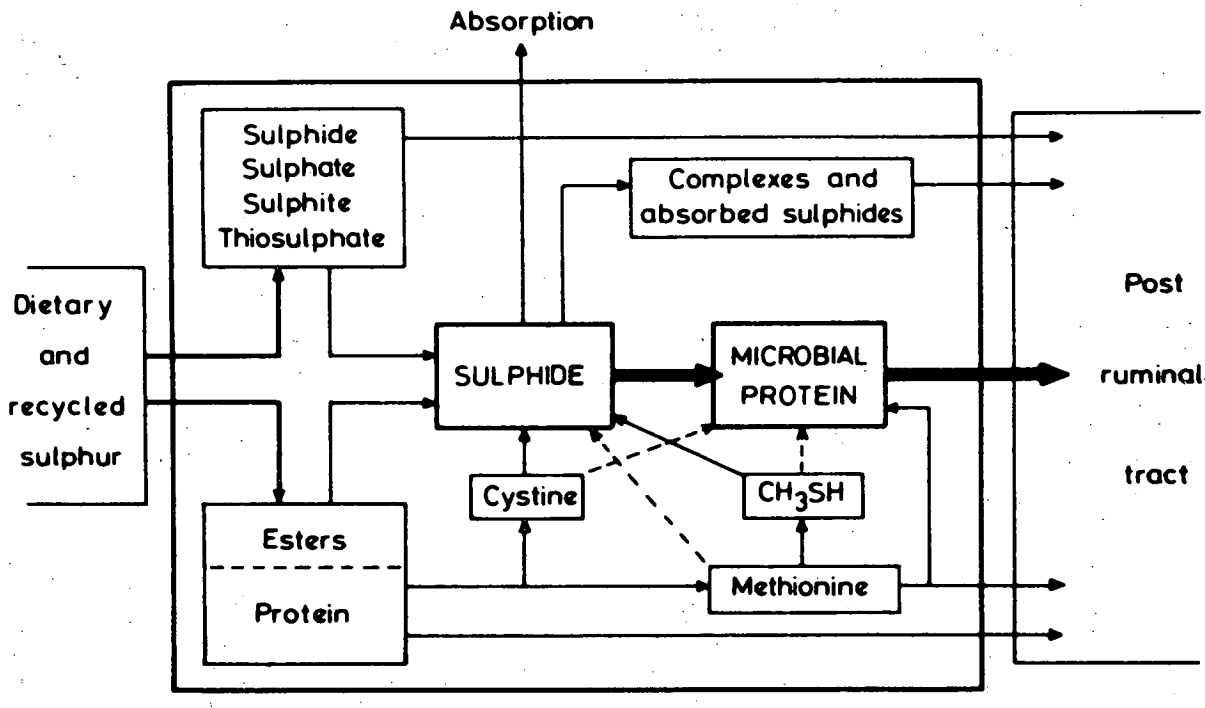


FIGURE 17. The major pathways of sulphur metabolism in the rumen (Bray and Till, 1975)

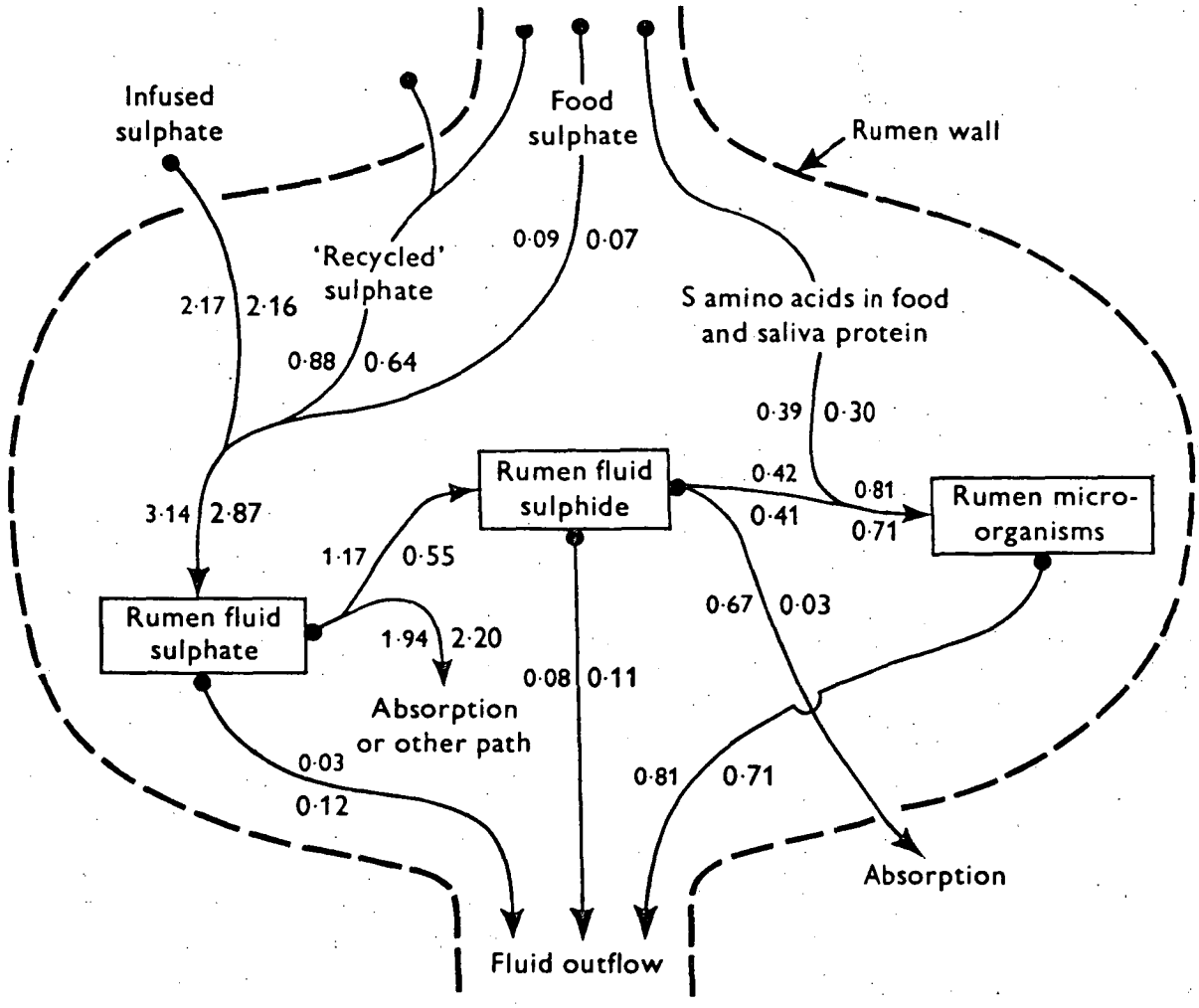


FIGURE 18. Schematic diagram of the daily turnover of sulphate, sulphide, and microbial-protein-S in the rumen of sheep (Gawthorne and Nader, 1976)

sulphate and sulphide in the rumen is presented in Figure 19 for sheep given brome grass or lucerne pellets at the rate of 33 or 66 g/h. Values in parentheses are for the lucerne diet (Kennedy and Milligan, 1978). Several points arose from the model results and may be summarised as follows:

(1) the concentration of inorganic sulphate in serum was increased to maximum values of 35 - 46 mg S/l by infusion of sodium sulphate (0 - 4 g S/d) into the rumen or abomasum;

(2) reabsorption of sulphate by the kidney reached a maximum of 0.69 - 1.1 mmol sulphate/l glomerular filtrate;

(3) the transfer of sulphate from blood to the rumen was related to the concentration of inorganic sulphate in serum, attaining maximum values of 133 (±13) mg S/d for sheep given brome grass plus sulphate, and 127 - 159 mg S/d for sheep given lucerne;

(4) bacteria derived 52% - 67% of organic S from rumen sulphide in sheep given brome grass, and approximately 45% of bacterial organic S was derived from sulphide for sheep given lucerne. Protozoa derived approximately 90% of organic S from bacteria; and

(5) it was estimated that endogenous organic S contributed 300 - 340 mg S/d to the rumen, and that 24% - 45% of S digested in the rumen was derived from endogenous sources.

Recently, Doyle and Moir (1979a) have used a block diagram (shown in Figure 20) to illustrate first approximations of the different sulphur pools in the reticulo-rumen, the interactions between them and first approximations of sulphur flows to the omasum. First approximations of the ruminal sulphur pools and of sulphur flows to the omasum were derived from the concentrations of sulphur in true digesta (see Faichney 1975; Doyle and Moir, 1979a) and the ruminal fluid volume or fluid flow. No attempt was made to distinguish between dietary and microbial protein, nor to subdivide the non-protein neutral sulphur fraction into peptide sulphur, free cyst(e)ine and methionine or other organic sulphur compounds present. Further, the reducible sulphur pools and sulphur flows are small in comparison with the sulphur in

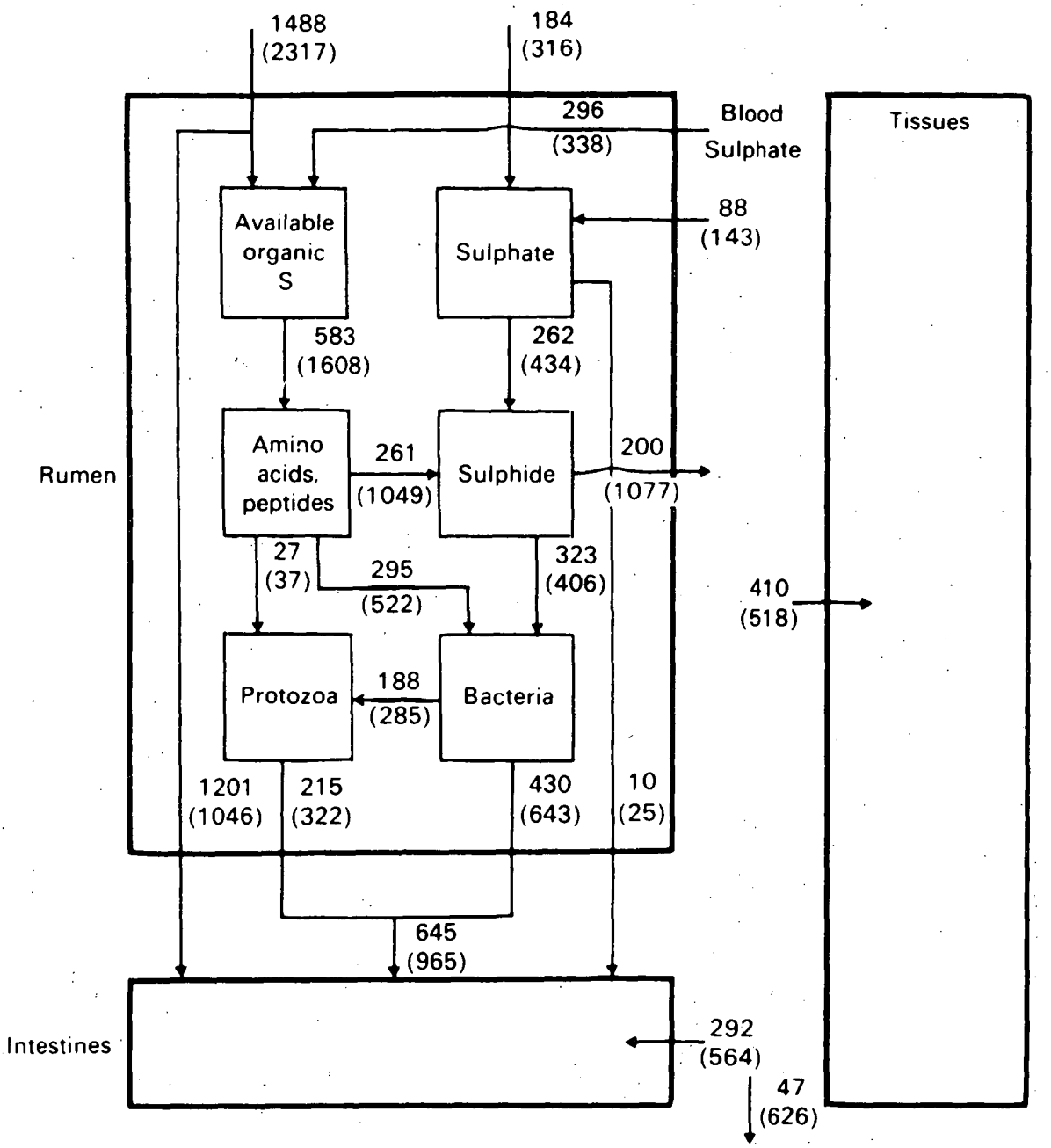
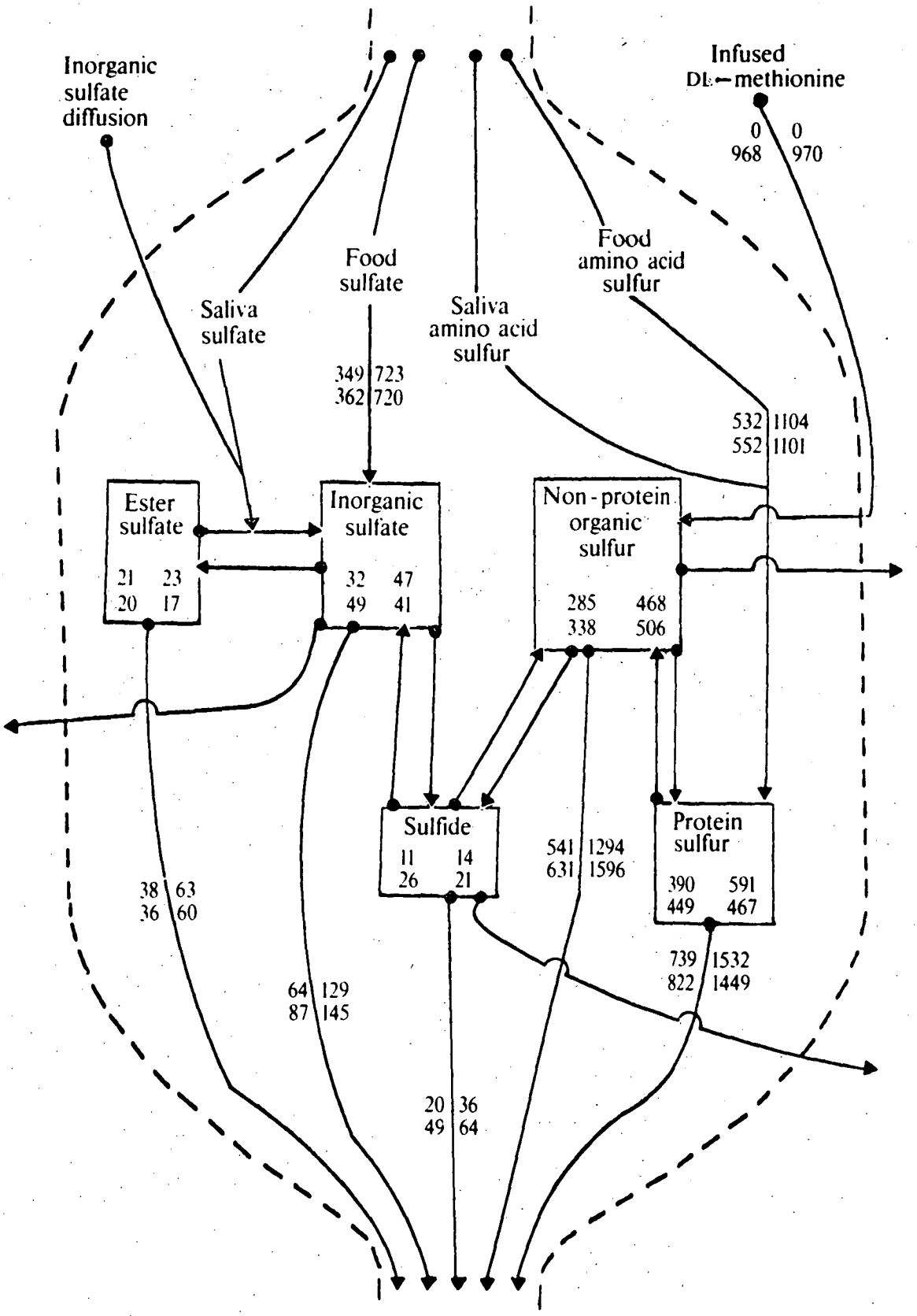


FIGURE 19. A model of sulphur metabolism showing the movement of S (mg S/d) between pools in the rumen and blood in sheep (Kennedy and Milligan, 1978)

FIGURE 20. Sulphur inputs (mg S/day) into the reticulo-rumen, first approximations of sulphur pools (mg S) and of sulphur flows to the omasum (mg S/day). The pool sizes and flows illustrated are the means for each of the four treatments in experiment 2 (see Doyle and Moir, 1979a). The convention used within each set of data is related to the treatments as follows:

500 g	1000 g
500 g + Met	1000 g + Met

(Doyle and Moir, 1979a)


Inorganic sulfate diffusion
Saliva sulfate
Food sulfate
Saliva amino acid sulfur
Food amino acid sulfur
Infused DL-methionine
0 0
968 970
349 723
362 720
532 1104
552 1101
Ester sulfate
21 23
20 17
Inorganic sulfate
32 47
49 41
Non-protein organic sulfur
285 468
338 506
Sulfide
11 14
26 21
Protein sulfur
390 591
449 467
541 1294
631 1596
38 63
36 60
64 129
87 145
739 1532
822 1449
20 36
49 64

organic fractions. In this regard the model places quantitative values on the relative contributions of different pools, without overlooking the potential importance of small rapidly changing pools. As Doyle and Moir (1979a) suggest, the first approximations of protein sulphur flows to the omasum are subject to errors. Overestimates occur as the sulphur content of particles in the rumen too large to flow through the reticulo-omasal orifice have been included in the estimated sulphur concentration of true digesta. Another error is that introduced by using fluid flow which is an underestimate of true flow. In addition, sequestration of non-protein sulphur compounds occurs in the rumen (Doyle and Moir, 1979a). The results of this model illustrate the importance of using true digesta in assessing pools in the reticulo-rumen of particular metabolites and the flows of these. The authors of the above model suggest that dry matter measurements would further improve estimates of pool size, as the concentration of dry matter in true digesta would enable estimates to be made of true digesta volume. However, it was suggested that, such measurements were not possible as the increased size of samples would have placed the animals under undue stress. As a conclusion, first approximations of sulphur flow illustrate that doubling the level of dry matter intake approximately doubled the flow of sulphur to the omasum, and that a very large proportion of the sulphur flowing from the reticulo-rumen was in an organic form, of which only half was protein sulphur. The dietary sulphur intakes of this model and the amounts of sulphur infused as DL-methionine into the rumen, together with the first approximations of sulphur flows from the reticulo-rumen and true sulphur flows from the duodenum, have been used to develop compartmental balance models (shown in Figures 21 and 22) of sulphur metabolism in the stomach of the sheep (Doyle and Moir, 1979b). Due to the method of calculation, the values for protein sulphur flow from the reticulo-rumen are probably overestimated. Also non-protein organic compounds may be precipitated by trichloroacetic acid and included in the organic protein fraction (see Annison 1956; Bird and Hume, 1971). Doyle and Moir (1979b) suggest that, as the magnitude of estimation errors is unknown, two extreme approaches can be adopted to complete the flow diagrams. Firstly, overestimates of the neutral sulphur flow from the reticulo-rumen can be considered as extremely small and therefore remain

FIGURE 21. A balance model of sulphur metabolism and absorption in the sheep's stomach under conditions of different dietary and supplemental sulphur inputs. First approximations of neutral sulphur flows from the reticulo-rumen are considered to be extremely small overestimates and therefore remain unchanged. Values in parentheses are estimated under the assumptions of the model and did not result from the analysis of digesta collected in experiment 2 (see Doyle and Moir, 1979b). All values are in milligrams of sulphur per day and the convention used within each set of data is related to the treatments as follows:

500 g	1000 g
500 g + Met	1000 g + Met

(Doyle and Moir, 1979b)

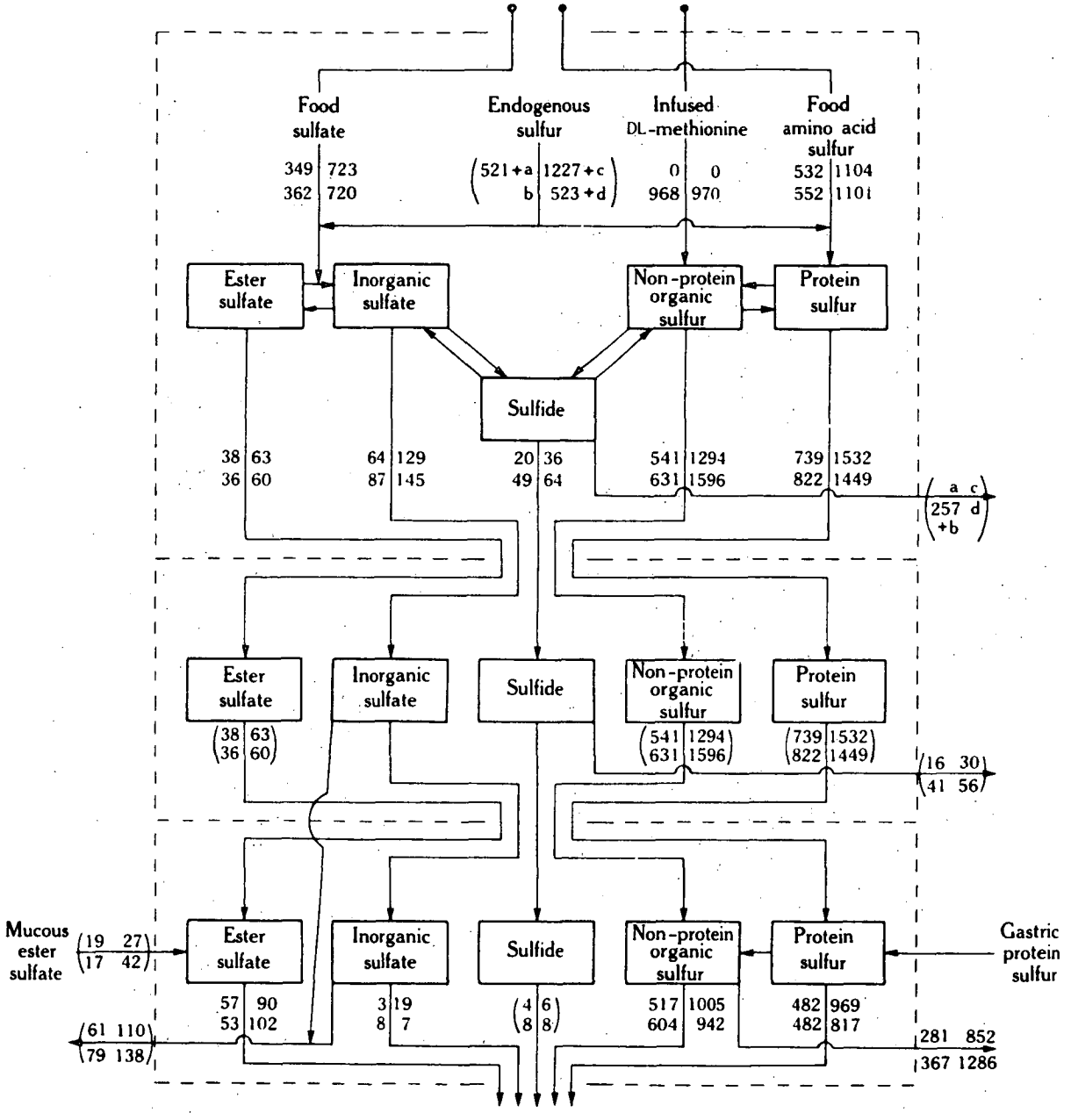
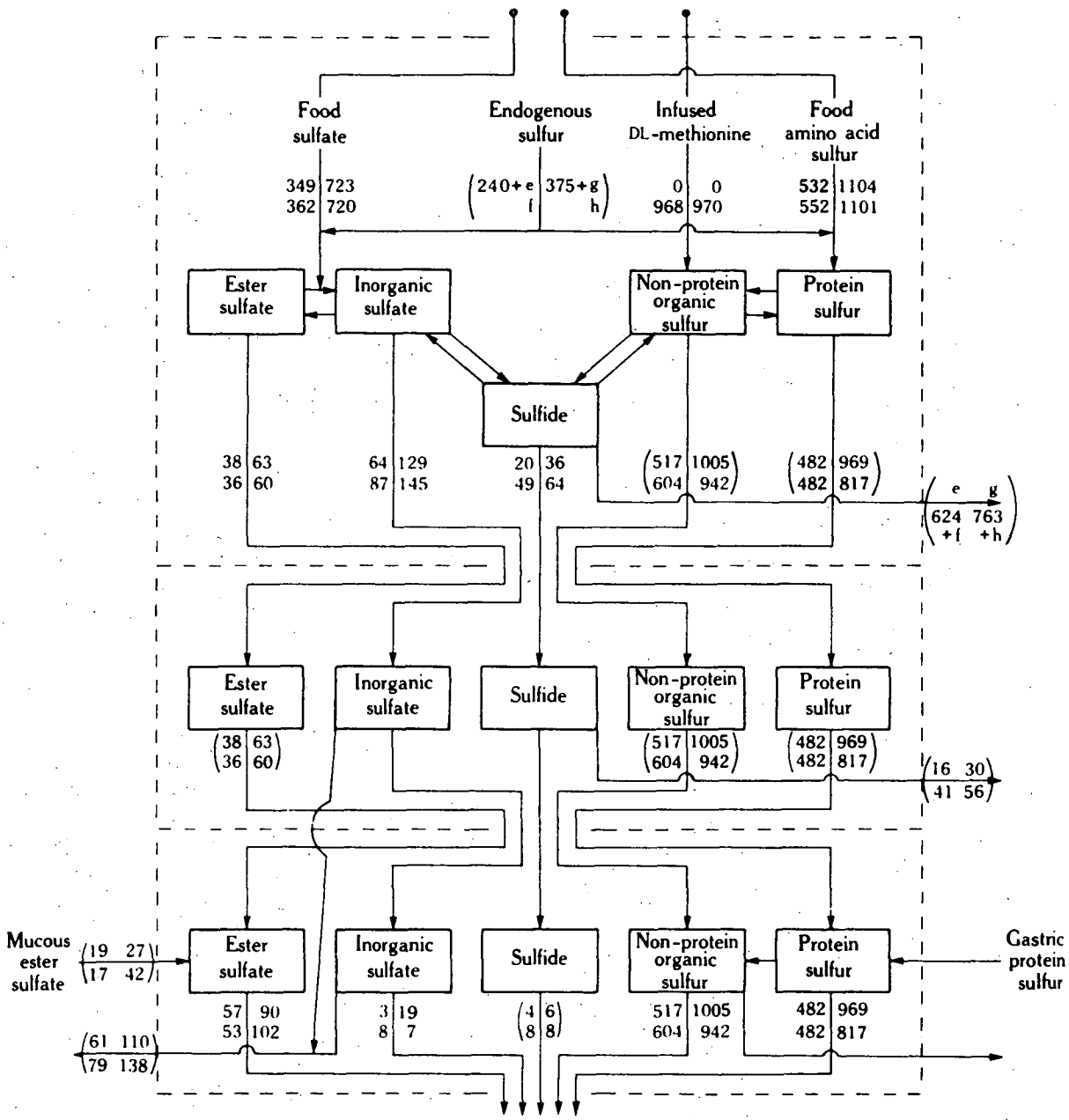

Food sulfate
349 723
362 720
Endogenous sulfur
(521+a 1227+c
b 523+d)
Infused DL-methionine
0 0
968 970
Food amino acid sulfur
532 1104
552 1101
Ester sulfate
Inorganic sulfate
Non-protein organic sulfur
Protein sulfur
Sulfide
38 63
36 60
64 129
87 145
20 36
49 64
541 1294
631 1596
739 1532
822 1449
a c
257 d
+b
Ester sulfate
Inorganic sulfate
Sulfide
Non-protein organic sulfur
Protein sulfur
(38 63
36 60)
(541 1294
631 1596)
(739 1532
822 1449)
16 30
41 56
Mucous ester sulfate
(19 27
17 42)
Ester sulfate
Inorganic sulfate
Sulfide
Non-protein organic sulfur
Protein sulfur
Gastric protein sulfur
57 90
53 102
3 19
8 7
(4 6
8 8)
517 1005
604 942
482 969
482 817
(61 110
79 138)
281 852
367 1286

FIGURE 22. A balance model of sulphur metabolism and absorption in the sheep's stomach under conditions of different dietary and supplemental sulphur inputs. First approximations of neutral sulphur flows from the reticulo-rumen are considered to be large over-estimates, and have been recalculated under the assumptions of the model. Values in parentheses are estimated under the assumptions of the model and did not result from the analysis of digesta collected in experiment 2 (see Doyle and Moir, 1979b). All values are milligrams of sulphur per day and the convention used within each set of data is related to the treatments as follows:

500 g	1000 g
500 g + Met	1000 g + Met

(Doyle and Moir, 1979b)


Food sulfate
Endogenous sulfur
Infused DL-methionine
Food amino acid sulfur
349 723
362 720
(240+e 375+g
f h)
0 0
968 970
532 1104
552 1101
Ester sulfate
Inorganic sulfate
Non-protein organic sulfur
Protein sulfur
Sulfide
38 63
36 60
64 129
87 145
20 36
49 64
(517 1005
604 942)
(482 969
482 817)
(e g
624 763
+f +h)
Ester sulfate
Inorganic sulfate
Sulfide
Non-protein organic sulfur
Protein sulfur
(38 63
36 60)
(517 1005
604 942)
(482 969
482 817)
(16 30
41 56)
Mucous ester sulfate
(19 27
17 42)
Ester sulfate
Inorganic sulfate
Sulfide
Non-protein organic sulfur
Protein sulfur
Gastric protein sulfur
57 90
53 102
3 19
8 7
(4 6
8 8)
517 1005
604 942
482 969
482 817
(61 110
79 138)

unchanged (see Figure 21), or alternatively the estimated protein sulphur and non-protein organic sulphur flows from the reticulo-rumen may be subject to large errors, and can therefore be equated to the flows from the abomasum minus any organic sulphur inputs into the abomasum (see Figure 22). The two flow diagrams represent two possible extremes of sulphur metabolism in the sheep's stomach. Firstly, there is the possibility of considerable protein digestion in and neutral sulphur absorption from the abomasum. The second approach suggests that the functions of the omasum and abomasum did not influence greatly the proportions of neutral sulphur flowing as protein or in a non-protein organic form. The two balance models present sulphur metabolism in the rumen in a light different from the recent model of Kennedy and Milligan (1978). The previous model looked at total organic sulphur flows in unavailable, protozoal and bacterial fractions, while the work of Doyle and Moir (1979a,b) illustrates the importance of non-protein organic sulphur. As Doyle and Moir (1979b) suggest, it is extremely important to compare these models in the light of both the measurements made and the assumptions made not only in the models but also in the flow estimates making up these models.

By comparison with the information available for nitrogen, there is little information on flows and uptake of the digestion products of sulphur compounds in the intestines particularly in the small intestine. The major pools of sulphur compounds may be illustrated by a 'box and arrow' diagram shown in Figure 23 (Kennedy and Siebert, 1975). The boxes represent pools of sulphur compounds, the arrows represent flows of sulphur. These pools of sulphur may be compared with the pools of nitrogen presented in a similar model by Nolan and Leng (1972) which was revised by Nolan (1975) (see Figures 14 and 15). Endogenous sulphur enters all compartments of the digestive tract as cell sloughings, mucin, and ester sulphates (including glycoprotein). Furthermore, sulphur enters the small intestine as plasma protein, bile, and pancreatic secretions. Flow of digesta also carries undegraded endogenous sulphur from the previous compartment, in addition to microbial protein, amino acids, undigested plant sulphur, and inorganic S. Sulphide, generated in the rumen by bacterial activity, may be incorporated into microbial protein, absorbed across the rumen wall (Bray, 1969a), or may pass into

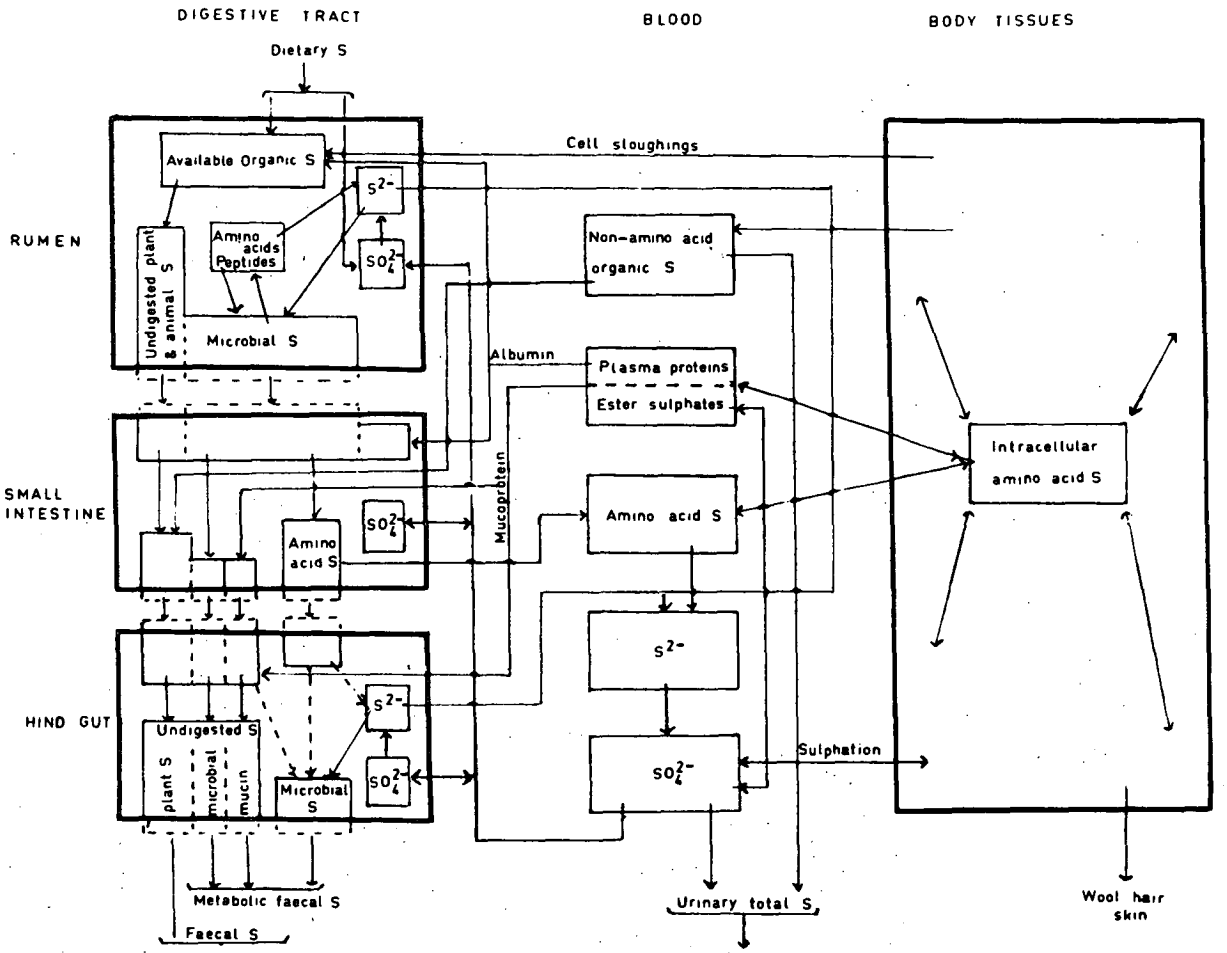


FIGURE 23. A model of sulphur metabolism in sheep (Kennedy and Siebert, 1975)

the duodenum in digesta. In the duodenum, sulphate, sulphide, and amino acids produced from breakdown of microbial protein, are absorbed. In the hind gut, sulphide may be generated by bacterial activity and incorporated into bacterial protein or absorbed. In the blood, sulphide is rapidly oxidised to sulphate, which may exist as the free ion, or attached as an esterified conjugate to plasma protein. The S-amino acids, absorbed from the small intestine, are incorporated into animal tissues as protein, or synthesised into wool, or hair. When protein is catabolised in the body, the S-amino acids are degraded to sulphate, and then are either excreted in the urine or recycled to the digestive tract. Sulphate *per se* is also required in the tissues, notably in connective tissue, where it is present as chondroitin sulphate (for a fuller discussion, see Whanger (1972)).

Section 5: THE METABOLISM OF NITROGEN AND SULPHUR IN THE RUMEN

(i) Introduction

The metabolism of sulphur and nitrogen is closely associated in ruminants as in other animal life (Garrigus, 1970); the relationship commonly expressed as nitrogen to sulphur ratio (N:S) will be discussed in detail elsewhere in this literature review.

The action of the rumen microflora may completely alter the dietary form of both of these elements; for instance, the degradation of dietary protein to yield ammonia and sulphide or the synthesis of microbial protein from dietary urea and inorganic sulphate.

Ruminants, through the medium of the reticulo-rumen and the symbiotic microbes, may satisfy their metabolic requirements with dietary inorganic nitrogen and sulphur compounds and cellulosic substances that are nutritionally inadequate for non-ruminant mammals (Garrigus, 1970). Apart from the effect of the fermentative reticulo-rumen there seems no reason to suggest that either nitrogen or sulphur metabolism in ruminants is greatly different from that of other animals. As ruminant metabolism combines a complex microbial system in both the rumen and large intestine with a mammalian system, any study of nitrogen and sulphur metabolism requires an understanding of the metabolic pathways in the micro-organisms as well as in mammalian tissues. A number of factors, including the type and amount of food consumed, the microbial population and the animal itself, determine the balance of this complex ecological system and thereby affect the utilisation of food by the animal.

Ruminants are distinguished from monogastric animals by their greater ability to utilise non-protein nitrogen in the synthesis of microbial protein. The success of ruminant feeding lies in the establishment of a careful balance between the requirements of the rumen micro-organisms and those of the host animal (El-Shazly and Abou Akkada, 1972). In many cases these requirements are antagonistic but in a few instances they are synergistic. It is therefore the task of the nutritionist to find a formula which decreases the wastes due to the decomposing activity of the rumen micro-organisms and also encourages the biosynthetic ability of these microbes.

Because of the anaerobic conditions predominating in the rumen, microbial protein synthesis is mainly limited by available energy. However, the degree of microbial protein synthesis may be limited by other factors. Chalmers (1961) considers that when sheep are fed a diet in which urea is the sole N source then microbial protein synthesis in the rumen may be limited by the availability of sulphur-containing amino acids.

(ii) Nitrogen Metabolism

There have been a number of extensive and intensive reviews of ruminant nitrogen metabolism (Reid, 1953; Chalmers and Synge, 1954a and 1954b; McDonald, 1958; Annison and Lewis, 1959; Barnett and Reid, 1961; Lewis, 1961; McDonald, 1962; McLaren, 1964; Phillipson, 1964; Blackburn, 1965; Hungate, 1965; Hungate, 1966; Cocimano and Leng, 1967; Conrad and Hibbs, 1968; Waldo, 1968; Chalupa, 1968; Smith, 1969; Allison, 1970; Church, 1970; Nolan *et al.*, 1963; Smith, 1975; Buttery and Lewis, 1976; Øroskov, 1977; Mehrez *et al.*, 1977; Thomas, 1977; Al-Rabbat and Heaney, 1978; Offer and Axford, 1978; Baldwin and Denham, 1979; Smith, 1979; Chamberlain and Thomas, 1979; Ganev *et al.*, 1979; Kennedy, 1980; Kennedy and Milligan, 1980 and Leibholz, 1980). This section however, will be largely centred around the ammonia production and utilisation of nitrogen within the rumen and microbial protein synthesis in the rumen.

The diet received by ruminants normally contains appreciable amounts of nitrogenous materials other than proteins; pasture plants, for example, contain about 20%-30% of their total nitrogen as non-protein nitrogen (NPN) (Ferguson and Terry, 1954; Hogan, 1964) and silage contains a much greater proportion (McDonald *et al.*, 1966). Nitrates may occasionally accumulate in plants and the concentration of nitrate may constitute a significant proportion of the total nitrogen. Although nitrate is readily reduced to nitrite in the rumen, and then to ammonia, the intermediate, nitrite, may accumulate to significant concentrations in the rumen (Lewis, 1951; Jamieson, 1959). Whether nitrite inhibits or competes with sulphate metabolism has not been determined (Moir, 1970).

Ruminant animals seem to have similar tissue requirements for essential amino acids to those of other animals (Black *et al.*,1957; Downes, 1961),

but the concept of protein quality has little value in ruminant nutrition owing to the synthesizing capabilities of rumen micro-organisms. Loosli *et al.*, (1949) have shown that the ten essential amino acids known to be required by the rat are synthesized in the rumen of the sheep and goats maintained on a ration in which the sole source of nitrogen was urea.

Urea is the most common source of non-protein nitrogen in use, although ammonium salts and biuret are other possible sources. Ammonium salts like urea are rapidly degraded in the rumen and provide ammonia for microbial protein synthesis. At the same levels of nitrogen intake, ammonium sulphate is less toxic than urea (Huston and Eng, 1974). However, urea is a cheaper source of non-protein nitrogen than the alternate ammonium salts. Other sources of NPN have been used to a limited extent in experimental work. One of these is uric acid which was found to be efficiently utilised by steers (Oltjen *et al.*, 1968).

(a) Ammonia Production

Nitrogen enters the rumen in food, mainly as protein, or in silage as protein and amino acids, in saliva, as urea, and possibly by the diffusion of urea across the rumen wall (Nolan *et al.*, 1973). The main nitrogenous end-product of degradation in the rumen is ammonia (the term will be used to include both unionised NH_3 and ionic NH_4^+) although peptide and amino acid intermediates are formed from proteins, and purine and pyrimidine bases from nucleic acids (Smith, 1975). The possible routes for proteins ingested into the reticulo-rumen are shown in Figure 24. The top arrows angled to the right in Figure 24 indicate the possibilities of these materials escaping from the rumen. Of the peptide and amino acid uptake by the rumen organisms a very appreciable proportion is initially returned to the free liquid phase as ammonia (Pilgrim *et al.*, 1970; Nolan *et al.*, 1973) which the majority of rumen micro-organisms can use as a major nitrogen source. Forrest and Walker (1971) have shown, by calculation, that the additional energy cost of synthesizing amino acids from ammonia and carbohydrate is very small. It is this fact that allows the replacement under appropriate conditions of part of the dietary protein by materials such as urea and biuret, which are readily hydrolysed to ammonia (Sutherland, 1976).

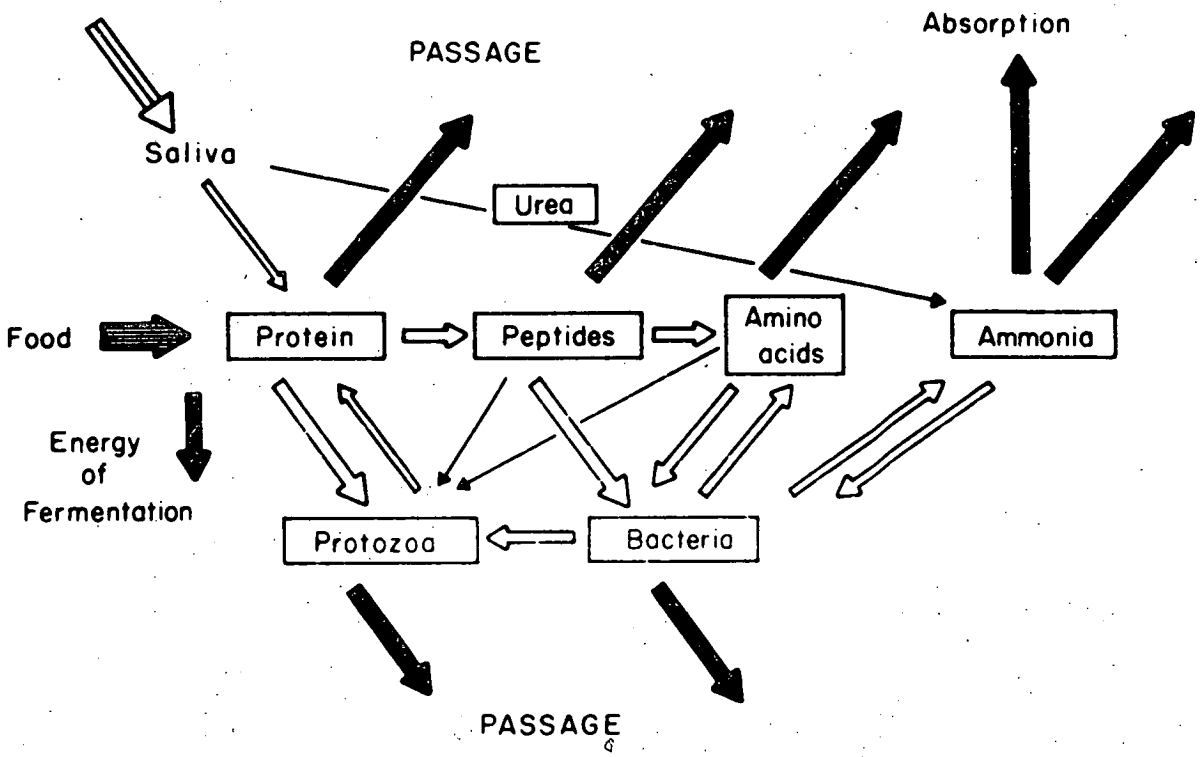


FIGURE 24. The major flows of nitrogenous material of the reticulo-rumen (Sutherland, 1976)

Urea entering the rumen is very rapidly hydrolysed to ammonia. In fact it is difficult to detect urea in rumen contents at any time after administration (Chalmers and Synge, 1954a). The rapid conversion of urea to ammonia in the rumen has been demonstrated many times; the powerful urease activity is apparently restricted to the rumen bacteria (Jones *et al.*, 1964; Abdel Rahman and Decker, 1966). Thus, for a wide variety of diets it appears that ammonia forms an important intermediate in the conversion of food nitrogen to microbial nitrogen.

Excessively high rates of ammonia production can occur, particularly if large amounts of urea, or proteins, such as casein, which are very soluble in rumen fluid, are eaten rapidly. The ingestion of spring grass, particularly the leafy parts grazed naturally, also tends to cause rapid ammonia production in the rumen (Smith, 1969). If the rate of production exceeds the rate at which the bacteria can utilise the ammonia, the concentration of ammonia in the rumen increases. This becomes most apparent if the diet is deficient in readily available carbohydrate such as starch; cellulose is of little value in enabling bacteria to make use of ammonia (Lewis and McDonald, 1958; Lewis, 1962; Hogan, 1964; Robertson and Hawke, 1965; Christian and Williams, 1966; Purser and Moir, 1966a; Davis and Stallcup, 1967; Deif *et al.*, 1968). Ammonia accumulation is also influenced by the composition of the microbial population.

A number of experiments have shown that, when the protozoa are suppressed, ammonia concentrations are reduced (Abou Akkada and El-Shazly, 1964; Purser and Moir, 1966b; Chalmers *et al.*, 1968). This is probably associated with a concomitant increase in the bacterial population (Eadie and Hobson, 1962; Giesecke *et al.*, 1966) and more efficient utilisation of ammonia. The adaptation which occurs with the continued feeding of diets containing large amounts of urea (Holzschuh and Wetteran, 1965; Schaadt *et al.*, 1966; Clifford and Tillman, 1968) has been shown to be related to the suppression of protozoa (Virtanen, 1967) although changes in the properties, and therefore probably the composition, of the bacterial population have also been implicated (Caffrey *et al.*, 1967; Wortham *et al.*, 1968). Chalmers and Synge (1954a) suggested that ammonia production in the rumen is governed by many factors, among them being the solubility of the protein ingested, the state of division of the protein particles, the degree of denaturation or the extent of processing

in the preparation of the foodstuff and the proportion of carbohydrate material.

The first suggestion of the significance of ammonia in the rumen was made by Pearson and Smith (1943) based on the finding that ammonia was produced in vitro during the incubation of rumen contents with protein. McDonald (1948, 1952) recognised the importance of ammonia in the rumen and studied the changes in the ammonia concentration in rumen contents following the feeding of various proteins: he demonstrated the production of ammonia *in vivo* from ingested protein and also the absorption of significant quantities of ammonia from the rumen. He suggested that urea derived from this ammonia in the liver would partly be excreted and partly returned to the rumen in the saliva. Following the work of McDonald (1948, 1952) in demonstrating the importance of ammonia production in the rumen, Chalmers *et al.*, (1954) were able to show convincingly that the extent of ammonia production in the rumen from a protein foodstuff is of considerable significance in the nitrogen metabolism of the ruminant. Their results indicated that the value of protein to the ruminant varied inversely with the rate at which it was attacked in the rumen. A number of similar experiments, in which purified proteins or protein-rich foodstuffs were included in the food, has been used to compare the concentration of ammonia produced in the rumen with the nitrogen balance of the animal, with a view to examining how far this is related to the nutritional value of the protein. These experiments are fully discussed by Chalmers and Synge (1954b) and Chalmers (1961) (see also Annison *et al.*, 1954).

There seems no doubt that the solubility of protein is related to the speed with which ammonia is produced but that wastage due to ammonia formation and absorption may be critical only when the nitrogen provided by the ration is marginal or inadequate for the optimal performance of the animal (Phillipson, 1964). A number of determinant factors control rumen ammonia pool size and turnover rate *in vivo*. Among these factors are (Baldwin *et al.*, 1972): (1) conversion of dietary nitrogen to ammonia, (2) ammonia assimilation by rumen microbes, (3) nitrogen recycling due to degradation of rumen microbes and ingestion of bacteria by protozoa, (4) ammonia absorption and passage, and (5) urea secretion into the rumen.

These and, perhaps, additional factors interact, *in vivo*, in a complex fashion in determining rumen ammonia pool sizes and rates of patterns of utilisation.

The formation of ammonia in the rumen leads to two opposing nutritional tendencies (McDonald, 1952). First, since substances such as urea, which are nutritionally valueless to the host, can be converted to ammonia and utilised for growth of bacteria, that is for synthesis of protein, which can be subsequently digested and used by the host, a gain of nitrogen accrues to the host animal. By contrast, the degradation of protein to ammonia, which can be directly absorbed from the rumen, implies a source of loss of nitrogen to the host animal. The interaction of these opposing tendencies is probably a major factor leading to the relative constancy of the biological value of food nitrogen (crude protein) for ruminants (Johnson *et al.*, 1942).

(b) Utilisation of Nitrogen within the Rumen

Essentially the processes in the rumen consist of, first, the conversion, by microbial action, of part of the dietary and endogenously secreted nitrogenous compounds into degradation products. Some of these products may be absorbed directly by the animal, either in the rumen itself or in the lower alimentary tract, but most are normally used in the formation of the bodies of micro-organisms. Thus, the nitrogenous substances presented to the abomasum and intestines of the ruminant consist, usually to a considerable extent, of those present in micro-organisms (bacteria and protozoa) rather than those in the diet. In general, the changes occurring in the rumen are advantageous to the animal when its diet contains mainly poor quality protein or a non-protein nitrogenous material such as urea, but may be disadvantageous when good quality protein is eaten (Smith, 1969).

Proteolysis of dietary proteins by rumen micro-organisms produces peptides and amino acids, which in turn are deaminated to ammonia, carbon dioxide, and short-chain fatty acids (Leng, 1970). The energy released during these reactions is used by the micro-organisms for movement, reproduction and growth. A large proportion of plant tissue (e.g. cellulose) cannot be digested by the ruminant animal, but the synthesised microbial matter is in general digestible and makes an important contribution to the nut-

rition of the host animal. Many individual species of rumen bacteria use ammonia as a nitrogen source in preference to amino acids or other more elaborate compounds and some species have an absolute requirement for it (Smith, 1969). The mixed bacterial population in rumen contents appears to digest starch more efficiently in the presence of ammonia than in the presence of amino acids (Acord *et al.*, 1966; Acord *et al.*, 1968). It seems probable, in fact, that ammonia is the main nitrogenous nutrient for bacterial growth in the rumen. On the other hand, the protozoa in the rumen are mainly ciliates (Hungate, 1966) and ciliates in general can rarely use ammonia as a major source of nitrogen (Smith, 1969). They usually have an absolute requirement for many of the amino acids essential to higher animals and for preformed purine and pyrimidine bases (Kidder, 1967). As Smith (1969) suggests there is little reason to suppose that the rumen protozoa are any exception. It seems likely that these organisms obtain their nitrogenous nutrients by engulfing and digesting bacteria or small particles derived from the food such as chloroplasts and by actively taking up free amino acids, purine bases and pyrimidine bases from the medium (see also Coleman, 1967). Coleman (1967) has shown that the rumen ciliate, *Entodinium caudatum*, incorporates nucleotides, derived from bacterial nucleic acids, directly into its own nucleic acids. The finding of Ulbrich and Scholz (1966) that ^{15}N, labelling rumen ammonia, accumulated rapidly in the bacteria and more slowly in the protozoa, is consistent with the view that the protozoa derive some of their nutrients by ingesting bacteria but the relative importance of bacteria, food particles and soluble nutrients in protozoal nutrition is likely to vary with the diet (Smith, 1969).

Recent studies have shown that in ruminants quantity and quality of ingested protein can be altered considerably before entering the small intestine, the main site of protein absorption (Hagemeister and Pfeffer, 1973; Hogan, 1975; Tamminga, 1975). This is the result of the simultaneous occurrence of microbial breakdown of dietary protein and microbial protein synthesis. If diets with a high protein content are fed, breakdown of protein usually exceeds microbial protein synthesis, resulting in high levels of ammonia in the rumen and in a net loss of nitrogen from the forestomachs. This nitrogen is regarded as apparently digested nitrogen, but has hardly any value in meeting the protein requirements

of the animal (Van't Klooster *et al.*, 1977). Otherwise, with feeding low protein diets microbial protein synthesis very often exceeds breakdown of dietary protein, which is possible because of microbial protein synthesis from recycled nitrogen.

Although the chemical and physical nature of the substrates supplied in the food is a primary selective influence determining the nature of the rumen microbial population (high concentrations of starch favouring the growth of amylolytic organisms, high fibre content increasing the number of cellulolytic organisms and so on) other factors such as pH, ionic composition and turnover rate are also important determinants of the occurrence of individual microbial species and so of the pattern of products (Sutherland, 1976). Hogan and Hemsley (1976) suggest that the extent of proteolysis is likely to be affected mainly by the concentrations of enzymes and substrate, the time available for the reactions and the presence of inhibitors.

Speculation on the role of rumen microbes in the first step of conversion of plant to animal protein by ruminant animals extends back at least to Zuntz (1891); however, many years passed before Schlottke (1936) and Sym (1938) showed that proteases in the rumen were in fact exclusively of protozoal and bacterial origin. Only a few microbial species are proteolytic but their occurrence is widespread and proteolytic activity has been considered to be independent of the composition of the microbial population in the rumen (Thomas, 1977). Recent evidence is not entirely consistent with this view (Hume, 1974). Proteolysis varies with the solubility of dietary proteins, and materials such as zein, soya bean and fish meal are only 30% - 45% degraded (Hume, 1974). It has been suggested that protein breakdown is reduced at high levels of feeding but soluble proteins are unaffected and even the results for insoluble proteins are equivocal (Miller, 1973; Ørskov and Fraser, 1973). Dietary additions of sodium chloride may reduce degradation by increasing the rate of passage of protein from the rumen (Hemsley, 1975).

A schematic summary of nitrogen utilisation by ruminants is presented in Figure 25. The amount of dietary true protein that escapes degradation varies, but an escape rate of 40% is probably an acceptable average for most dairy and beef animals (Satter and Roffler, 1977). The amount

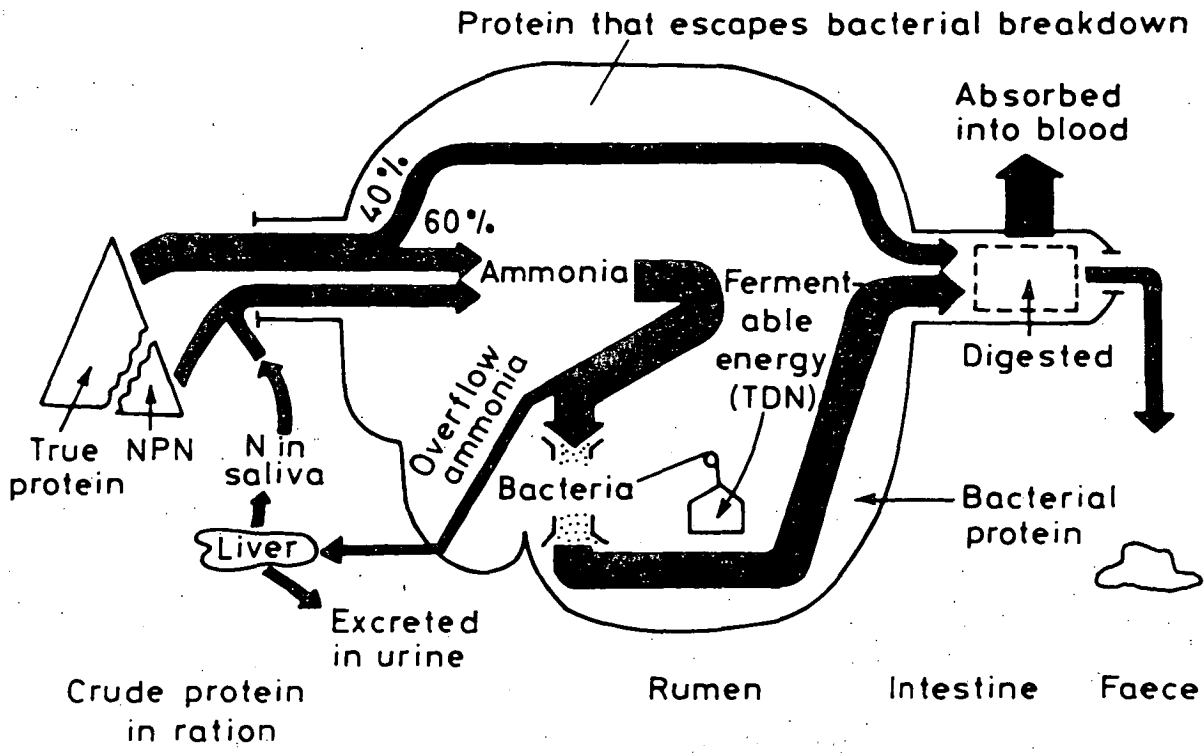


FIGURE 25. Schematic summary of nitrogen utilisation by ruminants

NPN = non-protein nitrogen
TDN = total digestible nutrient content

(Satter and Roffler, 1977)

of ammonia that can be utilised will depend on the number of bacteria and how rapidly they are growing, or in other words, the amount of fermentable energy. Satter and Roffler (1977) state that "it does not make much difference whether dietary true protein is degraded as long as the rumen bacteria are able to utilise all of the ammonia produced. Whether degraded or not dietary and recycled nitrogen ultimately ends up as protein in the intestine". This is not true, however, when ammonia production exceeds uptake by the rumen microbes. Nolan and Leng (1972) observed that in two experiments with many forages, including lucerne chaff, an average of 60% of the crude protein was broken down in the rumen and 40% passed unaltered to the small intestine where these proteins were hydrolysed into amino acids and then absorbed. The utilisation of nitrogen in the rumen has recently been studied using ^{15}N (see Nolan, 1975). The results, mainly for forage diets, show that 50% - 70% of bacterial nitrogen and 31% - 55% of protozoal nitrogen is derived from ammonia. Thus the uptake of preformed amino acids makes a substantial contribution to microbial synthesis. Nolan and Leng (1972) reported that in sheep fed 800 grams of lucerne daily, 80% of the microbial nitrogen passed through the ammonia pool and the remaining 20% came directly from amino acids. Baldwin *et al.* (1972) using a ^{15}N-ammonia tracer technique *in vitro* found that about 50% of microbial-N in the sheep was derived from ammonia-N. Pilgrim *et al.* (1970) estimated that 62% and 64% of the bacterial protein and 35% and 41% of the protozoal protein were derived from rumen ammonia in sheep given a lucerne diet. It appears that amino acids may make an appreciable contribution to microbial protein in sheep given lucerne diets.

Deficiencies in the supply of valine, leucine, isoleucine, phenylalanine and tryptophan to the microbes have been reported with diets containing little protein and a high proportion of non-protein nitrogen (see Thomas, 1973). But this apart, only the results of Hume (1970b) indicate that the mixture of amino acids supplied by dietary protein has an influence on microbial synthesis.

The ^{15}N results of Nolan (1975) also highlight the significance of engulfment of bacteria by protozoa. About 20% of the nitrogen incorporated into the microbes was recycled through the ammonia pool presumably due to protozoal activity or lysis of bacteria. With cereal diets where

the numbers of protozoa are high, engulfment of bacteria could account for about 9 g N/day in the sheep (Coleman, 1975) and, since a large proportion of the protozoa are sequestered in the rumen (Weller and Pilgrim, 1974), recycling of nitrogen could be extensive. Nolan and Leng (1972); Nolan *et al.* (1972) using forage diets have found that between 4.3 and 3.1 g N/day or about 30% of the ammonia continually being incorporated into microbial protein was recycled through the amino acid and ammonia pools in the rumen. They suggest that the recycling takes place largely within the rumen itself, namely, ammonia → other nitrogenous compounds → ammonia. This could occur as a result of lysis of bacteria in the rumen (Jarvis, 1968)due to the action of bacteriophages (Adams *et al.*, 1966; Hoogenraad *et al.*, 1967), or cytoclastic mycoplasma (Robinson, quoted by Hungate, 1970), or the engulfment of bacteria by protozoa which utilise bacterial amino acids (Coleman 1967) and produce ammonia as an end-product of their intermediary metabolism (McDonald, 1968), or lysis of bacteria after death (Hungate, 1966). One estimate has indicated that 40% of ruminal bacteria may be engulfed by protozoa (Abe and Kandatsu, 1969).

The accumulation of ammonia in the rumen generally has an adverse effect on the animal's nutrition, mainly because some of the ammonia passes directly into the blood. Toxic effects occur only in extreme cases and are apparently rare in practice although inhibition of rumen motility has been reported with ammonia concentrations in the rumen fluid of about 100 mg/100 ml (Juhasz, 1965).

Nitrogen is lost from the rumen as ammonia (Hogan, 1957) but there are additional non-dietary nitrogen inputs in the form of urea (McDonald, 1948) and protein in saliva and possibly trans-epithelial flow as ammonia originating from plasma urea (Houpt and Houpt, 1968) and some small transfer of amino acids. The total protein passing from the rumen to the omasum and abomasum may thus be less than or exceed the dietary input depending on the relative importance of ammonia losses to the additional nitrogen from saliva and trans-epithelial inflow (Sutherland, 1976).

Although ammonia appears to be the main end product of most degradation processes occurring in the rumen, other non-protein nitrogenous compounds

accumulate in rumen fluid to a relatively small extent, particularly in the first 1 or 2 hours after feeding (Stallcup and Davis, 1966). Amino acids, arising partly directly from the diet and partly as intermediates in protein degradation, are probably the most important and some consideration has been given to the possible nutritional importance of their uptake across the rumen wall. It has been shown that certain amino acids added in fairly large amounts to the rumen can enter the blood in this way (Cook *et al.*, 1965) but several workers (Leibholz, 1965; Wright and Hungate, 1967a), using both cattle and sheep, have found that, after normal feeds, the concentrations of individual free amino acids in rumen fluid rarely exceed about 0.3 m-mole/litre.

Amines, other than the common amino acids, have been identified in rumen fluid (Sjaastad, 1967). Some of these appear to originate only in the diet but some may be formed in the rumen by the decarboxylation of amino acids or in other ways (e.g. cadaverine, putrescine, histamine, phenylethylamine, δ-amino valeric acid, β-amino isotutyric acid) Sanford, 1963; Van der Horst, 1965; Clarke *et al.*, 1966; Hendriks *et al.*, 1966; Sjaastad, 1967). With normal feeding these substances are present in only small amounts in the rumen and clearly have no appreciable influence on the animal's nitrogen economy (Smith, 1969).

Nucleic acids (RNA or DNA), added in large amounts to the calf rumen, are rapidly degraded (McAllan and Smith, 1968). The degradation was shown (Smith and McAllan, 1970) to lead to temporary accumulations of low molecular weight polynucleotides (from DNA only), mono-and/or oligonucleotides, guanosine, adenosine, pyrimidine nucleosides and pyrimidine (but not purine) bases. These substances disappeared from the rumen much more rapidly than polyethylene glycol added as a marker and were not present in appreciable quantities in the rumen fluid of calves receiving normal diets (see also Smith, 1969). Smith (1969) suggests that about 40% - 50% of the microbial nucleic acid nitrogen produced in the rumen is not absorbed from the gut or is absorbed but excreted in the urine as allantoin and that most of the remainder enters the urea pool.

(c) Nitrogen Absorption from the Rumen

Ruminal ammonia which is not incorporated into nucleic or amino acids

and eventually into microbial protein is absorbed from the rumen or the lower gastro-intestinal tract. The extent of absorption in the rumen is influenced by pH (Hogan, 1961; Bloomfield *et al.*, 1963) and concentration gradient (Lewis *et al.*, 1957; Hogan, 1961). Ammonia entering the blood is rapidly converted to urea probably mainly in the liver although the results of Aliev and Kosarov (1967) suggest that some conversion may take place in the rumen mucosa. Apart from being converted to urea it is possible that ammonia absorbed into the blood may also be used in the liver for the synthesis of non-essential amino acids. Although this process has not apparently been demonstrated in ruminants it has been shown to occur to an important extent in man and the rat (Wrong, 1967). Anomalies in the relationship between rumen ammonia and blood urea have been reported (Abou Akkada and El-Shazly, 1965) and it has been pointed out that other factors may play a part in absorption (Pilgrim *et al.*, 1969) and that the effective pH, i.e. the pH at the site of absorption, may be different from that of the rumen fluid in general (Sperber, 1968).

Absorption of ammonia across the rumen wall may occur into the veins draining the rumen (Chalupa, 1972) and/or into the peritoneal fluid (Chalmers *et al.*, 1971), but there is no clear evidence that the absorption is sufficiently great to be of nutritional importance except under abnormal conditions (Smith, 1975). Smith (1975) states that when large, toxic doses of urea are given rumen pH may exceed 7.3 (Perez *et al.*, 1967; Chalmers *et al.*, 1971) and appreciable concentrations of unionised ammonia, which readily crosses the rumen wall, may be present (pKa for ammonia is about 9.25); when urea is given to provide a normal crude protein intake with adequate amounts of energy the pH generally remains below 6.5 (Chalupa *et al.*, 1970; Ciszuk, 1973) and then there is little evidence of appreciable absorption of ammonia from the rumen.

Observed correlations between concentrations of rumen ammonia on the one hand and of plasma urea or portal blood ammonia on the other (Lewis *et al.*, 1957; Smith, 1969), which are sometimes regarded as being evidence of such absorption, can also be explained by absorption of ammonia from digesta after passage from the rumen. The extent of nitrogen disappear-

ance from the digesta above the duodenum has been demonstrated in experiments with sheep (Hogan, 1965; Hogan and Weston, 1967a,b; Nicholson and Sutton, 1969). With low nitrogen intakes, and therefore low concentrations of ammonia in the rumen, there was usually a net increase of nitrogen between the diet and the duodenal digesta; with high nitrogen intakes, on the other hand, there was usually a loss of nitrogen in the stomachs which clearly varied with the composition of the diet but which was sometimes as much as 50% of the nitrogen intake. It was suggested that most of the nitrogen lost was absorbed as ammonia, the absorption probably occurs in the omasum as little absorption of ammonia has been found from the sheep abomasum (Chalmers *et al.*, 1972).

The fact that peak rumen ammonia concentrations often occur some hours before corresponding responses in blood composition (Hillis *et al.*, 1971; Ciszuk, 1973) suggests that absorption below the rumen plays an important part in the process; Nolan and Leng (1972) concluded, from a study of movements of ^{15}N-labelled compounds, that virtually all the ammonia leaving the rumen of sheep given a diet of dried lucerne left with the digesta flow. They estimated that only 2 g N/day as ammonia was absorbed from the forestomach of sheep (reticulo-rumen plus omasum); the remainder of the ammonia nitrogen (7.9 g N/day) was largely lost in microbial cells leaving the rumen.

Pilgrim *et al.* (1969), using ^{15}N introduced into the rumen of sheep, calculated that the amounts of ammonia-N produced in the rumen and absorbed there or at lower levels of the digestive tract were equivalent to 23% - 27% of the dietary nitrogen in lucerne hay chaff and 17% of that in lucerne hay pellets; 59% - 66% of the ammonia-N was absorbed from the rumen and the remainder passed to the omasum.

The general conclusion, that absorption of ammonia from the rumen is not an important factor in limiting the use of nitrogen from diets containing normal amounts of energy and nitrogen, even when the latter is derived largely from urea, agrees with that of Harmeyer *et al.* (1973) based upon studies of urea kinetics in sheep. Recently Nolan and Rowe (1976) suggest that some rumen ammonia nitrogen reaches blood urea by direct absorption and conversion in the liver, while some flows an indirect route as follows: rumen ammonia → microbial protein → absorbed

amino acids → blood urea. Some nitrogen also may pass by numerous other minor pathways. Nolan and McRae (1976) have approached the problem of partitioning these two major routes of transfer from the rumen ammonia pool to the plasma urea pool using sheep fitted with re-entrant duodenal cannulas; all digesta flowing from the rumen were collected, ^{15}N-labelled material from the rumen was thus prevented from passing into and being absorbed from the small intestine and the second route was thus curtailed. The only major transfer of ^{15}N to blood urea was therefore via absorbed ammonia; in sheep given a medium quality pelleted diet, Nolan and McRae (1976) found this was the major route for absorption of ruminal ammonia-N amounting to approximately 6 g N/day.

In addition to absorption of ammonia from the rumen, absorption was demonstrated by McDonald (1948) from the omasum, from the lower part of the small intestine to a limited extent, and from the cecum - in fact, from those parts of the alimentary tract where bacterial activity is appreciable.

(d) Transfer of Plasma Urea into the Rumen

Urea which escapes urinary excretion may pass to the rumen by way of the saliva (McDonald, 1948; Somers, 1961a,b,c,d; Houpt, 1959) and by diffusion across the rumen wall (Houpt, 1959; Houpt and Houpt, 1968; Moir and Harris, 1962). Urea was shown to be the most important nitrogen component in mixed and parotid saliva of sheep, making up 60% to 70% of the nitrogen (Somers, 1961a). The salivary route has been estimated to provide nitrogen equal to about one-tenth that in the diet (Kay, 1963). Under normal conditions, the urea concentration of the saliva is between 6 and 20 mg in 100 ml, and its ammonia level varies from 20 to 30 µg in 100 ml (Juhasz, 1972).

While there is general acceptance of the concept that the magnitude of nitrogen entry via saliva varies in relation to blood urea-N concentration (Somers, 1961b) the extent to which urea-N enters other than via saliva is not clear. Houpt (1959) suggests that transfer of urea from the blood to the rumen by diffusion through the rumen wall appears to be more important than by way of the saliva; Houpt (1959) reported that in sheep approximately 16 times as much urea-N reached the rumen

via diffusion than via the saliva but the tracer measurements of Nolan and Leng (1972) indicate that for sheep given lucerne diets, all the urea-N that entered the rumen could be accounted for by the salivary route. A similar conclusion was made by Kennedy and Milligan (1978a) who fed sheep lucerne hay or pellets, but in sheep given brome grass pellets, the transport of urea across the rumen epithelium contributed up to 9 g N/day or approximately 0.9 of the total transfer. Kennedy and Milligan (1978) found an inverse relationship between the rate of transfer of plasma urea-N to the rumen ammonia pool and ammonia concentration, and they suggested that this relationship may explain the differences obtained for estimates of urea transfer in sheep given brome grass or chaffed lucerne hay. In a recent experiment (Norton *et al.*, 1978), sheep were given diets of low nutritive value (native grasses) and the major rate of urea entry from the blood to the rumen was via saliva.

Juhasz (1972) suggests that urea passes from the blood into the rumen by diffusion across the rumen wall and the quantity of nitrogen reaching the rumen by diffusion is about ten times as much as nitrogen secreted with saliva. He estimated that at a urea concentration of 20 - 60 mg in 100 ml blood, 6 - 16 g urea will be recycled daily.

Quantitative measurements revealed that urea influx into the rumen is of quantitative importance (Table 1, Varady and Harmeyer, 1972). It may contribute more than 50% of the total nitrogen which is utilised in the rumen. Under conditions of low protein feeding a higher percentage is of endogenous origin (Varady and Harmeyer, 1972).

In vitro studies using isolated rumen epithelium there was apparently no movement of urea across the rumen wall (Norton, quoted by Nolan *et al.* 1972). Hecker and Nolan (1971) also came to similar conclusions from studies in which measurements of arteriovenous differences of urea across the rumen were made and it was suggested that the movement of urea into the rumen is mainly via salivary secretions. In other studies (see Nolan *et al.*, 1972) when sheep were given a low protein, high energy diet a greater proportion of the urea degraded actually entered the rumen even though plasma urea concentrations were low (4.4 mg N/100 ml). This suggested that in these animals there may have been some form of facilitated transport of urea into the rumen, since it was unlikely that saliva

TABLE 1. NITROGEN ENTRY (g) INTO THE RUMEN OF SHEEP PER DAY (Varady and Harmeyer, 1972)

Via saliva	Via rumen wall	Percentage of daily N-intake	Author
0.9 - 5.0		4 - 20	Somers (1961) Phillipson (1941)
	1.6 - 6.0	10 - 20	Houpt (1959)
2.5 - 3.5		37 - 45	Weston and Hogan (1967)
1.1 - 8.7		30 - 67	Coccimano and Leng (1967)

could have accounted for more than half of the 1 g urea apparently entering the rumen of these animals (Nolan *et al.*, 1972); it was concluded that there may be significant differences between animals on different diets and that, at times when nitrogen is limiting microbial growth, some mechanism of transport of urea into the rumen may be operative.

The first reliable measures of net endogenous urea transfer to the rumen of fed sheep (Weston and Hogan, 1967) and cattle (Vercoe, 1969) were approximately 5 g and 17 - 20 g N/day, respectively. These estimates were derived by comparing the increases in rumen ammonia concentration after urea was infused intravenously with the relationship previously established between ammonia concentration and intraruminal urea infusion rate. In these studies no further increase in rumen ammonia concentration was observed when plasma urea concentration increased above 120 mg N/L (Vercoe, 1969) for cattle, and 160 - 180 mg N/L (Weston and Hogan, 1967) for sheep.

More recently, Kennedy and Milligan (1980) suggest that for some roughage diets, the rate of urea transfer to the rumen of sheep was small and was attributable to salivary urea, whereas for other diets, substantial amounts of urea appeared to be entering the rumen across the rumen epithelium in addition to that carried in saliva; regression analysis indicated that the rate of transfer of endogenous urea to the rumen of sheep given those diets was associated with the concentration of rumen ammonia and of plasma urea, and with the amount of organic matter digested in the rumen. Kennedy and Milligan (1980) showed that the clearance of plasma urea to the rumen was inversely proportional to the concentration of rumen ammonia in both sheep and cattle, but that clearance was increased by the addition of grain or sucrose to the diet. They concluded that transfer of urea across the rumen epithelium may contribute significantly to the nitrogen economy of the micro-organisms, but the mechanism by which such transfer is controlled by the ruminant remains obscure.

Ruminants conserve nitrogen when dietary supplies are low by utilising endogenous urea and protein as sources of nitrogen for microbial protein synthesis (Houpt, 1970). Endogenous urea-N may be utilised by

the ruminant, because after transferral to the rumen, it is substantially broken down to ammonia (McDonald, 1948). Studies using ^{15}N have revealed that ammonia production in the rumen may be equivalent to 17% - 84% of dietary nitrogen intake (Mercer and Annison, 1976); these measurements included production of ammonia from endogenous protein and non-protein sources of nitrogen.

There is little doubt that entry of plasma urea into the rumen can provide a significant source of nitrogen for microbial growth and therefore has survival value where dietary nitrogen intake is low (Kennedy and Milligan, 1980). For sheep and cattle given low quality hay diets, endogenous urea may provide 25% of N available in the rumen (Nolan and Stachiw, 1979; Kennedy, 1980). For sheep given brome-grass pellets, 21% of the microbial nitrogen passing from the abomasum was derived from endogenous urea (Kennedy and Milligan, 1977). Continuous secretion of endogenous urea would maintain rumen ammonia levels allowing prolonged microbial digestion of the refractory fibrous components of the diet and maintenance of a viable microbial population during periods of dietary nitrogen-inadequacy or of fasting (Kennedy and Milligan, 1980). However, when a readily fermentable energy source is added to the diet, the endogenous supply of urea will often be inadequate and responses will be obtained with dietary supplements of urea or other non-protein nitrogen sources (Ørskov and Grubb, 1978). The response to dietary urea would be influenced by the amount of fermentable energy added and the concentration of plasma urea, those being the main factors affecting the entry of endogenous urea into the rumen at low concentrations of ammonia (Kennedy and Milligan, 1980).

The importance of readily available energy in the utilisation of circulating urea was shown by Houpt (1959); when carbohydrates (corn starch and sucrose) were supplemented to a low-protein ration, 52% of an ingested urea dose was utilised for rumen protein synthesis; without the supplemental carbohydrates, utilisation decreased to 22%.

Apart from the plasma urea-N transferred into the rumen, entry of endogenous N,other than urea,into the rumen has been suggested. Thus, Kennedy and Milligan (1977) and McRae *et al.* (1979), after comparison of the entry of dietary nitrogen plus endogenous urea N into the rumen with the

flow of non-ammonia N entering the duodenum, concluded that at least 1.7 - 3.0 g N/day of endogenous protein N was secreted into the reticulo-rumen, omasum and abomasum of sheep. Also, as a result of ^{15}N tracer studies, Havassy *et al.* (1974) concluded that non-urea nitrogenous substances, possibly glutamine and glutamic acid synthesised in the rumen epithelium, passed into the rumen. Indeed Kowalczyk *et al.* (1975) suggested that nitrogen derived from blood urea was utilised in the rumen wall and in other tissues to a larger extent than in the synthesis of microbial protein in the rumen.

(e) Some Aspects of Non-Protein Nitrogen Utilisation by the Ruminant

The feeding of non-protein supplements to ruminants is based on the knowledge that ammonia is the major end-product of the degradation of proteins in the rumen (McDonald, 1962), and on the belief, which appears to have been generally accepted, that most of the nitrogen utilised by micro-organisms comes from the ammonia pool in the rumen (Nolan and Leng, 1972). It has been established that ruminants can be maintained on diets containing no protein but only a non-protein source of nitrogen such as urea or various ammonium salts (Loosli *et al.*, 1949; Virtanen, 1966), indicating that all the essential amino acids for ruminants can be synthesised from ammonia by the ruminal micro-organisms.

It has been considered that the low concentrations of amino acids occurring in ruminal fluid result from rapid deamination of the free amino acids to keto acids and ammonia (El-Shazly, 1952; Lewis, 1955; Warner, 1956) rather than from rapid uptake of amino acids by micro-organisms even though some micro-organisms are known to have an obligatory requirement for peptides or amino acids *per se* (Hungate, 1966). The degrees to which ammonia, amino acids or even peptides may constitute starting points for snythesis of microbial-N in normally fed ruminants have not been ascertained, but could be expected to vary with the nature of the diet and possibly with the particular animals under observation (Pilgrim *et al.*, 1970). Some evidence is already available to suggest that in normal feeding (e.g. hay-fed cattle and sheep), ammonia-N may be the starting point to a very considerable extent (Portugal and Sutherland, 1966). However, as Nolan (1975) states, it is energetically and nutritionally wasteful for peptides and amino acids to be degraded and resynthesised, and microbial species that are known to assimilate amino acids when these are available (Portugal, 1963; Wright and Hungate, 1967b) would have a

competitive advantage over others without this capacity. It seems particularly likely that direct assimilation may occur in protozoa which have proteolytic enzymes but no deaminases and which appear to utilise amino acids (Coleman, 1967), Nolan (1975) has noted quite high levels of protozoa (5×10^5 protozoa/mℓ) in the rumen of sheep given a ration of 800 g/day lucerne chaff in equal portions every day and suggested that most proteolysis appears to take place inside the microbial cell or at the cell surface; it was suggested that this may enhance the possibility of direct assimilation of peptides and amino acids *in situ*, particularly if amino acids are slowly released.

Several reviews on different aspects of non-protein nitrogen utilisation by the ruminant have been published (Helmer and Bartley, 1971; Chalupa, 1972; Visek, 1973; Smith, 1975). The value of non-protein nitrogen to the ruminant generally depends upon the formation of ammonia in the rumen and the subsequent use of this ammonia by the rumen bacteria (Allison, 1970; Bryant, 1973). It has long been recognised that supplemental non-protein nitrogen is most efficiently utilised in rations low in protein and relatively high in digestible energy (Satter and Roffler, 1976).

There is evidence that increased levels of organic-N may reduce the microbial utilisation of ammonia (Pilgrim *et al.*, 1970; Mathison and Milligan, 1971). The relative low level of rumen ammonia for optimal utilisation, and the adverse effects of raised levels of organic-N on ammonia utilisation, may explain the poor responses obtained when normal diets are supplemented with non-protein nitrogen (Annison, 1975). Furthermore, the need to avoid sharp increases in ammonia concentration has important implications for the effective use of non-protein nitrogen. Annison (1975) clearly states that 'little and often' should be the objective when feeding sources of non-protein nitrogen that generate ammonia rapidly in the rumen, or if the system of husbandry calls for once or twice daily feeding, slow release ammonia sources might be useful. As emphasised by Loosli and McDonald (1968) the level of rumen ammonia is the simplest indicator of the likely usefulness of supplementary non-protein nitrogen. An ideal situation for the utilisation of non-protein nitrogen by ruminants is where the rate of ammonia-N production is balanced by the rate of utilisation by micro-organisms so that there

will be minimal escape of ammonia from the rumen (El-Shazly and Abou Akkada, 1972).

Satter and Roffler (1976) studying the relationship between ruminal ammonia and non-protein nitrogen utilisation by ruminants concluded that: (1) non-protein nitrogen may be utilised as well as plant protein when ruminal ammonia nitrogen concentration is low (<5 mg/100 mℓ; (2) supplementing non-protein nitrogen to animals whose ruminal ammonia concentration is in excess of 5 mg/100 mℓ would be without benefit; and (3) cattle may benefit from non-protein nitrogen added to high concentrate rations containing less than 12% - 13% crude protein (dry matter basis) or to all-forage rations containing less than 9% - 10% crude protein.

The results of Ranjhan *et al.* (1976) showed that milk yields and body weights were no different between cows given conventional concentrate and roughage-based rations and those receiving urea/molasses with a restricted amount of intact protein and cereal fodder. They also found that cows in experimental groups consumed both more energy and protein than their requirements but these higher intakes were not reflected in their milk yields or body weight gains, indicating poor utilisation.

Urea has been the most widely used non-protein nitrogen compound in ruminant rations. From the available evidence it seems likely that, for urea-containing diets of normal crude protein content, most of the ammonia rapidly liberated by ureolysis usually remains available to the rumen bacteria for some hours after feeding (Smith, 1975). This view is supported by findings that giving urea continuously or in frequent small doses, rather than twice a day, did not improve nitrogen utilisation in lambs (Knight and Owens, 1973; Streeter *et al.*, 1973). Similarly there have been very few reports in the literature of unequivocal improvements in nitrogen utilisation by ruminants due to inhibition of urease activity or to the replacement of urea by more slowly degraded non-protein nitrogen sources such as biuret or isobutylidene diurea (Smith, 1975).

Feeding a slow release extruded urea product containing urea, starch and carboxy resin improved performance and nitrogen retention in sheep (Huston *et al.*, 1974). Feeding 5% tallow with 1.5% urea tends to depress

nitrogen retention in sheep and rumen protein synthesis in vitro (Phillips and Church, 1975). These results may explain the depressed performance observed when urea was fed to steers in combination with tallow (Bradley *et al.*, 1966). Feeding branched-chain volatile fatty acids improved nitrogen retention in lambs fed a urea supplemented high roughage ration (Umunna *et al.*, 1975). An acid-resistant hemicellulose from corn cobs increased microbial protein synthesis *in vitro* and increased nitrogen retention of lambs fed a urea-supplemented high roughage ration (McLaren *et al.*, 1976). In beef cattle nutrition there is a trend toward using greater amounts of urea (Church, 1979). However, as pointed out by Chalupa (1968), even if 90% of the supplementary nitrogen is from urea, this will represent only about one-third of the protein equivalent in a typical ration. It appears, however, that some amino acids may be limiting for growth on certain diets. Methionine and threonine appeared to be limiting for growth of calves fed urea or soybean meal as a supplement (Leibholz, 1976) and sulphur containing amino acids were the most limiting for sheep fed urea containing diets (Owens *et al.*, 1973). Utilisation of urea may have a special application in areas where heat stress is a factor since a limited amount of information suggests that its inclusion will reduce the heat increment of feeding (Colovos *et al.*, 1963).

Ruminal urea hydrolysis usually proceeds at a faster rate than the ammonia assimilation reactions but there is better utilisation of nitrogen and ammonia toxicity is not a problem if ammonia production rates are more nearly equal to utilisation rates (Allison, 1970). Emphasis has thus been placed on feeding readily available carbohydrates with urea so that microbial growth, and ammonia assimilation, is rapid. Attempts have also been made to inhibit urease activity and to provide urea and other sources of non-protein nitrogen in forms that permit less rapid production of ammonia. Recent examples include: (1) development of a gelatinised starch product from which urea is slowly released (Deyoe *et al.*, 1968); Helmer and Bartley, 1971); (2) demonstrations that nitrogen can be supplied as uric acid (Oltjen *et al.*, 1968) and as biuret (but a period for 'adaptation' or selection of competent biuret-using microbes is important (Schroder and Gilchrist, 1969; McLaren, 1964); and (3) attempts to inhibit ruminant urease chemically or immunologically (Tillman

and Sidhy, 1969). The compound glucosyl ureide (Milligan *et al.*, 1972; Smith *et al.*, 1975) and the use of molasses with urea (Ramirez, 1972), also affect nitrogen utilisation by providing a suitable energy source to encourage microbial protein synthesis.

In Australia more than 90% of ruminants are grazed on pasture (Weston and Hogan, 1973) which at certain times of the year may be low in protein (<0.5% N/kg dry matter) and high in cell wall content. In sheep and cattle given these diets, dry matter intakes are low and the animals characteristically decline in body-weight (Kempton and Leng, 1979). In general, supplementation of these diets with non-protein nitrogen has not significantly improved production (Loosli and McDonald, 1968; Leng *et al.*, 1973). Egan (1965) and Egan and Moir (1965) however, have shown with mature wethers given dry low quality cellulose diets that organic matter intake could be substantially increased by providing amino acids post-ruminally. Recently, the effects of supplementation of a cellulose-based diet with either urea, casein or formaldehyde-treated (HCHO) - casein were studied in growing lambs (Kempton and Leng, 1979). The results from these studies indicated that supplementation of a low-protein-cellulose-based diet with such a protein increases feed intake and live-weight gain.

Finally, Ørskov (1977) suggests that non-protein nitrogen is likely to have little potential for utilisation in systems for high production if protein supplements are given conventionally so that they enter the rumen. When protein supplements are protected from rumen degradation non-protein nitrogen can be utilised in the basal feed if the microbial requirement is not met (Ørskov, 1977).

(f) Microbial Protein Synthesis in the Rumen

Microbial protein leaving the rumen ultimately provides a source of amino acids for the host animal as the protein is digested and absorbed in the small intestine (Stern and Hoover, 1979). Since rumen microbes are an important source of protein for the ruminant and since microbial growth rates can affect amino acid availability to the animal, it is important to study factors that may influence microbial protein synthesis and to optimise the yield of protein (Nikolic, 1977). This section will

examine the methods used to determine rumen microbial protein synthesis and discuss various factors affecting microbial growth.

(f1) Estimation of Microbial Protein Synthesis

Various methods have been used to estimate the quantity of microbial protein in digesta leaving the ruminant stomach. The majority of these techniques is based on determination of a single chemical marker believed to characterise the microbial components. Diaminopimelic acid (DAP), amineothylphosphonic acid (AEP), ribonucleic acid (RNA), and isotopes (^{35}S, ^{15}N, ^{32}P) incorporated into protein in the rumen have been used for this purpose. Differences in the amino acid profiles of individual components reaching the duodenum also have been used. Recently adenosine triphosphate (ATP) was proposed as a marker of microbial activity.

References

Diaminopimelic Acid

DAP was used by Weller *et al.* (1958) to estimate the rate of synthesis of bacterial protein. This technique takes advantage of the fact that DAP is present in the cell membrane of many types of rumen bacteria but is absent from plant material. The DAP method involves estimating the ratio of DAP:N in mixed rumen bacteria and the amount of DAP in digesta. From these values the amount of bacterial nitrogen in digesta can be calculated (Hogan and Weston, 1970). The ratio DAP:N has been reported to vary between species of rumen bacteria, from 0.6 to 3.4 g/100 g total amino acids (Purser and Buechler, 1966), but on fixed dietary regimes the ratio appears to be reasonably constant (Weller *et al.*, 1958) (Table 2). The reason for the high DAP:N ratio determined by Hutton *et al.*(1971) is not clear. Traces of DAP can probably be detected in protozoa, as bacteria in vesicles of entodiniomorphs and holotrichs have been observed by electron microscopy (Coleman and Hall, 1969; Stern *et al.*, 1977a,b).

Aminoethylphosphonic Acid

The main limitation to the use of DAP is that it measures only bacterial protein synthesis; data from Walker and Nader (1975) indicate that DAP-bacterial protein flows are 12% to 38% lower than ^{35}S-microbial protein flows (Table 3). A protozoal marker would therefore be a valuable adjunct to DAP. Ibrahim and Ingalls (1972) used DAP to measure bacterial protein synthesis and AEP, which is found in the lipid fraction of

TABLE 2. **DAP:N ratios in bacterial fractions** (Hume, 1976)

	mgDAP/g N
Weller, Gray and Pilgrim (1958)	38 – 44
Hogan and Weston (1970)	35 – 46
Hutton, Bailey and Annison (1971)	52 – 56
Ulyatt *et al.* (1975)	42 – 45

TABLE 3. **Comparison of estimates of microbial protein flow derived from ^{35}S and DAP** (Walker and Nader, 1975)

Ration	Microbial Protein Flow (g/day)		
	^{35}S (A)	DAP (B)	$(\frac{A-B}{A}) \times 100$
Wheaten straw	41.7	33.8	18.9
Wheaten hay chaff	49.8	43.9	11.9
Lucerne hay chaff	80.3	50.0	37.7
Clover hay	68.4	53.3	22.1
Perennial ryegrass	74.6	60.2	19.3
Tama ryegrass	75.7	60.1	26.0
White clover	74.5	63.6	14.6

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protozoa, to estimate protozoal protein synthesis. Abou Akkada *et al.* (1968) found this amino acid (AEP) to be consistently present in the ciliate protozoa *Isotricha* spp. and *Entodinium* spp., but absent from bacterial and plant protein.

Ibrahim and Ingalls (1972) found that the contribution of protozoa to the microbial protein in the rumen was 45% and 51% on two different diets; they assumed from this that the digesta leaving the rumen contained the same proportion of protozoa. There is evidence from Hungate *et al.* (1971), Weller and Pilgrim (1974) and Nolan (1975) that protozoa are preferentially retained in the rumen, and do not leave at the same rate as the bacteria. Thus, Ibrahim and Ingalls' (1972) protozoal and bacterial flow values are unlikely to be correct.

Czerkawski (1974) described methods for DAP and AEP determination that are relatively fast compared to other methods and require less elaborate equipment.

Ribonucleic Acid

Smith and McAllan (1970) used the ratio of RNA to total nitrogen in rumen fluid and rumen microbes to estimate the extent of conversion of dietary nitrogen to bacterial and protozoal nitrogen. However, the analytical procedure (McAllan and Smith, 1969) used to determine RNA is tedious. This technique relies on the assumption that nearly all dietary RNA is degraded in the rumen (McAllan and Smith, 1973). Buttery and Cole (1977) expressed doubt as to whether this is entirely true and suggest that microbial protein flow may be overestimated and that this problem may be amplified when large portions of the dietary protein and nucleic acid have been rendered insoluble by exposure to heat or chemical treatment. Recent evidence (Smith *et al.*, 1978) indicates that the RNA method does slightly overestimate the microbial contribution at the duodenum.

Another problem with the RNA method is the variability of the RNA:total N ratio of mixed bacteria due to diet and environment (Smith and McAllan, 1974). McAllan and Smith (1974) compared RNA and DAP as markers for determining the contribution of microbial nitrogen in duodenal digesta. Experimental animals included a protozoa-free calf and a faunated cow. They found good agreement between methods for the protozoa-free animal;

however, when the faunated cow was used they found a marked discrepancy between the two techniques. The microbial nitrogen to total non-ammonia nitrogen ratios in duodenal contents in the faunated cow were 0.78 and 0.40 for the RNA and DAP methods, respectively. Unless considerable amounts of dietary RNA survived rumen degradation in the cow experiment, it seems possible that the DAP method underestimated the microbial contribution in the cow because it did not account for the presence of protozoa (Stern and Hoover, 1979).

Amino Acid Profiles

A method for estimating quantities of microbial and dietary proteins in duodenal digesta based upon differences in the amino acid content of the protein reaching the duodenum was reported by Evans *et al.* (1975). Individual proteins passing to the duodenum were identified by their characteristic amino acid profiles. It was assumed that the profile of digesta was the weighted sum of the various profiles contributing to it. The method depends upon the computer generation of several profiles which represent mixtures of different proportions of the known profiles of the dietary and endogenous components that may be arriving at the duodenum (Stern and Hoover, 1979). The method assumes constant composition for microbial protein and that protein in each dietary component behaves as a single entity.

Evans *et al.* (1975) investigated the relationship between microbial total amino acids, estimated by their method, and DAP passing through the duodenum and found an r (correlation) value of 0.92 based upon 21 samples. Buttery and Cole (1977) suggest that since this method relies on the analysis of several different constituents (amino acids) it might be expected, with more development, to yield answers with the least bias. At present, this method is limited by a lack of knowledge relative to the differential degradation rates of different proteins present in conventional diets (Nikolic and Jovanovic, 1973).

ATP

Forsberg and Lam (1977) studied the use of ATP as a rumen microbial marker because of the following assumptions: (1) ATP is present in all living cells and absent from dead cells; (2) ATP concentration is similar in all microbes; and (3) extraction and assay of ATP is relatively

simple to perform and inexpensive. However, their experiments indicated that there are possible variations in the efficiency of extraction of ATP from rumen contents and they also observed differences in the concentration of ATP in rumen microbes. In contrast, Wolstrup and Jensen (1978) used similar methods to extract ATP from rumen contents and noted that variability was not as great as that found by Forsberg and Lam (1977). Wolstrup and Jensen (1978) indicated that choice of equipment was probably the source of variation. They concluded from their study that concentration of ATP reflected the level of activity of the biomass rather than the total amount of biomass. In addition, they showed concentration of ATP in the rumen biomass to be variable, ranging from 0.07% to 0.25% and dependent upon the nitrogen source fed to the animal. However, they indicated that many more investigations are required before the ATP method could be used in routine studies.

^{35}S Incorporation

Of the radioisotopes used as tracers to distinguish between microbial and dietary protein, ^{35}S has been used most frequently (Stern and Hoover, 1979). Use of ^{35}S as a microbial marker was first suggested by Henderickx (1961). The technique introduced by Henderickx is based on the following two assumptions: (1) the S/N ratio in microbial protein is constant; and (2) the ratio ^{35}S incorporated/mg protein-N synthesised is constant. However, Hume (1975) states that, if results obtained on this basis are taken as newly synthesised microbial protein only, another two assumptions are necessary: (3) there is no ^{35}S incorporation into microbial protein already present in the rumen; and (4) there is no significant breakdown of microbial protein in the rumen itself, e.g. by cell lysis. Walker and Nader (1968) used radioactive sodium sulphide ($Na_2{}^{35}S$) to label the sulphide pool of rumen contents *in vitro*. The basic premise of this method was that all of the sulphur incorporated into microbial protein was first passed through the free H_2S pool. While *in vitro* studies of Nader and Walker (1970) suggested the error introduced by sulphur incorporation from amino acids was negligible, *in vivo* studies revealed that considerable amounts of sulphur containing amino acids in microbial protein may arise from sulphur which has not passed through the H_2S pool (Gawthorne and Nader, 1976; McMeniman *et al.*, 1976a; Salter *et al.*,1979).

Walker and Nader (1975) subsequently described a method for the *in vivo*

measurement of rumen microbial protein synthesis which depends upon the incorporation into microbial protein of sulphur derived from ^{35}S-labelled inorganic sulphate infused continuously into the rumen. This method has the advantage of not relying on use of the highly labile sulphide and does not require that virtually all microbial sulphur be derived from hydrogen sulphide (Stern and Hoover, 1979). They compared estimates of microbial protein synthesis using the above ^{35}S method with values obtained using DAP from 27 individual experiments. They found a significant correlation ($r = 0.68$) between the two methods, with DAP estimates averaging approximately 30% less than ^{35}S estimates (see also Table 3). This was attributed to the inability of DAP to account for protozoal protein.

The predominant method of using ^{35}S as a microbial marker has been to measure differences in the ratio of specific activities of ^{35}S in either the cysteine (Leibholz, 1972), methionine (Beever *et al.*, 1974) or total sulphur amino acids (Hume, 1974) of duodenal digesta and a separated microbial fraction. Also, a similar ratio of sulphur amino acids to total protein for microbial and dietary material is assumed (Nikolic, 1977). In addition, the method is relatively expensive.

Although the ^{35}S technique based on differences in the ratio of specific activities of ^{35}S in sulphur amino acids in digesta and microbial fractions is laborious and may not be completely accurate, it does have definite advantages (Stern and Hoover, 1979). Quantitative recovery of the sulphur amino acids is not essential, as the technique is based on a ratio of specific activities. Also, the technique is capable of determining total microbial protein synthesis rather than just bacterial protein synthesis as with the DAP technique.

^{15}N Incorporation

Microbial protein synthesis has also been estimated by quantitating ^{15}N incorporation into microbes from either $(^{15}NH_4)_2SO_4$ (Pilgrim *et al.*, 1970) or $^{15}NH_4Cl$ (Mathison and Milligan, 1971). These methods are based on the incorporation of nitrogen from ammonia and do not account for microbial protein synthesised directly from amino acids or peptides. Besides being costly, the technique utilising ^{15}N as a marker is quite complic-

ated and as a result has not been extensively used (Stern and Hoover, 1979). However, because it deals directly with nitrogen it has proven to be a useful tool in studying the dynamics of nitrogen metabolism in the ruminant (Nolan *et al.*, 1976).

Kennedy and Milligan (1978b) compared the use of ^{15}N with ^{35}S as microbial markers in cold-exposed sheep. Estimates of microbial protein production determined by reference to ^{15}N were approximately 10% lower than estimates made using ^{35}S as a microbial marker. They suggested that the lower estimate from ^{15}N was a possible result of secretion in the omasum or abomasum of organic ^{35}S from endogenous sources, or passage of organic ^{35}S in bacterial exudates from the rumen.

Labelled Phosphorus Incorporation

Bucholtz and Bergen (1973) observed that phosphorus uptake and incorporation into microbial phospholipids was highly related ($r = 0.98$) to ruminal protein synthesis and proposed an *in vitro* method for estimating microbial protein production based on incorporation of ^{33}P into microbial phospholipids. Van Nevel and Demeyer (1977) expanded this approach to include incorporation of ^{32}P-labelled extracellular phosphate in total microbial P as the measure of microbial growth. This method required the following basic assumptions: (1) all P incorporated into the microbial fraction was derived from the labelled inorganic precursor pool; (2) the specific activity of the intracellular precursor pool equalled the specific activity of the extracellular pool; (3) there was no degradation of nonlabelled cells; and (4) cell composition remained constant during growth. Results from their *in vitro* experiments showed that assumptions 3 and 4 were not valid in incubations with rumen microorganisms.

Comparative studies of microbial protein synthesis using incorporation of the isotopic markers ^{35}S, ^{15}N and ^{32}P were conducted by Harmeyer *et al.* (1976). These experiments demonstrated that exchange and degradation processes occurred in rumen contents to a considerable extent. The isotope techniques did not appear to distinguish between conditions of no growth and negative growth. Negative net growth values occur in situations where degradation of microbial matter is greater than synth-

esis (Naga and Harmeyer, 1975).

Ling and Buttery (1978) assessed the use of RNA, ^{35}S, DAP and AEP as markers of microbial nitrogen in duodenal digesta. They found AEP to be present in substantial quantities in dietary and bacterial material as well as in isolated rumen protozoa and suggested that the use of AEP as a protozoal nitrogen marker was invalid. They indicated that one of the major problems with DAP as an index of total microbial nitrogen was it did not account for protozoal contribution to duodenal digesta nitrogen but concluded that it would probably continue to be widely used. Where total microbial values are required, the choice of method becomes either RNA or ^{35}S. They concluded that where microbial nitrogen estimates of a more general and comparative nature are required, the use of RNA would probably be adequate, however, where more accurate estimates are required, ^{35}S would be more appropriate.

It should be recognised that the procedures discussed have a common problem (Stern and Hoover, 1979). Sampling of rumen contents and the subsequent fractionation of the rumen contents into microbial fractions is one of the most difficult aspects of microbial investigations in the rumen. Microbes are generally obtained by differential centrifugation of rumen fluid, which usually includes a low-speed centrifugation step to remove food particles. Many protozoa, large bacteria, clumps of bacteria and bacteria which remain firmly attached to feed particles (Weller *et al.*, 1958) may be lost during this step. After high-speed centrifugation the bacteria obtained may form only a small part of the total rumen bacteria and may be less metabolically active than the greater numbers of bacteria that are associated with food particles (Smith, 1975).

It is obvious that for these methods some assumptions and simplifications have to be made which should be verified. Therefore there is a need for more experimental work with these methods. It should be realised that the knowledge of the rumen metabolism involves the measurement of many reactions and their parameters, and the final conclusion will always be limited by the largest degree of uncertainty of one of these reactions.

(f2) Factors Affecting Rumen Microbial Protein Synthesis

Numerous studies have been conducted to determine microbial protein synthesis in the rumen under varied conditions. Table 4 lists the results of several studies (see also Hume, 1976; Czerkawski, 1978; Stern and Hoover, 1979). Results are expressed as grams crude protein (CP, i.e. microbial N x 6.25) synthesised per 100 g organic matter digested (OMD) in the rumen, except where noted. Because of the difficulty in obtaining an accurate measure of true OM digestion, the majority of studies in Table 4 express microbial growth efficiency on an apparent OM digestion basis. Also there is a problem in estimating true microbial protein synthesis (i.e. [microbial N less microbial nucleic acid N] x 6.25) so, for this reason, microbial growth is generally expressed as grams CP or N synthesised. Hume (1976) suggests that microbial protein yields should be expressed either as quantities of microbial protein produced per mole volatile fatty acid (VFA) (or per mole ATP) produced in the ruminal fermentation, or as quantities of microbial protein produced per unit of OM fermented. The former is the more precise, (Walker *et al.*, 1975) but few workers have measured both microbial protein production and VFA production in the same experiment.

Sutton (1972) suggests that it does not seem useful to use as the denominator substrate truly digested, i.e. OM apparently digested plus the microbial OM synthesised in the rumen, as was reported by Hogan and Weston (1967b). Not only does this require some means of estimating the extent of microbial OM synthesis but it is theoretically unsound since the energy for protein synthesis comes only from fermentation (i.e. apparent digestion) of OM, the synthesis of microbial OM being energy-consuming (Sutton, 1972). He also suggested that it is more useful to measure true protein than crude protein as the 10% - 20% of the total nitrogen that comes from nucleic acids is of little or no value to the host. The data available suggest that, on forage diets, the yield of microbial protein is likely to be in the range of 15 - 20 g protein/100 g OM fermented. However, on non-forage diets the yield could vary more widely. For example, low digesta flow rates from the rumen of animals fed restricted amounts of concentrate diets could result in an increase in the extent of cell lysis and resynthesis within the rumen, and hence

a lowered net yield (Hume, 1976). Therefore yields outside the above range are not necessarily in error, although it is important that explanations should be found for such values.

The mean (±SD) of 95 observations shown in Table 4 indicates that approximately 17.8 (±5.19) g CP are synthesised per 100 g OMD, with values ranging from 6.2 to 31.6. Also, the mean (±SD) of 16 observations shown in Table 4 indicates that approximately 10.6 (±3.02) g CP are synthesised per 100 g dry matter digested (DMD), with values ranging from 5.2 to 16.3. Values by Leibholz (1972) seem low, possibly because digesta flows were uncorrected. Some of the variability between studies can be attributed to the microbial markers used. However, there are several factors that probably induced real differences; these factors include concentration and source of nitrogen and carbohydrates, rumen dilution rate, dietary sulphur and frequency of feeding (Stern and Hoover, 1979).

Nitrogen Concentration

Rumen microbial protein synthesis requires an adequate supply of nitrogen to achieve maximum efficiency. If nitrogen level is not adequate, uncoupled fermentation may occur and this will result in fermentation without useful ATP production (McMeniman *et al.*, 1976b; Buttery, 1977). Smith (1979) also states that if nitrogen supply to the rumen bacteria is inadequate, rumen function is affected in a number of ways. Thus, digestion of starch and cellulose and other polysaccharides in the 'fibre' fraction are depressed to differing extents (McAllan and Smith, 1976). Bacterial synthetic effort is diverted from protein to storage polysaccharide (Walker and Nader, 1970; McAllan and Smith, 1977) and ATP may possibly be dispersed in other ways (McMeniman *et al.*1976b). The proportion of microbial organic matter turning over probably increases (Smith and Smith, 1977) and the reduced growth rate means that a greater proportion of ATP is likely to be used for bacterial maintenance and less for protein synthesis (Isaacson *et al.*, 1975). In contrast, if the nitrogen level is excessive, energy may be the limiting factor for efficient utilisation of nitrogen. Therefore, for maximal efficiency of microbial growth to occur, nitrogen and energy availability in the rumen must be balanced (Stern and Hoover, 1979).

TABLE 4

Efficiency of Microbial Protein Synthesis in the Rumen

Reference	Animal	Method	Diet	Protein Yield g CP/100 g OM Digested
Hogan and Weston (1967b)	Sheep	Non-ammonia N	Lucerne hay + maize + peanut meal	15.0
			Wheaten hay + maize	15.6
Conrad *et al.* (1967)	Cattle	^{35}S	Chopped dried lucerne	20.0
Hume *et al.* (1970)	Sheep	Protein-free[a]	Semipurified diet + urea	13.3
Hume (1970a)	Sheep	Protein-free[a]	Urea with:	
			VFA added	13.4
			No VFA (Volatile fatty acids)	12.5
Hume (1970b)	Sheep		Semipurified diet supplemented with:	
		Protein-free[a]	Urea	17.1
		Substantial degradation[b]	Gelatin	19.8
		Substantial degradation[b]	Casein	23.3
		Direct zein determination[c]	Zein	22.5
Hume and Bird (1970)	Sheep	Substantial degradation[b]	Gelatin + urea with:	
			No sulphur	18.5
			Sodium sulphate	19.0
			Cystine	17.7
			Sodium sulphate + cystine	20.2
Hogan and Weston (1970)	Sheep	DAP	Dried forages	23.1
Hogan and Weston (1971)	Sheep	DAP	Straw + urea	27.5
Mathison and Milligan (1971)	Sheep	^{15}N	Barley	16.3[d]
			Hay	12.5[d]
Lindsay and Hogan (1972)	Sheep	DAP	Lucerne hay	22.0
			Dried red clover	25.0
Leibholz (1972)	Sheep	^{35}S	Straw supplemented with:	
			Lucerne meal	6.3
			Starch	9.5
			Lucerne meal + casein	6.2
			Starch + casein	10.5
			Wheat gluten	6.9
			Starch + wheat gluten	11.6
Ørskov *et al.* (1972)	Sheep	DAP	Rolled barley supplemented with:	
			0 urea	16.3
			0.7% urea	16.3
			1.4% urea	15.2
			2.1% urea	14.4
Hagemeister and Pfeffer (1973)	Cattle	DAP	Untreated casein	20.8
			Formaldehyde treated casein	19.7
			Untreated + treated casein	29.7
			Soybean meal	25.3
			Soybean meal + treated casein + urea	20.1
			Urea	20.8
Miller (1973)	Sheep	^{35}S	Rolled barley	16.3
			Protein concentrate	18.8

TABLE 4 (continued)

Reference	Animal	Method	Diet	Protein Yield g CP/100 g OM Digested
Hogan (1973)	Sheep	DAP	Dried subterranean clover	18.4
Hagemeister and Kaufmann (1974)	Cattle	DAP	Forage	15.4
			Mixed rations	21.2
			Coconut	17.6
Pitzen (1974)	Sheep	DAP	Corn + urea	20.1
			Corn + soybean meal	16.5
Hume and Purser (1975)	Sheep	^{35}S	Subterranean clover at four stages of maturity:	
			Pre-wilting	19.3
			At wilting	22.3
			Post-wilting	20.2
			Mature	17.8
Sutton *et al.* (1975)	Sheep	RNA	Rolled barley	13.5
			Protein concentrate	17.3
Nolan (1975)	Sheep	^{15}N	Chopped lucerne hay	16.3
Walker *et al.* (1975)	Sheep	^{35}S	Dried forages	15.1
			Fresh forages	24.6
Ulyatt *et al.* (1975)	Sheep	DAP	Ruanui ryegrass	16.2
			Manawa ryegrass	30.7
			White clover	19.8
Cole *et al.*(1976)	Cattle	RNA	Whole shelled corn with cottonseed hull levels of:	
			0	7.5[d]
			7%	10.3[d]
			14%	11.8[d]
			21%	12.7[d]
			Steam flaked and dry rolled corn with cottonseed hull levels of:	
			0	5.2[d]
			7%	8.7[d]
			14%	7.3[d]
			21%	13.8[d]
Chamberlain *et al.* (1976)	Sheep	DAP	Sugar beet pulp + barley	14.3
Hagemeister *et al.* (1976)	Cattle	DAP	Forages:	
			Ryegrass, 200 Kg N/ha	16.3
			Ryegrass, 400 Kg N/ha	14.0
			Mixed rations (45% N supplied by:)	
			Casein, untreated	21.0
			Casein, treated with foraldehyde	19.4
			Coconut pellets	12.4
			Soybean meal	20.6
			Fish meal	22.3
			Yeast	22.0
			Rapeseed meal	20.6
			Peanut meal	19.5
			Horsebean meal	19.0
			Urea	20.8
Kropp *et al.* (1977a)	Cattle	RNA	Grass + soybean meal	9.9[d]
			Grass + soybean meal + urea	11.6[d]
Kropp *et al.* 1977b)	Cattle	RNA	Cottonseed hulls + soybean meal	23.0
			Cottonseed hulls + urea	23.5

TABLE 4 (continued)

Reference	Animal	Method	Diet	Protein Yield g CP/100 g OM Digested
Beever *et al.* (1977)	Sheep	^{35}S	Silage	16.7
			Formaldehyde treated silage	6.6
			Dried formaldehyde treated silage	7.7
Crooker (1978)	Sheep	^{35}S	Semipurified diet supplemented with:	
			Peanut meal	17.4
			Peanut meal + urea	19.7
			Heated peanut meal	24.3
			Heated peanut meal + urea	31.6
Kelly *et al.* (1978)	Sheep	DAP	Ryegrass silage + formic acid (cutting):	
			Wilted spring silage	15.6
			Early direct-cut autumn silage	7.5
			Late direct-cut autumn silage	10.0
Prigge *et al.* (1978)	Cattle	RNA	Dry rolled corn	7.5[d]
			Steam flaked corn	9.4[d]
			High moisture corn, whole shelled and treated with propionic acid	14.8[d]
			High moisture corn, ground prior to ensiling	9.5[d]
Smith *et al.* (1978)	Cattle		Flaked maize and straw supplemented with:	
		DAP	Peanut meal	10.6
			Fish meal	18.1
			Soybean meal	17.5
			Heated soybean meal	16.3
		RNA	Peanut meal	11.9
			Fish meal	16.9
			Soybean meal	15.6
			Heated soybean meal	18.1
Allen and Harrison (1979)	Sheep	^{35}S	Dried grass nuts and ground maize	15.3
			Dried grass nuts, ground maize and monensin	12.6
M.D. Stern (unpublished data)	Cattle	DAP	Corn silage, hay and corn gluten meal	21.8
			Corn silage, oat straw and ground corn	27.7
Chamberlain and Thomas (1980)	Sheep	DAP	Hay + concentrate and given intraruminal infusions of:	
			Water	16.3
			Urea solution	20.4
			Artificial saliva	16.9
			Artificial saliva + urea	21.5

[a] Assumed that with a protein-free diet, the amount of protein N flowing from the rumen may be equated with the daily production of microbial protein in the rumen.

[b] Assumed that protein was substantially degraded in the rumen, therefore nearly all the protein leaving the rumen was of microbial origin.

[c] Zein was separated from microbial protein by precipitating the latter with 80% ethanol (McDonald, 1954)

[d] Efficiency expressed as g CP/100 g dry matter digested.

Hume *et al.* (1970) showed with sheep fed protein-free purified diets, containing urea as the nitrogen source, that increasing the dietary nitrogen concentration from 0.54 to 1.82% (or 2.6 to 9.2 g N/day) increased ruminal protein production from 32.5 to 50.0 g/day. There was no further increase in protein synthesis when dietary nitrogen concentration was raised to 3.29% (or 16 g N/day). They concluded that microbial protein synthesis was depressed when the dietary crude protein concentration was below 11%. Satter and Roffler (1977a) indicated that the dietary crude protein concentration at which maximum microbial growth occurs in cattle is about two percentage units higher than in sheep fed similar diets. Therefore, it has been suggested that microbial protein synthesis will peak when typical dairy diets contain approximately 12% to 13% crude protein (Burroughs *et al.*, 1975; Satter and Roffler, 1975). Above these levels of dietary crude protein, ammonia-N concentrations will increase without a concurrent increase in protein production(Stern and Hoover, 1979). The 12% to 13% dietary crude protein level is not a fixed point, however, and will vary with the fermentable energy content of the diet, amount of dietary non-protein nitrogen, extent of dietary protein degradation, efficiency of rumen microbial growth and salivary nitrogen input into the rumen (Satter *et al.*, 1977).

Microbial nitrogen is derived from ammonia-N and (or) preformed amino acids, with the latter highly dependent on the dietary nitrogen source (Stern and Hoover, 1979). The percentage of microbial nitrogen derived from ruminal ammonia-N has been reported to range from 40% to 100% under various conditions (Pilgrim *et al.*, 1970; Mathison and Milligan, 1971; Al-Rabbat *et al.*, 1971; Nolan *et al.*, 1976; Al-Rabbat and Heaney, 1978). Mercer and Annison (1976) suggested that maximum microbial protein synthesis occurs at relatively low rumen ammonia-N concentration, even when energy is not limiting.

Several *in vitro* studies have shown maximum microbial growth to occur when the ammonia-N concentration was 5 to 8 mg/100 mℓ (Allison, 1970; Annison, 1975; Nikolic *et al.*. 1975; Satter and Slyter, 1974). Hume *et al.* (1970) observed, *in vivo*, that microbial growth attained a maximum level when rumen ammonia-N concentration reached approximately

9 mg/100 mℓ. In contrast, Miller (1973) found a considerably higher value of approximately 29 mg/100 mℓ. Results of a more recent *in vivo* study (Okorie *et al.*, 1977) indicated that maximal protein synthesis was achieved when the rumen ammonia-N concentration reached 5 mg/100 mℓ, an observation consistent with the *in vitro* observations of Satter and Slyter (1974).

Nikolic (1976) showed that when the nitrogen content of the diet was reduced to 1.59%, by exclusion of the urea supplement, the protein-N (trichloroacetic acid insoluble) concentration of the rumen dry matter became significantly lower than with a diet containing 2.04% nitrogen both before and after feeding. The mean rumen ammonia concentration with the nitrogen-deficient diet over a four-hour post-feeding period was 4.9 mg NH_3 - N/100 mℓ. Addition of a urea supplement in increasing amounts progressively increased rumen ammonia concentration but the protein content of the rumen dry matter was not increased by dietary-N levels above 2.04% (1.0% urea). It was suggested that either increased lysis of susceptible micro-organisms or increased proteolysis of feed proteins may occur when ammonia concentrations are low, namely below 5 mg-N/100 mℓ. This may decrease both the concentration of protein in the rumen dry matter and the amount passing daily to the duodenum (Nikolic, 1976).

Mehrez *et al.* (1977) used the dacron bag technique *in situ* to predict that the rumen ammonia-N concentration for maximum rate of fermentation was 23.5 mg/100 mℓ. Discordant with the results of Mehrez *et al.* (1977), Ortega *et al.* (1979) found *in situ* that progressively increasing rumen ammonia-N from 6.3 to 27.5 mg/100 mℓ did not result in any significant changes in rate of fermentation. However, in studies examining the rumen ammonia-N concentration required for maximal rate of fermentation, Stern and Hoover (1979) emphasise that this measure does not necessarily equate to maximal protein synthesis.

Mehrez *et al.* (1977) suggest that the optimal ammonia concentration of rumen fluid may be defined as that which results either in the maximum rate of fermentation in the rumen or that which allows the maximum production of microbial protein per unit of substrate fermented. The two definitions may not always coincide; for instance Ørskov *et al.*(1972)

showed with a barley feed that the microbial protein produced per unit of substrate fermented was not altered as a result of urea supplementation while the extent of rumen fermentation and digestibility was increased.

Nitrogen Source

Although the nitrogen concentration in a diet may appear to be adequate for maximum microbial growth, resistance of the protein to ruminal degradation may result in nitrogen deficiency (Stern and Hoover, 1979). McMeniman and Armstrong (1977) determined that 2.0 g of available nitrogen per 100 g OMD is the minimum amount required for efficient microbial protein production for low-roughage diets. With starchy cereal diets there are distinct possibilities of nitrogen inadequacy, particularly with corn in which the protein is highly resistant to ruminal degradation (Thomas, 1977). Depending upon various factors such as species, stage of maturity and drying, forages can also result in deficiencies of available nitrogen to the rumen microbes (Hume, 1975; Ulyatt *et al.*, 1975; Walker *et al.*, 1975).

Microbial proten synthesis can occur in the rumen on diets in which urea is the only nitrogen source, however, efficiency of microbial growth may be limited by a deficiency of preformed amino acids (Stern and Hoover, 1979). Hume (1970b) found that nitrogen provided from urea, gelatin, casein and zein resulted in microbial synthesis of 17.1, 19.8, 23.3 and 22.5 g CP/100 g OMD in the rumen of sheep, respectively. It is possible that urea resulted in the most inefficient microbial growth due to a lack of preformed amino acids (Stern and Hoover, 1979). Hume (1970b) suggested that since degradation of both gelatin and casein in the rumen approached completion, microbial protein production on the gelatin diet may have been limited by the rate of synthesis of one or more amino acids by the rumen bacteria, since gelatin is deficient in several amino acids, including methionine.

Salter *et al.* (1979) examined the origin of nitrogen incorporation in rumen bacteria of steers fed protein and urea-containing diets. They found that when an adequate dietary supply of preformed amino acids was available that proline, arginine, histidine, methionine and phenylalanine were derived from the medium to a greater extent than other

amino acids. While synthesis of proline, arginine and histidine increased on the urea-containing diet, that of methionine and phenylalanine did not. Therefore, methionine and phenylalanine may be limiting for bacterial growth on diets low in protein and high in non-protein nitrogen.

Amos and Evans (1976) ascertained that the addition of sunflower protein to a control diet containing low quality Coastal bermudagrass increased microbial protein synthesis. However, there appeared to be no benefit from the addition of urea to the control diet. In contrast, microbial protein production increased in steers fed a low quality roughage, when urea replaced equal amounts of supplemental soybean meal nitrogen (Kropp *et al.*, 1977a). These results are consistent with the observations of Pitzen (1974), who found that replacement of soybean meal with urea in a corn based diet increased the efficiency of protein synthesis from 16.5 to 20.1 g/100 g OMD.

Ben-Ghedalia *et al.* (1978) observed that adding maize gluten to a purified diet in which urea was the only source of nitrogen improved efficiency of microbial protein production in sheep, however, adding casein or fish meal to the purified diet did not elicit a response. They concluded from these observations that for ruminant diets which contain little or no preformed protein, a slowly degradable protein may be beneficial.

Stern and Hoover (1979) suggest that nitrogen source and the extent of degradability in the rumen play a major role in determining the efficiency of microbial protein synthesis. The extent to which protein degradation occurs and the various factors contributing to this degradation have been discussed in several recent reviews (Blackburn, 1965; Smith, 1969; Chalupa, 1975; Sutherland, 1976; Satter *et al.*, 1977; Sniffen and Hoover, 1978; Tamminga, 1979).

Carbohydrate Source

The two major reactions of the ruminal fermentation are the degradation of carbohydrates to volatile fatty acids (VFA) as a method of energy (ATP) generation and the concomitant production of microbial cells. The overall scheme of ruminal activity is depicted in Figure

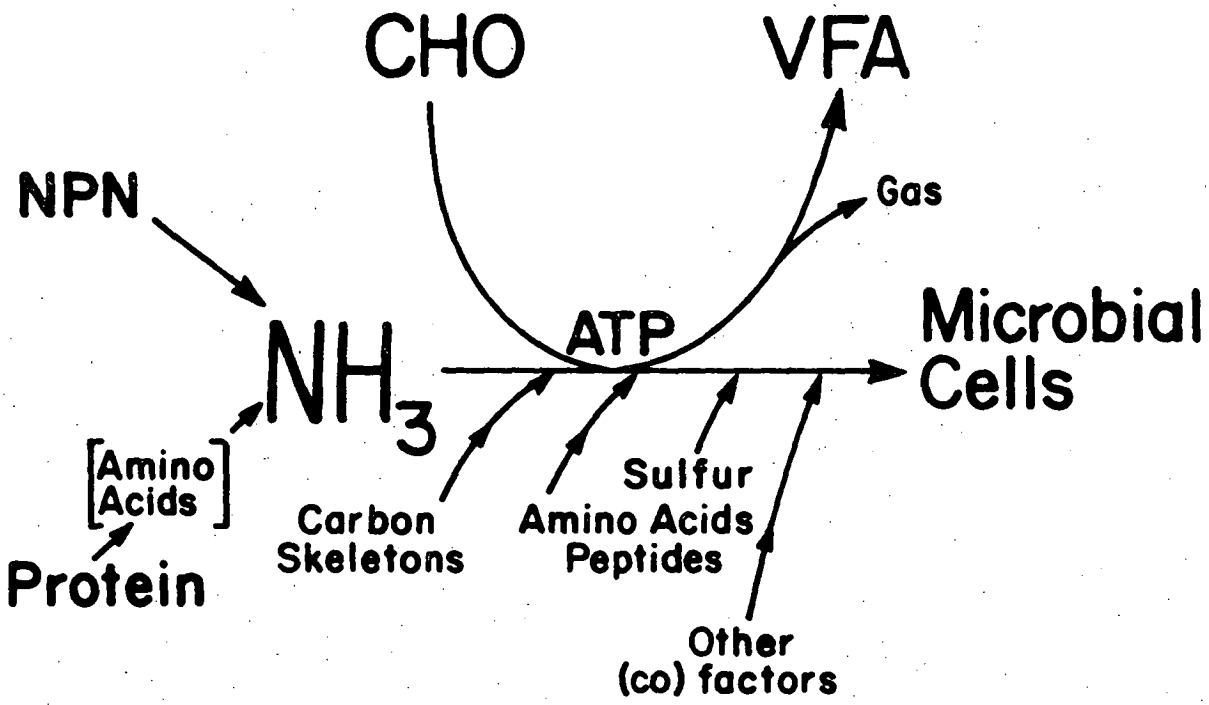


FIGURE 26. A flow diagram of the interrelationship between carbohydrate fermentation (ATP generation) and microbial cell growth in the rumen (Bergen and Yokoyama, 1977)

26 (Bergen and Yokoyama, 1977).

The process of VFA production from carbohydrates and cellular growth to a high degree is a coupled process. A deficiency of any one of the components in the overall scheme (Figure 26) will impose limits and depress the output (VFA and microbial cells) of the ruminal fermentation (Bergen and Yokoyama, 1977).

Oldham *et al.* (1977) suggest that efficient utilisation of degraded dietary nitrogen requires that the energy from the fermentation of dietary OM must be supplied at a rate which matches the synthetic abilities of the rumen microbes. Readily available carbohydrates such as starches and sugars were found to be more effective than other carbohydrates in increasing utilisation of degraded dietary nitrogen and (or) increasing microbial growth both *in vitro* and *in vivo* (McDonald, 1952; Chalmers and Synge, 1954b; Lewis and McDonald, 1958; Phillipson *et al.*, 1962; Robertson and Hawke, 1965; Offer *et al.*, 1978; Stern *et al.*, 1978). When starch has been added to high cellulose diets, or replaced part of the cellulose, increased nitrogen utilisation and decreased fibre digestion has been reported (Slyter *et al.*, 1971; Offer *et al.*, 1978; Stern *et al.*, 1978). The efficiency of starch to promote nitrogen utilisation may be related to energy yield during fermentation. Compared to other carbohydrates studies, McAllan and Smith (1976) found that fermentation of starch provided the greatest amount of energy for rumen bacteria. Although ruminal degradation of starch is generally found to be at a high level (Sutton, 1971), differences in digestion due to source of starch (such as corn vs barley) have been reported (Ørskov *et al.*, 1971; Durand *et al.*, 1975, 1976). Thus Durand *et al.* (1975) using starches from three different varieties of maize (normal, waxy and amylomaize) differing in their amylose content and in their type of crystallinity, and three tropical tubers (cassava, sweet potato and yam) found *in vitro* that starches from amylomaize and yam are poor available sources of energy. Whether this poor availability was related only to the B-type crystallinity or to the amylose level, is not known. On the other hand, the above authors have shown that cassava or sweet potato is an adequate source of energy for urea utilisation; a weak process, such as pelleting, increased the availability further.

Kameoka (1962) noted that nitrogen utilisation in the rumen was markedly reduced as the amount of soluble carbohydrate decreased in feedstuffs labelled with ^{15}N (however, the soluble protein was also reduced) and Schwartz *et al.* (1964) reported that soluble starch remained in the rumen two to three times as long as did glucose.

Meyer *et al.* (1967) have demonstrated some effect of feed processing methods on nitrogen utilisation. Finely ground hay and expanded grain were compared to unprocessed feed. The expansion process results in almost complete gelatinisation of starch. The results showed that the processed feed lowered rumen ammonia concentration about 50%, yet resulted in an increased bacterial nitrogen level of about 50%, thus indirectly indicating more efficient utilisation of nitrogen due to greater carbohydrate utilisation.

Stern *et al.* (1978) noted that dietary energy level is not the only factor limiting microbial growth. They found an increase in microbial growth in continuous cultures (15.0 to 19.5 g microbial CP/100 g OMD) in response to increased dietary non-structural carbohydrate levels, even though diets were isocaloric and VFA production and DM digestibilities did not differ markedly among diets. It was concluded that a major factor affecting the utilisation of degraded dietary nitrogen was the type and rate of availability of carbohydrates. It is apparent that the extent and rate of degradation in the rumen of both the nitrogen and carbohydrate sources are quite important in determining the efficiency of microbial growth.

Dilution Rate

Several studies have demonstrated a positive correlation between increased dilution rate and increased microbial growth (Hobson, 1965; Hobson and Summers, 1967; Stouthamer and Bettenhaussen, 1973; Isaacson *et al.*, 1975; Harrison *et al.*, 1976; Kennedy *et al.*, 1976). Rumen dilution rate is defined as the proportion of total rumen volume leaving the rumen per hour (Harrison *et al.*, 1975). Several factors such as diet, intraruminal buffer infusion, level of intake and environmental conditions have been found to alter dilution rate. Dilution rate of the rumen liquid phase is generally greater in animals fed forage vs concen-

trate diets (Topps *et al.*, 1968; Bauman *et al.*, 1971; Hodgson and Thomas, 1975) and was increased by inclusion of the mineral salts of the artificial saliva into the diet (Thomson *et al.*, 1975), ingestion of saline (1.3% w/v NaCl) drinking water (Potter *et al.*, 1972), and inclusion of high levels of salt (ca. 20%) in the diet (Hemsley, 1967), but not by ruminal infusion of water (Harrison *et al.*, 1975).

Cole *et al.* (1976) observed increases in rumen dilution rate (0.03 to 0.05/hr) along with increases in protein synthesis (7.5 to 11.8 g/100 g DMD) when steers were switched from an all concentrate diet to one containing only 14% roughage. A recent investigation by Allen and Harrison (1979) revealed that feeding monensin to sheep decreased rumen dilution rate from 0.07 to 0.04/hr, with a concomitant decrease in microbial synthesis from 24.5 to 20.2 g N/kg OMD.

Intraruminal infusion of artificial saliva in sheep increased dilution rate from 0.03 to 0.08/hr (Harrison *et al.*, 1976). Concurrently, there was an increase of total amino acids synthesised per mole hexose fermented from 25.4 to 29.8 grams. Kennedy *et al.* (1976) found that sheep maintained at an environmental temperature of 18 to 21 C were less efficient in synthesising microbial protein than were those maintained at -1 to 1 C (47.9 vs 54.9 g N/kg OMD, respectively). The increased efficiency of microbial production in cold-exposed sheep was positively correlated with dilution rate (0.1 vs 0.14/hr, respectively). In a subsequent study, Kennedy and Milligan (1978b) found that when sheep were maintained at 22 to 25 C and 2 to 5 C, there was an increase in dilution rate from 0.07 to 0.12/hr and a concurrent increase in microbial synthesis from 35.9 to 50.9 g N/kg OMD, respectively. Similar trends were observed by Hogan and Weston (1970), who showed that increased dilution rates in sheep from 0.06 to 0.1/hr were associated with microbial production rates of 31 and 37 g N/kg OMD, respectively.

Kennedy *et al.* (1976) suggested that any one, or more likely a combination, of the following factors may be involved in causing efficiency of microbial synthesis to be positively related to dilution rate: reduced autolysis of bacteria; reduced engulfment of bacteria by protozoa; changes in microbial population structure induced by a change in sub-

strate or possibly due to washout of slow generation time organisms.

Hobson and his co-workers at the Rowett Research Institute (Hobson, 1965; Hobson and Summers, 1967; Hobson *et al.*, 1968; Hobson and Summers, 1972) have over the last decade, made some very careful studies on the effect of dilution rate on the behaviour of individual species of rumen micro-organisms in continuous culture. For the species which they have studied, growth yield, the apparent efficiency of growth and the pattern of metabolic products obtained were functions of dilution rate. Extending this to the rumen ecosystem, rumen liquid turnover (passage of liquid per unit volume of rumen liquid per unit time) may be expected to be an important influence in terms of the redistribution of microbial species between overlapping niches, the pattern of metabolites produced and the efficiency of microbial protein synthesis (Sutherland, 1976). This concept gives a ready explanation of changes, such as those observed by Eadie *et al.* (1970), obtained on alteration of feeding level. Ishaque *et al.* (1971) have observed large changes in the nitrogen flow to the duodenum with changes in fermentation pattern.

Sutherland (1976) summarised the possible changes in rumen organisms and products due to change in liquid turnover and these are: (1) change in growth yield; (2) change in fermentation pattern of individual organisms; (3) change in distribution pattern of micro-organisms, i.e. redistribution of biomass; (4) change in fermentation pattern due to 3; (5) change in conditions of pH and Na^+ and Na^+/K^+ ratios; (6) changes in energy/protein ratios; (7) changes in glucogenic precursors/total absorbed energy.

Other Factors

In addition to the factors discussed previously, other factors affecting microbial protein synthesis are dietary sulphur, frequency of feeding and mineral concentrations. Sulphur is required by rumen micro-organisms for synthesis of methionine and cysteine and intake of sulphur may limit protein synthesis when large amounts of non-protein nitrogen are used (Buttery, 1977). Studies by Hume and Bird (1970) showed that when sheep were fed a diet supplying 0.6 g sulphur per day (N:S = 34.3), 82 g microbial protein were produced daily in the rumen. Raising sulphur intake to 2.0 g/day (N:S = 10.9) increased protein production to 94 g/day,

but there was no further increase when intake was raised to 3.4 g/day (N:S = 6.4). Based on this type of information an optimum nitrogen: sulphur ratio of 10:1 has been suggested for maximum microbial growth.

Feeding sheep at two hour intervals resulted in greater protein synthesis than feeding once daily (Al Attar *et al.*, 1976). Jensen and Wolstrup (1977) investigated the effect of feeding dairy heifers at frequencies of two and twelve times daily. Frequent feeding compared to twice daily feeding resulted in a decrease of microbial metabolites in rumen fluid and an increase in the microbial ATP pool. More recently, Michalowski (1979) showed in sheep that the changing of feeding frequency from once to twice daily caused an increase of microbial protein synthesis in the rumen.

Nikolic *et al.* 1976) have investigated the effect of different concentrations of sulphide, magnesium and zinc on the overall rate of protein synthesis and net ammonia utilisation by rumen micro-organisms incubated in vitro under conditions approximating to those found in the rumen. It was found that the rate of protein synthesis and net rate of utilisation of ammonia-N were not affected by mean sulphide concentrations from 3.6 - 8.0 mg sulphur/litre, which is in accord with the finding of Nippo and Hinkson (1974). Moreover, the rate and efficiency of protein synthesis were not significantly affected by increasing the concentration of total magnesium from 8.4 to 15.3 mg/100 mℓ, although the rate tended to be higher when the concentration of magnesium was 10 - 12 mg/100 mℓ. Similarly, zinc concentrations varying from 5.2 - 12.4 mg/litre did not affect the overall rate of protein synthesis, although the efficiency tended to be higher when the concentration of zinc was greater. Durand and co-workers (1975) also found that addition of zinc did not affect VFA production and rate of protein synthesis by ovine rumen content.

Protein synthesis is also influenced by the microbial population and conditions in the rumen (Thomas, 1977). These factors were highlighted by the finding that in sheep given a high-concentrate diet, the efficiency of protein synthesis was directly correlated with the molar proportion of propionic acid in the rumen (Ishaque *et al.*, 1971a). Similar results have also been obtained under other dietary conditions (see Thomas, 1973). Subsequently it was shown (Hodgson and Thomas, 1972)

that with the diet used by Ishaque *et al.* (1971a) the molar proportion of propionic acid was inversely correlated with the clearance rate of the rumen liquid phase. This relationship has now been confirmed to operate with a wide range of mixed forage and concentrate diets (Harrison *et al.*, 1973; Hodgson *et al.*, 1976) although it does not apply with moderate or poor-quality forages (Hodgson and Thomas, unpublished data quoted by Thomas, 1977). However, it is now clear that protein synthesis is not always correlated with the proportion of propionic acid (Chamberlain *et al.*, 1976) and that where correlations occur they can be positive or negative (Harrison *et al.*, 1976; Kennedy, *et al.*, 1976). Where the latter arise there is a direct relationship between protein synthesis and the clearance rate. In the work of Ishaque *et al.* (1971) low propionic acid fermentations were very high in butyric acid and in ammonia concentration. In contrast, in the experiments of Harrison *et al.* (1976) and Kennedy *et al.* (1976) all fermentations were low in butyric acid and rumen ammonia concentrations varied little with the type of fermentation. The simultaneous occurrence of high butyric acid proportions and high ammonia concentrations is a characteristic of protozoal activity (Abe *et al.*, 1973) and this may explain the low efficiency of this type of fermentation.

Due to the variety of factors already known to affect microbial growth, Stern and Hoover (1979) suggest that investigators should make an effort to report all details on the conditions imposed during their studies. These may include: (1) diet composition and analysis; (2) level of intake; (3) rate of passage of solids and liquid through the rumen; (4) rumen ammonia concentrations; (5) analysis of soluble or non-structural carbohydrates (sugars, starches and fructosans) in diet; (6) nitrogen: sulphur ratio of diet; (7) feeding rate; (8) protein degradability. Also researchers studying rumen microbial growth should express their results uniformly in regard to efficiency of microbial protein synthesis, since this would enhance the interpretation of results from various studies and may lead to a better understanding of the factors affecting microbial growth. Consequently, as a conclusion, with a knowledge of these factors it might be possible to manipulate rumen microbial protein synthesis and subsequently increase protein utilisation in ruminants (Stern and Hoover, 1979).

(iii) Sulphur Metabolism

A detailed and comprehensive coverage of many aspects of sulphur metabolism in micro-organisms may be found in reviews by Peck (1962) and Trudinger (1969), in treatises by Roberts *et al.* (1955), Roy and Trudinger (1970) and Greenberg (1975), and in symposia proceedings edited by Shapiro and Schlenk (1965) ("Transmethylation and Methionine Biosynthesis"), Muth and Oldfield (1970) ("Symposium : Sulphur in Nutrition") and by Excerpta Medica (1980) ("Sulphur in Biology"). Similarly, the metabolic pathways of inorganic and organic sulphur that may occur in the rumen have been recently reviewed (Moir, 1970; Whanger, 1972; Schiff and Hodson, 1973; Bray and Till, 1975; Schiff, 1980).

(a) Dietary Sulphur

The amount and possible forms of plant sulphur that may ultimately be ingested by ruminant animals have been reviewed by Thompson *et al.*, 1970; Moir (1970); and Garrigus (1970). The plant normally synthesises all of its organic sulphur compounds from sulphate (Thompson *et al.*, 1970). The sulphur content of plants tends to be positively related to the sulphur content of both the soil and air environment in which they are grown (Allaway and Thompson, 1966). At least 90% of the organic sulphur is in the form of cysteine and methionine (Thompson *et al.*, 1970). Mertz and Matsumoto (1956) reported that sulphur deficient alfalfa plants have poor protein synthesis which results in increased content of water-soluble, non-protein nitrogen compounds within these plants.

The total sulphur content of a plant changes more than does its sulphur-amino acid content with changes in the level of available sulphur in the soil (Allaway and Thompson, 1966). Sulphur-adequate plants generally contain more sulphur-amino acids and are presumably of better nutritional quality for animals than are sulphur-deficient plants. Generally, diets deficient in the sulphur-containing amino acids have caused a reduction in voluntary food consumption by other animal species (Kumata and Harper, 1962; Sanahuja *et al.*, 1965).

In fodder plants most of the sulphur is in the protein component which

has an average nitrogen to sulphur (N:S) ratio of about 15:1 (Dijkshoorn and Van Wijk, 1967) but the total ratio has been found to vary from 4:1 up to 55:1 in maize and bean plants when grown on N-deficient, S-adequate soils or S-deficient, N-adequate soils (Stewart and Porter, 1969). Begg and Freney's (1960) data for a number of pasture species show a N:S ratio between 10:1 and 23:1 over a range of plant sulphur contents. Thus the plant material ingested by ruminants contains sulphur principally in the form of protein sulphur together with variable inorganic sulphate, but includes such diverse metabolically important compounds as glutathione, biotin, lipoic acid, coenzyme A, sulpholipids, glycosides, choline sulphate, thetins, penicillin and derivatives of methionine and cystine (Thompson *et al.*, 1970).

The sulphur content of plant dry matter varies widely in both the organic and inorganic sulphur fractions, the amount of inorganic sulphate present in a plant generally being indicative of the supply of sulphur to the plant during growth (Odelien, 1963).

Begg and Freney (1960); Thompson *et al.*(1970); and Jones *et al.* (1971) suggest that there is a steady decline in the nitrogen and sulphur concentration in plant material throughout growth. Differences in the sulphur content of pasture plants, due to seasonal changes and species differences, may cause sulphur intake of ruminants to drop resulting in a deficiency of sulphur for the rumen micro-organisms; under such conditions no benefit will be obtained from supplementing the diet with urea and/or energy sources. Supplementation of such diets with some form of sulphur may be necessary to provide optimum dietary N:S ratios (Playne, 1969; Kennedy and Siebert, 1972). The desirable dietary N:S ratio is about 10 to 13.5:1 for sheep (Moir *et al.*, 1967-1968; Bird 1972) and about 13.5 to 15:1 for cattle (Bird, 1974); thus the use of non-protein nitrogen, e.g. urea to supplement low protein diets, may require the provision of additional sulphur (Moir *et al.*, 1967-1968). Sulphur supplements commonly used are elemental S, various sulphate salts, and to a lesser extent S-amino acids and the hydroxy analogue of methionine; in addition, some feed additives such as molasses may contain appreciable amounts of sulphur (Stewart, 1976).

Because of their solubility, sulphates are present in most water supplies.

Bray (1965) indicated the possibility that sheep drinking highly sulphated waters could consume up to 1.5 gm of sulphur per day. However, the nutritional value of sulphur in drinking water has not been investigated, yet an animal's sulphur intake from this source may be large enough to produce adverse effects (Weeth and Hunter, 1971).

(b) The Production of Sulphide in the Rumen

Although mammals can oxidize reduced sulphur compounds and incorporate sulphate into various organic molecules, they are unable to reduce sulphate to the sulphide level in significant quantities (Huovinen and Gustafsson, 1967) and must depend upon plants and bacteria to provide them with their reduced sulphur compounds. Because the rumen micro-organisms can reduce oxidized forms of sulphur to forms which can be incorporated into organic compounds, ruminants have the ability to obtain their sulphur supply from inorganic sources of sulphur. This is in contrast to non-ruminant animals which must have their dietary supply of sulphur in an organic form.

Sulphate reduction is known to occur in ruminants (Lewis, 1954; Anderson, 1956) and two genera of bacteria, *Desulphotomaculum* (Campbell and Postgate, 1965; Coleman, 1960) and *Desulphovibrio* (Postgate and Campbell, 1966), producing sulphide from inorganic sulphate have been isolated. There were two postulated mechanisms for the reduction of sulphate by micro-organisms (Peck, 1962). Those organisms that reduce sulphate to the sulphide level and incorporate the sulphur into cellular materials without the production of any free detectable sulphides (H_2S, unless from the fermentation of reduced sulphur compounds or following death and autolysis of the micro-organisms) are termed assimilatory sulphate-reducing micro-organisms. The initial activation of sulphate requires adenosine triphosphate (ATP) from which adenosine-5'-phosphosulphate (APS) is formed. APS is then phosphorylated in the 3' position by ATP to yield 3'-phosphoadenosine-5'phosphosulphate (PAPS), which is the activated form reduced to the sulphide level (see Peck, 1970). Another group of micro-organisms utilises sulphate as the terminal electron acceptor and produces massive amounts of hydrogen sulphide, and these are termed dissimilatory sulphate-reducing organisms. APS, rather than PAPS, is the activated form of sulphate reduced by these micro-organisms and only a small proportion of the sulphide produced by these bacteria is incorporated into cellular constituents (Thompson, 1967).

It was previously thought that the reduction of APS was restricted to the dissimilatory process while assimilatory reduction utilised PAPS. However, Schiff and Hodson (1973) reported that APS was shown to be the substrate for reduction in assimilatory reducers such as *Chlorella* and spinach chloroplasts as well, the apparent utilisation of PAPS being due to a 3' nucleotidase which converts PAPS to APS. Recently, Schiff (1980) proposed two pathways of assimilatory sulphate reduction. One, found in some blue-green algae (cyanobacteria) and in all oxygen-evolving eukaryotes, begins with APS where the sulpho group is transferred via APS sulphotransferase to a thiol acceptor (glutathione ($G\text{-}S^-$) in *Chlorella*) to form the organic thiosulphate ($G\text{-}S\text{-}SO_3^-$). The organic thiosulphate appears to be reduced further by an organic thiosulphate reductase employing reduced ferredoxin to form $G\text{-}S\text{-}S^-$. The terminal sulphur is then thought to be reductively transferred to O-acetylserine via O-acetylserine sulphydrase to form cysteine. A second pathway, found in bacteria and fungi, begins with PAPS where the sulpho group is transferred via PAPS sulphotransferase to an acceptor thiol to form an organic thiosulphate. Since thioredoxin is indispensable, this molecule may be the carrier or may serve to reduce the carrier. NADPH via thioredoxin reductase or glutathione and glutathione reductase reduces thioredoxin. These reactions release sulphite which is further reduced to sulphide by sulphite reductase, employing NADPH. Sulpide is then thought to react with O-acetylserine to form cysteine via O-acetylserine sulphydrase.

Since rumen micro-organisms can incorporate sulphate sulphur in cellular materials and can produce hydrogen sulphide from sulphate (Block *et al.*, 1951; Lewis, 1954; Anderson, 1956; Emery *et al.*, 1957 a, b; Muller and Knappen, 1960; Henderickx, 1961 a, b; Halverson *et al.*, 1968), presumably both assimilatory and dissimilatory sulphate reducing organisms are present in the rumen. These processes can be disrupted by competitive inhibition of APS formation by molybdate, selenate, tungstate or chromate and, although the results are variable, this inhibition has been used to show that microbial reduction is the major, if not sole, method of sulphate reduction in the rumen (Bray and Till, 1975). Figure 27 shows the relation of sulphate to the many reactions which sulphur undergoes in the biosphere.

The dissimilatory sulphate reducers have been found in concentrations of

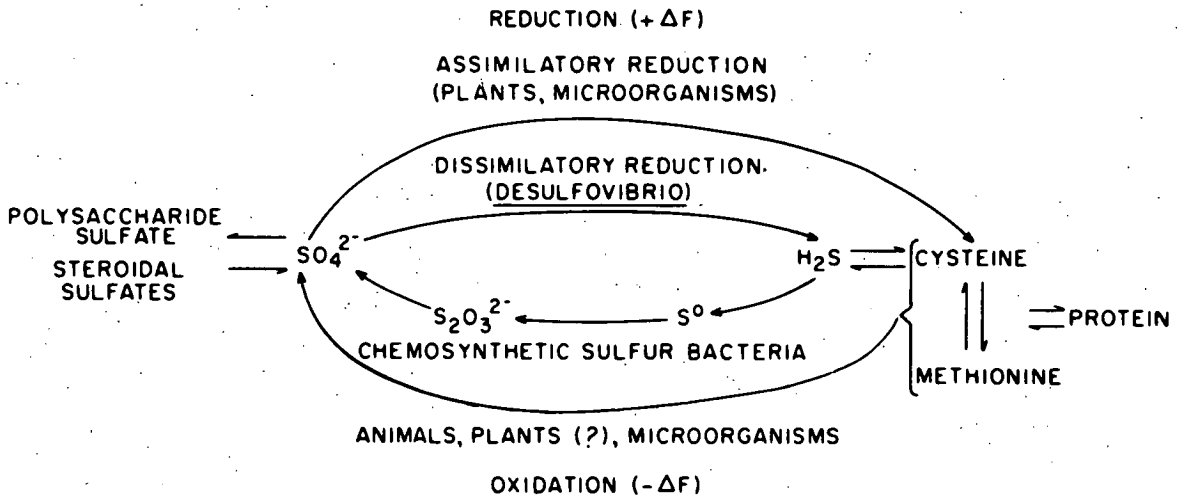


FIGURE 27. The sulphur cycle in nature showing the oxidation-reduction reactions that sulphur undergoes in various organisms (Schiff and Hodson, 1973; Schiff, 1980)

10^2-10^8/mℓ of rumen fluid (Gutierrez, 1953; Coleman, 1960; Bryden, 1972; Huisingh, 1973). The presence of assimilatory sulphate-reducing bacteria in the rumen was demonstrated in a study of ten different rumen bacterial isolates (Emery *et al.*, 1957 a, b); five could use inorganic sulphate for protein synthesis but in the presence of cystine only two did so. The existence of bacteria that cannot reduce sulphate has subsequently been confirmed by the finding of two strains of *Streptococcus bovis* that were unable to use sulphate or sulphite for growth.

Anderson (1956) added 90 g of sodium sulphate to the rumen of sheep and found a steady reduction of sulphate over a period of 10 h. Four to 5 h after addition of the sodium sulphate an accumulation of sulphide occurred, but this soon disappeared due to the very rapid absorption and bacterial utilisation of sulphide in the rumen. Other workers have also shown a transient accumulation of sulphide in the rumen from sulphate administration (Bray, 1964; Spais *et al.*, 1968; Hume and Bird, 1970) or from various feeding regimens (Matsumoto and Shimoda, 1962; Spais *et al.*, 1968). The optimum pH for the reduction of sulphate was 6.5 (Lewis, 1954; Anderson, 1956) but the reduction rate was not very sensitive to pH changes. Hydrogen gas served as the best hydrogen donor in sulphate reduction (Lewis, 1954), but glucose, formate, fructose, lactate, pyruvate, succinate, ethanol, citrate and malate were also effective hydrogen donors.

The level of sulphide in the rumen after dosing sheep with sulphate or S-containing amino acids is shown in Table 5 with the time taken to reach that level. The very high levels of sulphide in the work of Lewis (1954) and Anderson (1965) did not result in any toxic symptoms, nor did the addition of sulphide to give rumen concentrations of 330 µg S^{2-} per mℓ (10 g of sodium sulphide was administered into rumen daily, Spais *et al.*, 1968) or 113 µg per mℓ (Anderson, 1956). Bray (1969a), however, observed respiratory difficulty in sheep when sulphide levels of approximately 330 and 266 µg per mℓ were added to buffers replacing rumen contents, and sulphide could be smelled on the animals' breath in 10 to 15 minutes. As rumen concentration fell, the animals recovered without incident.

Sulphite and thiosulphate are also reduced to the sulphide level by rumen microbes (Lewis, 1954), and cyanide can be detoxicated to form thiocyanate by rumen micro-organisms in the presence of sulphide (Anderson, 1956). It

TABLE 5. Accumulation of sulphide in rumen liquor after dosing with sulphate (Moir, 1970)

Feeding Conditions	Dietary S%	Sulfur Given	Rumen Sulfide µg/Ml	Time (Hr.)	Reference
Concentrates + hay	—	80 gm $Na_2SO_4 \cdot 10H_2O$	160	4	Lewis (1954)
Concentrates + hay[1]	—	80 gm $Na_2SO_4 \cdot 10H_2O$	288	4–6	Lewis (1954)
Lucerne hay	0.28	90 gm $Na_2SO_4 \cdot 10H_2O$	97	5	Anderson (1956)
ditto		60 gm $Na_2SO_4 \cdot 10H_2O$	72	3	ditto
ditto		30 gm $Na_2SO_4 \cdot 10H_2O$	38	2	ditto
ditto		nil	10	0–7	ditto
Halophytes	0.6DM	nil	7–8	2–4	Spais *et al.* (1968)
Lucerne	0.146	nil	5	4	
ditto	0.146	10 gm SO_4	9.8 ± 2.2	6	
Lucerne fasted	0.146	nil	1.7	constant 0–12	
ditto	0.146	10 gm SO_4	10.5 ± 2.4	6	
Oat hulls	—	3 gm cystine-S	44	2	Bird (1969)
+ lucerne	—	3 gm cystine-S	47	2	ditto
	—	3 gm meth.-S	2	35	ditto
	—	3 gm meth.-S[2]	5	6	ditto
Oat hulls,	0.075	nil	0.54	constant	Hume and Bird (1970)
starch, urea	0.237	1.6 gm SO_4-S	1.9	ditto	ditto
	0.231	1.6 gm cystine-S	4.3	ditto	ditto
	0.423	1.6 gm SO_4-S + 1.6 gm cystine-S	3.5	ditto	ditto
Oat hulls,	0.058	nil	0.00	4	Bray and Hemsley (1969)
urea	0.143	0.38% Na_2SO_4	2	2	ditto
(800 gm)	0.318	1.16% Na_2SO_4	6	8	ditto

[1] + 40 gm $Na_2SO_4 \cdot 10H_2O$ for 15 days.
[2] Fed for 20 days.

has been suggested that thiosulphate or a bound derivative may be involved in sulphite reduction (Dreyfuss and Monty, 1963; Findley and Akagi, 1969).

The rate of sulphate reduction and/or incorporation of sulphate sulphur into cellular materials is influenced by the type of diet (Whanger, 1972). Knappen (1963) showed that the reduction of sulphate by rumen micro-organisms increased considerably when they were taken from an animal after reversal from pasture feeding to dry fodder and grass; substitution of grass by sugar beet leaves caused the rate of reduction to decrease again. In addition, the rate of incorporation of inorganic sulphate sulphur into organic compounds was faster with rumen fluid from cattle fed high-concentrate rations than from cattle fed low-concentrate rations (Emery *et al.*, 1957a). Whanger (1965) suggests that "the rate of reduction of sulphate and the incorporation of sulphate sulphur into bacterial protein is about 10 times faster in rumen micro-organisms from sheep fed a sulphur-deficient, purified diet than those from sheep fed a sulphur-adequate diet".

Lewis (1954; Anderson (1956); Spais *et al.* (1968) and Bird and Moir (1971) have shown that ruminal populations readily adapt to sulphate loading as evidenced by an increased production of H_2S. This appears to be due to an increased activity of dissimilatory sulphate-reducing bacteria.

There is now substantial evidence that sulphate sulphur, when adaptation to give reduction is established, is an adequate source of sulphur for the rumen microbiota (Moir, 1970). Sulphide is the key intermediate between the breakdown of ingested and recycled sulphur and its subsequent utilisation and/or loss from the system, as is ammonia in nitrogen metabolism in the ruminant (Moir, 1970; Bray and Till, 1975). The utilisation of dietary sulphur depends not only on the quantity and the nature of available sulphur, but upon (1) the rate of sulphide production, (2) the uptake of sulphide by micro-organisms, and (3) the loss of sulphide from the rumen (Moir, 1970). Based on known pathways, rumen micro-organisms may potentially obtain their essential sulphur supplies (a) entirely from cyst(e)ine directly; (b) partially from methionine directly; (c) partially or wholly from inorganic sulphates by assimilatory reduction (see Peck, 1962); (d) partially or wholly from sulphide accumulating from dissimilatory reduction of inorganic sulphates; or (e) from sulphide originating from cystine,

cysteine or other organic substances (Moir), 1970).

The rate of sulphide production is rapid whether it is from sulphate (Lewis, 1965; Anderson, 1956; Bray, 1969b; Bird and Moir, 1971), from more reduced inorganic sulphur substrates, i.e. thiosulphate and sulphite (Lewis, 1954; Henderickx, 1961b), or from cysteine and cystine (Nader and Walker, 1970; Bird and Hume, 1971; Bird, 1972a). Sulphide concentration rises steeply and usually reaches peak values within two hours of feeding (Moir, 1970).

Elemental sulphur has been used as a sulphur source in ruminant rations (Starks *et al.*, 1953; Chalupa *et al.*, 1971), but little is known about the mechanism of its reduction. In research using ^{35}S from elemental S, sodium sulphate and L-methionine, Johnson *et al.* (1971) found that true digestibility of sulphur from these three sources in sheep was 36.0, 77.8 and 77.8%, respectively, and true retention values were 26.8, 56.0 and 70.0% for the three respective sulphur sources. In general, sulphate appears to be a better S source than elemental S (Bray and Till, 1975), but the only reported cases of acute S (sulphide) toxicity occurred following large intakes of elemental S (Coghlin, 1944; White, 1964). As with sulphate, rumen microbial populations take some time to adapt to the reduction of elemental S, but once adapted they produce sulphide at a greater rate than from sulphate (Warner, 1969). Albert and co-workers (1956) fed supplemental S as sodium sulphate, methionine or elemental S to growing lambs receiving a purified ration containing 4% urea; their data indicated much more efficient utilisation of S from the methionine and sulphate than from elemental sulphur. It has been suggested that cyst(e)ine is degraded much more rapidly than methionine (Bird 1972a, Bird and Moir, 1972). However, degradation of cyst(e)ine in the rumen is not always complete; for example, when cyst(e)ine was fed to sheep at the rate of 5.28g/800g DM, 200-400 mg cyst(e)ine flowed from the rumen to the omasum per day (Hume and Bird, 1970). However, large amounts of free cyst(e)ine are unlikely to occur when protein is the major dietary source of S and it appears that most of the S in the microbial S-amino acids is derived from the sulphide pool (Hume and Bird, 1970). DL-methionine and its hydroxy analogue are equivalent in supporting the growth of rumen bacteria (Gil *et al.*, 1973), while dietary supplements of the analogue have given increases in weight gain (Brown *et al.*, 1960) and milk production (Griel *et al.*, 1968). Most of

the experimental evidence suggests that the rate of sulphide formation is highest with cyst(e)ine, then inorganic S, then methionine (Bray and Till, 1975).

Most forms of ingested sulphur pass through the rumen sulphide pool. However, information is lacking on the proportions of protein S and methionine S that by-pass the pool (Bray and Till, 1975). It has been shown that dietary methionine and its analogues or derivatives are extensively degraded to sulphides prior to resynthesis by the microflora in ruminants (Zikakis and Salsbury, 1969; Downes *et al.*, 1970; Amos *et al.*, 1974), and for this reason methionine is now regarded as the first limiting amino acid for growing sheep (Nimrick *et al.*, 1970; Schelling *et al.*, 1973).

The addition of 0.66 per cent cystine to a low sulphur diet (0.075 per cent) fed at two-hourly intervals raised the ruminal sulphide level from 0.54 µg/mℓ to 4.26 µg/mℓ (Hume and Bird, 1970); only a small amount of cystine passed out of the rumen without degradation. Infusions of 3 g of cystine, cysteine or methionine sulphur as a single dose into the rumen of sheep, immediately after feeding a restricted (200g) diet, gave peak ruminal sulphide levels of approximately 45 µg/mℓ for both cysteine and cystine; although sulphide rose significantly with methionine, the level remained below 5 µg/mℓ (Bird, 1972a). In addition, however, thiomethane was also released. Bird (1972a) suggests that the thiomethane may be demethylated to H_2S. Adaptation for twenty days with methionine infusions did not alter the situation. It may be inferred from several feeding studies that orally fed methionine is almost completely degraded, as either most of the amino acid sulphur, whether fed as methionine alone or as protein (Downes *et al.*, 1970), has appeared shortly in the urine or has had little, if any, effect upon wool growth (Reis, 1969; Downes *et al.*, 1970). In another experiment (Bird and Moir, 1972) where 2g methionine was given daily per rumen or per abomasum, together with ^{35}S-methionine, 22.8 per cent of the ^{35}S appeared in wool with abomasal infusion, but only 5.6 per cent with ruminal infusion. Also sulphide and methyl mercaptan levels in ruminal and omasal contents were very low, indicating a slow rate of degradation. It has been suggested that some methionine may escape the rumen because of the slower rate of degradation (Moir, 1975), particularly where the outflow of digesta is rapid (Bird, 1972a). Doyle (1977) suggests that the amount of unaltered methionine supplement available for absorption by

the host animal might be improved by feeding methionine analogues or encapsulated methionine which may be less readily degraded.

(c) Absorption of Sulphur from the Rumen

Almost all of the sulphide which was added to the rumen disappeared within 2 hours and at the same time an increase in blood sulphate occurred (Anderson, 1956). This suggested direct absorption of sulphide from the rumen, which has been confirmed subsequently by other workers with radio-sulphur. Bray (1969a, b) showed that there was very little absorption of ^{35}S-sulphate, whereas the absorption of ^{35}S-sulphide was very rapid when the normal contents of the rumen were replaced by a buffer solution. The pattern of sulphide disappearance from the rumen followed a first order reaction and the estimated half-life of rumen sulphide ranged from 10 to 22 minutes. Therefore, this suggests that under natural conditions the sulphate has to be reduced to the sulphide level before absorption can occur.

The size of the rumen sulphide pool is usually small, due to the rapid absorption of sulphide from the rumen (Bray, 1969a, b). The absorption rate is controlled by sulphide concentration and ruminal pH (Bray, 1969a; Bray and Till, 1975). The sulphate ion *per se* is apparently not absorbed from the rumen (Bray, 1969a). However, Gawthorne and Nader (1976) suggest that there may be some sulphate absorption from the rumen, but their evidence is inconclusive.

Sulphide is also rapidly absorbed from the duodenum and post-duodenal intestinal tract (Bray, 1969b). An estimated 40% - 90% of the dose was absorbed within 60 minutes, but sulphate absorption from the same region was much slower over the same time period, i.e. 25% or less. After oral dosing of lambs with ^{35}S elemental sulphur, sodium sulphate or L-methionine via gelatin capsule, the greatest amount of radioactivity appeared in the plasma from sodium sulphate at 1 hour, whereas the greatest amount from the other two sources appeared in the plasma 42 - 54 hours after dosing (Johnson *et al.*, 1970a, b). Apparent absorption of sulphur when sodium sulphate was infused continuously into the rumen of sheep was 93, 95 and 96%, respectively, when the amounts infused were 1.5, 3.0 and 6.0 g/day (Bird and Moir, 1971).

Undissociated hydrogen sulphide is absorbed from the rumen much faster than the sulphydryl ion (Bray and Till, 1975), and a parallel situation exists with ammonia and the ammonium ion (Hogan, 1961). However, the normal rumen pH range would favour the retention of ammonia relative to sulphide since, from published data (Hogan, 1961), it can be calculated that more than 99% of the rumen ammonia would be present as the ammonium ion which has a rumen half-life of about 150 minutes. In contrast, 35% to 95% of the total sulphide would be in the form of hydrogen sulphide which has an estimated half-life of about 10 minutes (Bray and Till, 1975).

During periods of low fermentative activity in the rumen (see Walker and Nader, 1970) the sulphide produced would not be as rapidly incorporated by bacteria, and would therefore be lost by absorption from the ruminal pool (Bray, 1969a). Doyle (1977) states that "whenever production of sulphide exceeds demand by the bacteria, even at the most active fermentative phase, sulphide absorption will occur". However, all of the rumen sulphide is not available for absorption across the rumen wall. At rumen liquor sulphide levels of less than 0.6 µg S/mℓ most of the sulphide was held in the microbial fraction (Bray and Hemsley, 1969). In other experiments approximately 20% of tracer amounts of ^{35}S-sulphide were bound non-enzymatically to acid-insoluble materials in the rumen (Walker and Nader, 1968), and some sulphide is present as insoluble copper sulphide (Mills, 1960; Bird, 1970). However, Bray and Till (1975) estimate that all of these processes together would render less than 1 µg sulphide S/mℓ unavailable for absorption from the rumen.

Doyle (1977) states that the absorption of organic sulphur from the rumen as sulphur amino acids would appear to be limited by the low concentration of free amino acids in the ruminal liquor. It is suggested that amino acids such as methionine and cysteine, are poorly absorbed across the rumen wall (Annison, 1956; Leibholz, 1971). However, Kurilov *et al.* (1969) suggested that ^{35}S-methionine was absorbed and retained in the rumen wall and was subsequently either absorbed into the blood or returned to the rumen. Lazarov and Ivanov (1970) found in goats that 0.5% of the ^{35}S-methionine activity transported from rumen to blood. In addition Lazarov and Ivanov (1970) showed that cystine is transported through the rumen epithelium to a much lesser extent than methionine.

It is clear that quantitatively the most important absorption of sulphur is as sulphide and Doyle (1977) states that "quantitatively the most important absorption of sulphur must be largely dependent on the rate of reduction of organic sulphur and inorganic sulphate to sulphide; the rate at which free sulphide is utilised by the micro-organisms; the amount of sulphide flowing from the rumen, and environmental factors which affect sulphide absorption".

(d) The Incorporation of Sulphur into Microbial Protein

Rumen micro-organisms can utilise both inorganic and organic sulphur to synthesise sulphur-containing amino acids in the production of microbial protein. Loosli *et al.* (1949) first reported that sulphate-sulphur was incorporated into cysteine and methionine of microbial protein. Further, they reported that these specific amino acids were subsequently incorporated into the proteins in milk and wool produced by the animal. When rumen micro-organisms are incubated with radioactive sulphate, radioactive sulphur can be found in cysteine, cystine and methionine of the bacterial proteins (Block *et al.*, 1951; Emery *et al.*, 1957a; Muller and Knappen, 1960; Henderickx, 1961a, b; Knappen, 1963; Henderickx *et al.*, 1964). Henderickx (1961a, b) reported that rumen micro-organisms used ^{35}S-labelled sulphate, sulphite or elemental sulphur for the synthesis of sulphur containing amino acids. In addition to cysteine, cystine and methionine, radioactivity was found in homocysteine, which he suggested was an active metabolite in the synthesis of the sulphur-bearing amino acids (Henderickx, 1961a; Henderickx *et al.*, 1964). Sulphide added to an incubation mixture of rumen microbes was reported to lower the incorporation of sulphate sulphur into bacterial protein more than the addition of methionine or cystine (Halverson *et al.*, 1968). This suggested that sulphate was reduced to the sulphide level before incorporation into the bacterial proteins. Conrad *et al.* (1967, 1967a) studied the rate of ruminal synthesis of methionine in cows using ^{35}S-sodium or barium sulphide and found that the level of dry matter, the level of nitrogen intake, and the grams of alfalfa nitrogen significantly affected methionine synthesis which increased at a rate of 1.5 gm per kg of feed consumed and 6.7 gm per 100 gm of alfalfa nitrogen consumed. Other workers have shown that the diet apparently affects the rate of synthesis on the basis of findings which indicated that the methionine content in bacterial protein was significantly

greater in "green-fed" sheep than "dry-fed" sheep (Johanson *et al.*, 1949).

Although it has been shown that a few rumen bacteria require amino acids which contain sulphur for growth (Pittman and Bryant, 1964), the current opinion is that sulphide is the primary source of S for bacterial protein synthesis and that amino acids containing S are synthesised *de novo* from this substrate (see Moir, 1970). Evidence in support of this view was provided by Nader and Walker (1970) who showed in rumen studies *in vitro* a maximum of 11% and 1% direct incorporation of ^{35}S-methionine and ^{35}S-cystine respectively. In an earlier estimate Landis (1963) using sulphur-labelled sulphate, methionine and cystine orally administered to three lactating goats found that the direct incorporation of methionine was of the order 75%-83%, but that little direct incorporation of ^{35}S-cysteine occurred. Recently, however, Gawthorne and Nader (1976) reported the extent of direct incorporation of sulphur amino acids by rumen micro-organisms, *in vivo*, to be greater than generally believed. Thus, Gawthorne and Nader (1976) found in sheep that only 53%-57% of the S in microbial protein originated from the sulphide pool. They suggest that approximately half the S-amino acid content of microbial protein in ruminal digesta was synthesised *de novo* from sulphide, and the remainder resulted from the direct incorporation of amino acids from digested plant and salivary proteins. However, Gawthorne and Nader (1976) were unable to account for 62% to 77% of the daily sulphate-S turnover (i.e. sulphate turnover - [sulphide turnover + sulphate outflow in rumen fluid] = unaccounted sulphate). McMeniman *et al.* (1976), using ^{35}S-sodium sulphate infused into the rumen of sheep consuming a variety of diets, showed that when diets containing appreciable quantities of pre-formed amino acids were fed to sheep, up to 44% of the total S and 72% of the cystine S incorporated into rumen bacteria were not derived from the rumen sulphide pool. More recently, Kennedy and Milligan (1978) used ^{35}S-sodium sulphate with sheep and found that bacteria derived 52%-67% of organic S from rumen sulphide in sheep given brome grass, and approximately 45% of bacterial organic S was derived from sulphide for sheep given lucerne. Protozoa derived approximately 90% of organic S from bacteria. Nolan and Leng (1972) used ^{15}N with sheep fed lucerne chaff and found that approximately 29% of the N digested in the rumen was directly incorporated as amino acid N into bacteria and similar levels of incorpora-

tion can be inferred from other reports (Pilgrim *et al.*, 1970; Mathison and Milligan, 1971).

Coleman (1967) has shown that protozoa demand pre-formed amino acids and many digest bacteria to obtain these. Nolan and Leng (1972) suggest that around 30% of the ammonia continually being incorporated into ruminal microbial protein may have recycled through the amino acid and ammonia pools as a result of lysis of viable bacteria in the rumen due to bacteriophage activity or the engulfment of bacteria by protozoa (see Section 4). Walker and Nader (1968) using ^{35}S for the estimation of microbial protein synthesis *in vitro* found that there was no reversibility of sulphide incorporation, i.e.there was no exchange of incorporated ^{35}S with the sulphide pool. Recently, Walker and Nader (1975) using ^{35}S for the measurement *in vivo* of ruminal microbial protein synthesis in sheep did not observe distortion of the plot for decline of microbial ^{35}S in the rumen after cessation of isotope infusion and suggested that sulphur recycling within the rumen is not a factor significantly affecting the results obtained. However, the internal recycling of sulphur within the rumen needs more investigation.

Bray and Till (1975) suggest that the relationship between the rumen sulphide pool and microbial protein synthesis can be considered from three aspects: (1) the limitation of microbial fermentation and thus the limitation of cell production through sulphide deficiency; (2) the generation rate of sulphide required to satisfy observed microbial growth rates; and (3) the quantity of sulphide required for the total microbial organic matter synthesised in the rumen.

Species of ruminal bacteria differ in their content of methionine and cystine (Purser and Buechler, 1966) and the methionine or cystine content of mixed ruminal bacteria may vary amongst diets (Leibholz, 1972). The nitrogen:sulphur ratio of protein from mixed ruminal bacteria might therefor alter with change in diet, since the composition of bacterial species change with diet changes (see Warner, 1962).

Moir *et al.* (1967-1968) and Arora *et al.* (1977) have suggested a dietary N:S ratio of 10:1 while Bird (1972) has suggested 10 to 13.5:1, as the optimum ratio for microbial protein synthesis. Recently, Walli and Dudgal (1978) have shown that sulphur plays an important role in the utilisation

of urea based diets in cattle and buffaloes and its relative proportion to nitrogen in such diets is also equally important with special reference to protein synthesis in the rumen in general and the synthesis of thiamine acids in particular; out of the three N:S ratios studied 10:1 ratio seems to be adequate as regards the protein and thioamino acid synthesis in the rumen.

Walker and Nader (1975) have used ^{35}S incorporation to estimate microbial protein production *in vivo*. The rate of dilution of isotope in the rumen after withdrawing the ^{35}S-sulphate infusion is used to calculate the flow rate of microbial S from the rumen and the flow of microbial protein is then estimated from the N:S ratio of the microbial fraction. Walker and Nader (1975) report this ratio to be 12.3 to 13.8. In their *in vitro* work Walker and Nader (1968) reported N:S ratios of 10.7 and 11.0 for the bacterial and protozoal fractions, respectively. Bird (1973) reported much higher N:S ratios (20.2 to 24.5) for microbial fractions. The reason for these different ratios is not clear. However, if Walker and Nader's (1968, 1975) methods for measuring microbial protein synthesis are to be used, microbial N:S ratios should be determined in each experiment, as the authors suggest.

Bray and Till (1975) state that "the precise level at which rumen sulphide concentration limits bacterial growth or fermentation has not been determined but it appears to be about 1 µg sulphide-S per mℓ of rumen liquor".

(e) Metabolic Interrelations of Sulphur with Other Elements

It has been well established that a metabolic interrelation between sulphate, molybdenum and copper exists. Molybdenum apparently exerts its limiting effect on copper retention in the presence of inorganic sulphate and neither molybdenum nor sulphate alone interferes with copper retention (Whanger, 1972). However, the effectiveness of sulphate of molybdenum is increased to a maximum as the intake of the other is increased (Underwood, 1962).

Miller and Engel (1960) have shown that in molybdenum-fed sheep, the addition of sulphate to the diet increased the urinary and faecal excretion of molybdenum and reduced the level of this element in various tissues. Molybdenum inhibits the reduction of sulphate to sulphide *in vitro* (Huisingh

and Matrone, 1972) and *in vivo* (Gawthorne and Nader, 1976). However, Mills (1960) found that in sheep the *in vivo* sulphide levels of the rumen increased with molybdate supplements (50 ppm Mo). Likewise, Bosman and Deijs (1969) and Hartmans and Bosman (1970) reported that additions of molybdate (50 ppm Mo) increased the *in vivo* sulphide levels in the rumen of cattle. Results from sheep-feeding experiments (Bryden, 1972; Bryden and Bray, 1972) showed that dietary molybdate up to 48 mg Mo/kg feed enhanced rumen sulphide levels, whereas levels of 76 mg Mo/kg feed and greater, depressed rumen sulphide concentration. Huisingh *et al.* (1975) found that dietary sodium molybdate (50 ppm Mo) significantly inhibited the production of sulphide from sulphate, but enhanced the production of sulphide from methionine and suggested that the enhanced rumen sulphide levels measured by some workers were due, in part, to an increase in the rate of sulphide production from the sulphur amino acids. Gawthorne and Nader (1976) found that the addition of 50 mg Mo/day with 10 g Na_2SO_4/day decreased the rate of reduction of sulphate to sulphide by 50% but although the rate of sulphide production was slower, the concentration of sulphide in rumen contents was increased; they suggested a second action of Mo in inhibiting the rate of apparent absorption of sulphide from the rumen. Similar effects of molybdate on sulphate reduction were found when suspensions of *Desulfovibrio desulfuricans* and an enriched culture of a rumen *Desulfovibrio* species were incubated in Warburg respirometers (Bryden, 1972; Bryden and Bray, 1972). A molybdate concentration of 3.5 ppm Mo stimulated sulphide production, whereas concentrations of 4.2 ppm Mo and greater, were inhibitory. The other group VI anions, tungstate, chromate and selenate inhibited sulphate reduction at all concentrations.

Dowdy and Matrone (1968a, b) using purified diets showed that the rate of decline of plasma copper was similar for lambs fed diets with additions of 0.03% sulphate, 2 ppm molybdenum and 0.03% sulphate, 2 ppm molybdenum and 0.06% sulphate, and 4 ppm molybdenum and 0.12% sulphate, but was significantly slower than lambs fed this diet with 4 ppm molybdenum and 0.03% sulphate. However, this did not correlate very well with the hemoglobin patterns; the hemoglobin levels of lambs fed the basal diet (no molybdenum plus 0.03% sulphate) and those fed the diet of 2 ppm molybdenum and 0.03% sulphate remained rather constant at normal levels. All of the lambs on the other diets developed anaemia. Their data suggest that, at low levels of moly-

bdenum the effect of increased dietary sulphate was synergistic with molybdenum, whereas at high levels the main depressing factor on hemoglobin appeared to be related with the level of molybdenum. The same workers (Dowdy and Matrone, 1968a, b) showed that copper present in a Cu-Mo complex was less available than soluble copper salts, and suggested that high molybdenum may render copper unavailable by forming such a complex *in vivo*. Prentice and Matrone (1970) suggested that sulphate may be related to the formation of this complex and in their data, copper was found to significantly decrease molybdate inhibition of sulphate reduction. Sulphur has also been shown to reduce the depression in cellulose digestion caused by copper with rumen microbes (Evans and Davis, 1966).

Huisingh and Matrone (1972) found that copper alleviated the molybdate inhibition of sulphate reduction and explained these effects firstly through competitive inhibition of APS reductase by MoO_4^{2-} and secondly the formation of a Cu-Mo complex which decreases the availability of MoO_4^{-2} for inhibition of sulphate reduction. Huisingh and Matrone (1972) also suggest that the inhibition of sulphate reduction in the rumen will result in quantities of sulphate passing from the rumen down the tract and in the intestines competing with MoO_4^{2-} for a common carrier system during absorption and that molybdate and sulphate will also compete for reabsorption pathways in the kidney tubules. Bishara and Bray (1978b) observed that the intravenous infusion of high levels of molybdate inhibited tubular reabsorption of sulphate; similarly, infusion of high levels of sulphate inhibited molybdate reabsorption. Other workers have observed decreased copper levels in livers of sheep (Hogan *et al.*, 1968) or cattle (Vanderveen and Keener, 1964) when molybdenum and sulphate were added to the diet.

The presence of high sulphide levels in the rumen depressed the concentration of soluble copper (Mills, 1960) and a progressive fall in the flow of soluble copper to the omasum has been observed with increasing rumen sulphide levels up to about 3 µg S/mℓ, beyond which the movement of soluble copper (2 mg/day) was not depressed (Bird, 1970). Possibly the latter represents water-soluble organic ligand-bound copper; however, the capacity of the rumen to produce H_2S materially affects the availability of copper to the animal, presumably through the formation of cupric sulphide (Hartmans,

1969; Bosman and Diejs, 1969).

Suttle (1974), using a copper repletion technique, identified the rumen as the major site of the Cu-Mo-S interaction in normal diets. He found that total dietary sulphur is more important than dietary inorganic sulphate alone and that cupric thiomolybdate formation in the rumen may be an important process in lowering the availability of copper. Suttle (1974) also suggested that as dietary molybdenum levels increase there is also a systemic effect on the distribution of blood copper fractions resulting in increased urinary copper excretion.

Various sulphur compounds have been shown to influence the metabolism of selenium by rumen micro-organisms (Whanger, 1970). Cysteine and methionine inhibited the uptake of selenomethionine by rumen microbes more than did sulphite. Sulphite also inhibited the uptake of selenite and selenomethionine, but did not show the specificity for selenite as did methionine for selenomethionine. However, conflicting reports have been presented on the effect of sulphur on selenium metabolism in the animal. The addition of sulphate to the diet was reported to decrease the effectiveness of dietary selenium as selenite in the prevention of white muscle disease (WMD) (Muth *et al.*, 1961; Schubert *et al.*, 1961; Hintz and Hogue, 1964), while other workers could not find evidence for sulphate contributing to WMD (Boyazoglu *et al.*, 1967; Whanger *et al.*, 1969). Sulphate was reported to only slightly effect the metabolic fate of ^{75}Se-selenate in gestating (Roffler *et al.*, 1965) or lactating ewes (Paulson *et al.*, 1966). Schubert *et al.* (1961) reported an increase in the incidence of WMD in lambs from ewes grazed on alfalfa pasture after the field was treated with gypsum, but Allaway and Hodgson (1964) were unable to demonstrate a consistent effect of increased sulphur content of forages in contributing to selenium deficiency in livestock. Whanger *et al.* (1969) found that the addition of 1.0% potassium sulphate to a selenium-deficient diet for ewes resulted in a significant increase in the number of WMD lambs with cardiac lesions, but the addition of 0.5% methionine did not have this effect. This effect of sulphate, however, was prevented by additional selenium (Whanger *et al.*, 1970).

Somers and Underwood (1969) found that both nitrogen and sulphur retention were significantly lowered in zinc-deficient sheep; faecal excretion of

nitrogen and sulphur was similar between normal and deficient sheep, but the urinary excretion of both elements was significantly elevated in zinc-deficient animals. These results suggest impaired protein metabolism in zinc-deficient animals (Whanger, 1972). Goodrich and Tillman (1966) reported that phosphorus retention was significantly reduced in lambs by sulphate in the presence of 100 ppm copper. Sulphate has also been shown to lower the retention of calcium, and the possibility of calcium sulphide formation in the rumen was suggested as a probable reason for this effect (Goodrich and Tillman, 1966).

(f) Recycling of Sulphur

Sulphide and sulphate form a recycling system (Figure 28, Bray and Till, 1975) that is in many ways similar to the ammonia-urea system described by McDonald (1948). In the rumen and, to a lesser extent, the large intestine, many S-containing materials are reduced to sulphide which is either rapidly absorbed or used for synthesis of microbial protein. Absorbed sulphide is oxidised to sulphate in blood and liver and becomes distributed in the extracellular fluid (Bray 1969c), together with any sulphate absorbed from the intestines and SO_4^{2-} from organic sulphur catabolism. Sulphate is recycled directly to the large intestine or to the rumen via salivary secretion (see Table 6), and some is excreted in the urine. In sheep, the sulphate returned in parotid saliva and the total sulphate recycled is related to plasma sulphate levels (Bray and Hemsley, 1969; Kennedy *et al.*, 1975), and in cattle a strong positive correlation exists between mucid salivary sulphate and blood sulphate (Moir, 1970). Bray and Till (1975) state that "the plasma sulphate level depends on the combined effects of the rumen (and large intestine) sulphide incorporation and absorption rate constants, the oxidation rates in liver and blood, and the amount of recycling". The upper level of sulphate in the blood is regulated by the kidney, but little is known about the maintenance of minimum sulphate levels; Feland *et al.* (1973) suggest that plasma sulphate concentrations may be involved in regulation of sulphur amino acid catabolism. Kennedy (unpublished data, cited by Kennedy *et al.*, 1975) suggested that the degree of sulphate recycling appears to be more closely related to the concentration of that part of the sulphate in plasma that is not bound to plasma proteins than to the total concentration of sulphate in plasma.

Bray (1969c) concluded that the direct flow of sulphate across the rumen

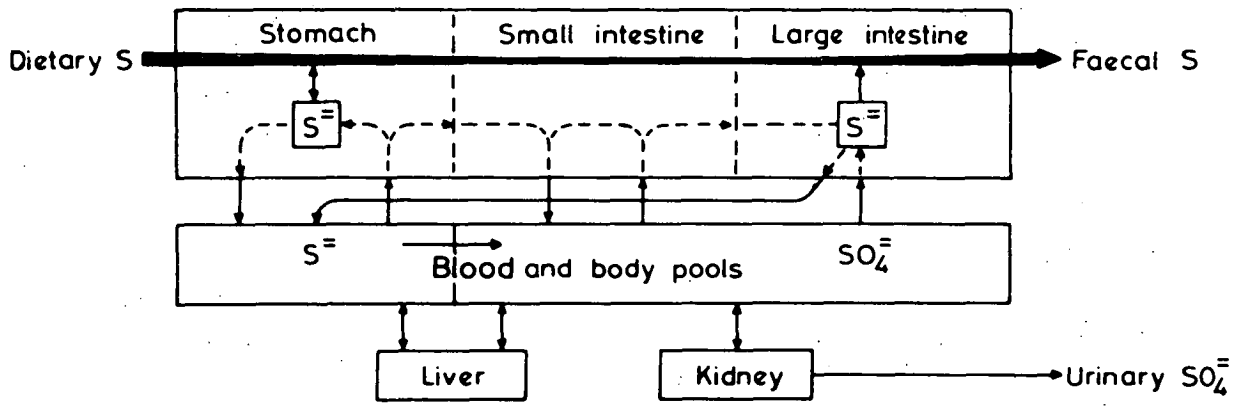


FIGURE 28. The sulphide-sulphate cycle (Bray and Till, 1975)

TABLE 6. The published estimates of sulphur recycled to the rumen of sheep per day (Bray and Till, 1975)

Animal	BW (kg)	Dietary S (%DM)	Recycled sulphur (mg/kg BW)			Plasma Sulphate-S (μg/ml)	Ref.
			From	Total S	Sulphate-S		
Sheep	46	0.2-0.25	mixed and parotid saliva	3.0-3.9	–	20-58	*19*
Sheep	45	high	across rumen wall	–	0.44	40	*22*
Sheep	45	low	"	–	0.11	10	*22*
Sheep	35	0.2-0.3	calculated† total	1.6-3.9	0.71	21	*22*
Sheep	40	0.058	parotid saliva	0.11	0.025	3.2-7.6	*23*
Sheep	40	0.318	"	0.17	0.1	27-51	*43*
Sheep	40	0.075	calculated total	11.2	–	–	*12*
Sheep	40	0.023	"	4.8	–	11.5	*8*
Sheep	35	0.025-0.035	"	3.3-4.4	0.77-1.06	–	*
Sheep	33	0.07	"	–	0.14	16	*63*
Cattle	200	0.07	"	–	1.2	23	*63*
Sheep	33	0.16-0.34	"	–	3.2	62	*63*
Cattle	200	0.16-0.34	"	–	2.5	54	*63*

*P.M. Kennedy (pers. comm.)

† Calculated from radiotracer dilutions

19 = Bray (1964)

22 = Bray (1969c)

23 = Bray and Hemsley (1969)

43 = Gutierrez (1953)

12 = Bird and Hume (1971)

8 = Bird (1972)

63 = Kennedy *et al.* (1975)

wall was of minor importance since estimated values for diets adequate and deficient in sulphur were only 20 and 5 mg sulphate-S/day respectively. Recently, Kennedy *et al.* (1976) suggest that sulphate constitutes only a small part of the sulphur returned from blood to rumen with sheep fed a diet low in sulphur, and that saliva is probably the major source of recycled sulphur; these authors calculated the transfer of sulphate from plasma to the rumen as 29 mg S/day. Of this only 12 mg S/day passed as organic sulphur in digesta from the stomach. As the net gain of sulphur in the stomach in this experiment was 153 mg/day, sulphate transferred from the plasma contributed only a small amount of sulphur derived from endogenous sources in the stomach. This situation is similar to that existing with salivary urea and protein (Nolan and Leng, 1972) as relative sources of recycled nitrogen. Salivary proteins thus play a vital role, by acting as a source of sulphide, ammonia and amino acids, in maintaining bacterial growth and activity with sheep fed low sulphur, low protein diets. The effectiveness of salivary proteins in this role will be modified not only by the relative rates at which the metabolites are released, but also, as Bray and Till (1975) have indicated, by the relative rates of absorption of sulphide and ammonia from the rumen.

The sulphate recycled to the rumen is reduced and is then available for protein synthesis, in much the same way as is ammonia released from recycled urea, thus providing a valuable conservation mechanism when feed is low in S (Bray and Till, 1975). Values for the S recycled in sheep and cattle are presented in Table 6 together with blood sulphate concentrations. From these data it was estimated that recycling to the reticulo-rumen would contribute about 2-5 mg S/day/kg body weight (Bray and Till, 1975), but depending on the N:S ratio, may do little to provide additional S for the microbial fixation of recycled nitrogen. The transfer of endogenous S to the rumen may result in a net gain in the stomach of up to 0.44 g S/day (Bird and Hume, 1971). Kennedy and Milligan (1978) estimated that 130-160 mg S/day was added to the rumen as endogenous sulphate in sheep fed brome grass plus sulphate and lucerne respectively, and suggested that 110-140 mg of this entered via salivary secretions; these authors also estimated that endogenous organic S contributed 300-340 mg S/day to the rumen, and that 24% - 45% of S digested in the rumen was derived from endogenous sources. Gawthorne and Nader (1976) estimated that the apparent

recycling of sulphate to the ruminal contents by way of the saliva or across the rumen wall was between 640 and 880 mg S/day, but these estimates were derived by difference and could be subject to appreciable errors. Recently, Doyle and Moir (1979b) estimated that the minimal endogenous sulphur inputs for unsupplemented (no methionine) sheep fed 500 and 1000 g dry matter per day was 521 and 1227 mg S/day respectively [minimum endogenous sulphur inputs were estimated from the known inputs of sulphur and the estimated flows of sulphur from the reticulo-rumen].

Values of 70-80:1 have been reported for the ratio of recycled urea-N to recycled sulphate-S in sheep fed low quality diets (Moir *et al.*1967-1968; Bray and Hemsley, 1969; Moir, 1970) and these may not allow efficient use of recycled nitrogen. However, many other factors contribute to the variations in the N and S recycling (Moir, 1970) and, although the N:S ratios were calculated on daily flows, a proper assessment requires more knowledge of the relative utilisation and recycling rates of N and S (Bray and Till, 1975). In sheep it appears that, in spite of the S recycling, supplementation is frequently necessary, but for cattle the recycling of S appears to be more favourable (Moir, 1970; Kennedy and Siebert, 1975; Kennedy *et al.*, 1975). Kennedy *et al.* (1975) found that the daily recycling of sulphate to the rumen of sheep was 98 mg sulphur on the lucerne diet,and 3.9 mg sulphur on the spear grass diet, while for cattle the estimates were 533 mg sulphur for the lucerne diet and 234 mg sulphur for the spear grass diet. Expressed on the basis of body weight, daily recycling of sulphate was 3.2 and 2.5 mg sulphur/kg for sheep and cattle respectively when fed lucerne, and 0.14 and 1.2 mg sulphur/kg respectively when fed spear grass. The small quantity of sulphate recycled in sheep fed spear grass is a reflection of the amount of sulphate in the body pool, which is reduced due to the demand for S of wool growth (Kennedy and Siebert, 1975).

Kennedy *et al.* (1975) suggested that a major rate of excretion of ^{35}S from the blood sulphate pool was via the post-ruminal tract. Sulphate recycling could thus be analogous to the recycling and degradation of blood urea which in the sheep appears to occur mainly in the post-ruminal tract (Nolan and Leng, 1972). The passage of both urea and sulphate into the post-ruminal tract may seriously reduce the amounts available for recycling to the rumen and incorporation into microbial protein. Kennedy *et al.*(1976) using isotope dilution techniques with ^{35}S-Na_2SO_4 found that appreciable

quantities of ^{35}S, present in the plasma as sulphate, crossed the intestinal wall and were found in the intestines in non-sulphate forms (26% of the dose in small and large intestine was derived from plasma sulphate). The net gain of ^{35}S in the intestines was equivalent to approximately 18% of the daily infusion of ^{35}S-sulphate. Sulphur influx into the intestines may be attributed to the secretion of bile and pancreatic fluids into the small intestine (Bird 1972) and to the secretion by goblet cells of sulphated glycoproteins and mucopolysaccharides (Hecker, 1973). The gain of organic ^{35}S in the large intestine was probably due to synthesis of microbial protein from urea and sulphate secreted into the large intestine (Bird and Thornton, 1972).

The combined bile-pancreatic secretions (CBD) in sheep contribute 142-245 mg S/day, with total S concentration inversely related to fluid flow rate, and comprised of 88% carbon-bonded S (10% as protein S) and 6% each of ester and inorganic sulphate (Bird, 1972b; Bird and Thornton, 1972). Intraduodenal infusion of ^{35}S-Na_2SO_4 has shown that losses of S from the bile-pancreatic system can be replaced from inorganic sources, either from recycling via rumen micro-organisms or perhaps by direct taurine synthesis in the liver similar to that observed in rats (Martin *et al.*, 1972). However, the limited data available are insufficient to allow quantitative estimation of S inputs and the ultimate fate of secretions (Bray and Till, 1975).

The net entry into the omasum and abomasum of S in digestive juices can be regarded as negligible in relation to that from other sources (Hume, 1974). Apart from inorganic S, the major inputs of S into the intestines are CBD secretions, plasma proteins (which contribute about 80 mg S/day, Campbell *et al.*, 1961), and the turnover of mucosal cells (Bray and Till, 1975). The gut wall contains about 70 mg S/kg BW and the net turnover is about 2-7 mg S/day/kg BW, but the proportion directly entering the gut is unknown (Till *et al.*, 1973).

(g) The Metabolism of Sulphur in the Post Ruminal Tract

The Omasum and Abomasum

The flow of digesta into the omasum of sheep is about 10-16 litres/day/kg organic matter intake (Weston and Hogan, 1971; Bird and Thornton, 1972;

Grovum and Williams, 1973), and it is probable that the flow is similar in other ruminants.

In sheep, diets that provided 0.6 to 6.6 g S and had N:S ratios from 34:1 to 6.5:1 (Bird and Hume, 1971; Bird and Moir, 1971) produced digesta flows of about 10 litres/day/kg organic matter intake and transferred 1 - 1.5 g S/day into the omasum. This sulphur was comprised of inorganic sulphate (3%-5%), sulphide (0.4%-2.8%), ester sulphates (3%) and neutral (carbon-bonded) S (92%). Protein S was 68% of the neutral S and, especially in the basal diet used, would be virtually all microbial protein (Hume and Bird, 1970; Hume *et al.*, 1970). Most of the plant protein degradation occurs within the rumen (Hogan and Weston, 1967a), although the actual amount depends on factors such as stage of maturity and mineral content (Weston *et al.*, 1970).

Published amino acid compositions reveal a large range of N:S ratios in the protein of bacteria 17-28:1 (Weller, 1957; Purser and Buechler, 1966; Leibholz, 1972) and protozoa 15-31:1 (Weller, 1957; Purser and Buechler, 1966; Williams and Dinusson, 1973), when calculated assuming microbial protein contains 16% N and no preferential destruction of amino acids during hydrolysis. Reported values of total N to total S ratios for rumen bacteria range from 11:1 (Warner, 1969) to 22:1 (Bird, 1973).

The N:S ratio in the digesta reaching the intestines is about 14:1 (Bray and Till, 1975) and is much narrower than many values reported for total and protein N:S ratios of rumen micro-organisms. Bray and Till (1975) also estimated that the digesta flowing into the intestines would provide the equivalent of 1-2 g of amino acid sulphur/kg organic matter intake.

Apart from that in mucous, the net flow into the omasum and abomasum of sulphur in digestive juices can be regarded as negligible in relation to that from other sources. Hume (1974) found no significant additions of endogenous sulphur to the digesta in the abomasum of sheep.

The Small Intestine

Apart from the digesta flowing into the intestine (1-2 g of amino acid S/kg organic matter intake), the combined bile and pancreatic secretions may contribute 142-245 mg S/day (Bird, 1972b); ester sulphate and inorganic

sulphate each accounted for 6%, protein was 10%, and soluble organic sulphur 78% of the total. Other major inputs of sulphur into the intestine are plasma proteins which contribute about 80 mg S/day (Campbell *et al.*, 1961), mucous and the turnover of mucosal cells.

The apparent digestibility of sulphur in the stomach can range from negative values for dietary sulphur (e.g. Hume and Bird, 1970) to 100% for sulphate infusions (Leibholz, 1972) but from consideration of data on nitrogen digestion it appears that for a wide range of herbaceous feeds the majority of the digestion and absorption takes place in the intestines.

For a range of 23 diets the mean calculated true digestibility of non-ammonia nitrogen (NAN) was 76% (Hogan and Weston, 1970). The amino acids probably accounted for about 80% of NAN; and the remaining NAN was mainly present as nucleic acids (see Hogan and Weston, 1970). Since it has been established that the N:S ratio in the digesta leaving the stomach covers a fairly narrow range (Bray and Till, 1975), it is probable that similar proportions of N and S are digested in the intestines. This suggestion is further supported by studies of the intestinal digestion of ^{35}S-labelled microbial protein (74%) (Bird, 1972e) and *in vitro* digestion of microbial and protozoal protein from four different diets (70%-79%, mean 74%) (Bergen *et al.*, 1968).

There is little information on the digestion of sulphur in the intestines, in particular the small intestine, or on its flows and uptake, by comparison with that available for nitrogen. The total N:S ratio changes from about 14:1 to 8:1 as the digesta passes through the intestines, but as there can be considerable flows of S into the small intestine (Hogan, cited by Bray and Till, 1975), the NAN:NSS (non-sulphate sulphur) ratio may not change greatly. Bray and Till (1975) provided evidence that much of the N data will provide useful estimates of S digestion. The digestibility of microbial sulphur should be similar to microbial nitrogen, since most of the cellular sulphur is contained in protein as cyst(e)ine or methionine (Roberts *et al.*, 1955). However, the cell wall often constitutes a substantial part of the total dry weight of the bacterial cell; for *E. coli* the cell wall accounts for 15% of the dry weight (Salton, 1964). As the bulk of the bacteria found in the rumen are Gram-negative, cell walls from these bacteria may be expected to account for a similar proportion of dry

weight. Differential digestion of microbial cell components may occur in the intestines with the cell wall component being more resistant than the remaining protein (see Allison, 1970). The data of Hoogenraad *et al.* (1970), however, suggest a differentially greater digestion of cell wall ^{14}C than of cell content ^{14}C. Hogan (1973) considered that very little digestion of bacteria cell walls occurred in the small intestine of sheep. Mason and Milne (1971) have shown that in normal sheep mucopeptide, a component of microbial cell walls, was not digested in the small intestine, but was extensively degraded by bacteria in the caecum and colon.

The small intestine of the sheep normally contains 0.9-1.4 g dry matter/kg body weight and the dry matter content increases from about 6% to 8% along its length. Sulphate is absorbed at up to 4 mg S/hour/kg body weight (Bird and Moir, 1971), a value well in excess of the amount likely to by-pass the rumen on most diets. In addition, SO_4^{2-} infused into the duodenum and not absorbed in the small intestine may be reduced in the large intestine to hydrogen sulphide which is then absorbed (Bird, 1971).

Substantial amounts of N are absorbed along the whole length of the small intestine (Kay, 1969) and it is probable that the S-amino acids released by the action of the proteolytic enzymes are also absorbed along the whole length. *In vitro* studies with sheep have indicated that the ileum is the most active and efficient site of amino acid absorption (Johns and Bergen, 1973; Phillips *et al.*, 1976). The relative order of affinity by three amino acids for the transport site(s) in the jejunum (based on Km data) was lysine > methionine > glycine; the relative order for rate of transport (based on Km and Vmax data) was methionine > lysine > glycine (Johns and Bergen, 1973; Williams, 1969). Phillips *et al.* (1976) found that the jejunum and ileum of sheep were more active sites than the duodenum for the removal of L-methionine from mucosal fluid.

The Large Intestine

The sulphur entering the large intestine will be comprised of mainly organic sulphur in the form of undigested and microbial protein residues, plus small amounts of tauro-conjugated compounds, and sulphate from the blood (Bird and Thornton, 1972). In the small intestine, goblet cells in the mucosa secrete sulphated glycoproteins and mucopolysaccharides (Hecker, 1973) and some may pass into the large intestine. Bird and Thornton (1972)

suggested that 30% of the flow of sulphur to the ileum was mucoprotein in their experiment. Mucin-secreting goblet cells are also present in the colon and the caecum, however, Hecker (1973) observed little mucin in the large intestine which suggested that the bacteria of the hindgut degrade the mucin.

The caecum of the sheep possesses an active microbial population (e.g. Faichney, 1969; Ørskov *et al.*, 1970; Hecker, 1971) and therefore might account for a considerable portion of the organic matter not digested within the rumen. However, although the hindgut has the potential to digest almost as much cellulose as the foregut under normal circumstances, the actual amount digested is limited by the short retention time of digesta in the hindgut compared to that in the rumen (Doyle, 1977).

The microbial activity largely determines the proportion of total sulphur that is excreted or absorbed. Under most dietary conditions, bacterial growth in the large intestine is limited by a supply of fermentable carbohydrates; in sheep, the infusion of glucose (0-90 g/day) into the distal ileum changed the balance between ileal S input and faecal S output from a net loss of 132 mg S/day to a net gain of 25 mg/day resulting from the synthesis of microbial protein (Bird and Thornton, 1972). The increased faecal output of organic sulphur was compensated by a decreased urinary excretion of sulphur. The glucose treatment had no effect on the overall N and S balance of the animal, which agrees with the reports that there is little absorption of amino acids from the hindgut (Henderickx *et al.*, 1972). On examining the results from experiments using labelled ileal digesta, long term infusions of ^{35}S-Na_2SO_4 into the colon and labelled micro-organisms infused into the caecum, Bray and Till (1975) observed that 30%-60% of the ^{35}S was absorbed, 1%-10% retained in tissue and wool, and from 0%-40% recycled to the rumen. Under most circumstances, it is likely that the sulphide resulting from microbial degradation of various sulphur compounds in the hindgut (endogenous pancreatic protein, tauro-conjugated bile acids or other sulphur secretions within the caecum and colon) would not be extensively used for protein synthesis but would be largely absorbed as such.

Recently, Doyle and Moir (1980) suggest that the disappearance of neutral sulphur between the ileum and anus is probably due to fermentation of organic sulphur compounds in the hindgut and subsequent absorption of the

end products of fermentation. However, the extent to which protein sulphur in the ileal digesta is degraded and the sulphide-S either absorbed from the hidgut or converted into microbial protein is not known. Ørskov *et al.* (1970) suggest that extensive breakdown of protein may occur during caecal fermentation on the basis of the high proportions of isobutyric and isovaleric acids detected. Judson *et al.* (1975) found that ^{35}S appearing in the urine from ^{35}S-labelled bacteria injected into the caecum of sheep accounted for 21% of the dose, and as negligible amounts of sulphur amino acids are absorbed from the ovine large intestine (Judson *et al.*, 1975; Elliott and Little, 1977), the sulphur was presumably absorbed as sulphide. Bird and Thornton (1972) found that from 26.9% to 53.6% of the ^{35}S infused via the ileum was excreted in the faeces, therefore from 46.4% to 73.1% (mean 59.8%) of ^{35}S from ileal digesta labelled *in vivo* with ^{35}S-Na_2SO_4 was absorbed from the hindgut. Doyle and Moir (1980) found that the disappearance of organic sulphur accounted for 33%-51% of the sulphur flowing from the terminal ileum. As with the small intestine, estimates of net absorption from the large intestine differ substantially from true absorption, as there may be considerable inputs of inorganic sulphate into the large intestine (Kennedy *et al.*, 1976). As Doyle and Moir (1980) suggest, further data on the digestion in the intestines of dietary and microbial sulphur components and on the quantitative pathways of hindgut fermentation are necessary to establish the amount and form in which sulphur is absorbed, and its availability for production processes.

(h) Some Aspects of Sulphur Utilisation by the Ruminant

An excellent review of the need of sulphur in the diet of ruminants was made by Garrigus (1970). The need for sulphur in the diet is particularly important when non-protein nitrogen makes up a large percentage of the protein equivalent in the diet. Sulphur deficiency has been shown to limit non-protein nitrogen utilisation (Thomas *et al.*, 1951; Moir *et al.*, 1967-1968) and dietary inorganic sulphur can correct this deficiency. The reduced transaminase activity of rumen micro-organisms from sulphur-deficient sheep (Whanger and Matrone, 1970) is probably a factor contributing to inefficient non-protein nitrogen utilisation. Proof that sulphur in various forms can be utilised by the ruminant has been obtained by feeding with ^{35}S and by observing the labelling of various body tissues and milk. For example,

Hale and Garrigus (1953) recovered ^{35}S in blood proteins and wool of sheep fed inorganic-S and sulphate-S. In sheep on forage diets, apparent digestibility of sulphur decreased linearly with the reciprocal of dietary S content and was predicted to be zero when dietary S was 0.81 g S/kg organic matter (Landlands *et al.*, 1973).

Whiting *et al.* (1954) concluded that the S requirement for sheep did not exceed 0.1% of the ration. Starks *et al.* (1953) found that in lambs when the basal ration containing 0.06% S was increased to 0.705% S, N retention and wool growth were increased and weight loss reduced. The work of Bray and Hemsley (1969) with sheep indicated no improvement in performance of sheep fed diets with 0.143% total S as compared to rations with 0.318%. Maintenance requirement for S, calculated from metabolic urinary and faecal losses and S content of wool growth showed that 0.48 g of retainable S was required daily in sheep (Johnson *et al.*, 1971). Langlands and Southerland (1973) reported that approximately 70 g S were secreted to produce 2 kg clean wool, 50 g S in a lactation yielding 100 litres of milk and 8 g S in birth of a single lamb. Joyce and Rattray (1970) calculated the daily maintenance S requirement of 20 to 40 kg growing sheep to be 0.54 g/day.

Yearling wethers were used by Rendig and Weir (1957) in determining that a level of 0.155% total dietary sulphur resulted in good growth rates. Perhaps the highest sulphur requirement was reported by Evans and Davis (1961) who found that on the basis of cellulose digestion in the rumen the optimum level of sulphur in the diet was 0.29%. This higher level based upon *in vivo* results is similar to some of the optimum levels reported based upon *in vitro* results (Barton *et al.*, 1971; Bull and Vandersall, 1973). Bray (1965) concluded that a sulphur level of 0.14% was adequate for maximum nitrogen retention in sheep.

Some studies using dairy cattle have failed to show any improvement when natural diets were supplemented with sulphur. Jacobson *et al.* (1969) reported no improvement in dairy cows when natural diets containing about 0.10% sulphur were supplemented with sodium sulphate to raise sulphur to 0.18%. Grieve *et al.* (1973a) found no improvement in feed intake or milk yield when sodium sulphate was added to corn silage diets containing between 0.11% and 0.13% S. Nitrogen utilisation was not improved by the addition of sodium sulphate (Grieve *et al.*, 1973b). In these studies it

appeared that the sulphur requirement was met by the 0.11%-0.13% sulphur present in the basal diet. Supplementing a diet composed of hay containing 0.13% S and a grain mixture with 0.28% S did not increase performance of dairy cows (Burgess and Nicholson, 1971). On the other hand, Bouchard and Conrad (1973) found that the addition of sulphur to a low sulphur diet improved the status of dairy cows as measured by various criteria. Thus, based upon regression analysis they concluded that a S level of 0.12% would approximate S balance and 0.18% would allow for a positive S balance of 4 g/day in cows producing 8 to 37 kg milk/day. Chalupa *et al.* (1973) reported improvement in Angus steers when sodium sulphate or elemental sulphur were added to bring dietary sulphur from 0.05% to 0.13%, but there was no improvement measured at higher sulphur levels. Thus, the 0.13% dietary sulphur appeared to meet fully the steers' needs.

Low-S forages generally contain large amounts of lignin and fibre and small amounts of protein and soluble carbohydrate. Such forages are of low organic matter digestibility, and energy intake is likely to be small even when S-deficiencies are corrected. As a result, the response to S is generally restricted to either a reduction in the rate of liveweight loss or a small gain in liveweight (Downes *et al.*, 1975). When the sulphur content of the diet is reduced there is a depression in the concentration of sulphide in the rumen liquor and of sulphate in serum (Kennedy and Siebert, 1972a) and saliva (Bray and Hemsley, 1969). Ruminal microbial activity is also inhibited, and this has several undesirable consequences (Downes *et al.*, 1975): (1) microbial protein synthesis in the rumen is reduced (Hume and Bird, 1970); as a result, the utilisation of dietary and particularly urea N is reduced, and the concentration of urea in blood and saliva and the excretion of N in urine are increased (Bray and Hemsley, 1969); nitrogen retention therefore declines; (2) less organic matter is digested in the rumen, and this results in an overall reduction in the animal's ability to digest dietary organic matter; energy retention is therefore depressed; (3) intake is reduced (Coombe *et al.*, 1971); and (4) the conversion of ruminal lactate to propionate through the acrylate pathway is inhibited, and lactate accumulates in the rumen (Whanger and Matrone, 1967).

Symptoms of a sulphur deficiency are not specific and might be difficult

to identify with sulphur (Elam, 1975). Thomas *et al.* (1951) reported that sheep fed purified diets which were sulphur-deficient showed symptoms such as loss of appetite, loss of weight, excessive lacrimation, weakness, dullness, emaciation and death. Various ruminal factors are exhibited by animals fed a sulphur deficient diet which reflect the effects of insufficient sulphur on the rumen micro-organisms. Sulphur deficiency affects the kind as well as the number of rumen micro-organisms present in the rumen. Gall *et al.* (1951); Whanger and Matrone (1965) found that the predominant micro-organisms present in the rumen of sheep fed sulphur-containing purified diets with urea as the sole nitrogen source were gram-positive, whereas those present in the rumen fluid of sheep fed this diet without sulphur were mostly gram-negative. The bacterial population was found to be about double in rumen contents of animals fed the diet containing sulphur (Gall *et al.*, 1951), which may have contributed to the greater viscosity of the rumen fluid observed in sheep fed the sulphur-containing diet than in those fed this diet without sulphur (Whanger and Matrone, 1965). Additional ruminal effects have been found in the form of end products of microbial action. Whanger and Matrone (1965) showed that rumen micro-organisms from sheep fed a sulphur-deficient diet formed more acetate and propionate from glucose, lactate or a purified diet than those from sheep fed the diet with sulphur added. Further, there was an accumulation of lactate in the rumen of the deficient sheep. The cause of these effects on lactate and VFA production has not been fully clarified. The addition of 160 g of sodium sulphite to daily rations for lactating cows significantly reduced the molar percentage of ruminal acetate from 65% to 55% and significantly increased the molar percentage of propionate from 18% to 23% (Alhassan *et al.*, 1969). The ruminal butyrate and valerate levels were also significantly increased by the sulphite feeding. Krabill *et al.* (1969) have shown that methane production was significantly decreased and the ratio of carbon-dioxide to methane was widened from 1.98 to 4.49 when 75 mg of sodium sulphite were added to 100 ml of ingesta in *in vitro* studies. The mechanism of action of sulphite on rumen fermentation is unknown, but one analogous to the effect of this compound on alcohol fermentation in yeast has been proposed (Alhassan *et al.*, 1969).

One of the most common indications reported of a sulphur deficiency is reduced feed consumption. Reports of lower feed intake by animals fed diets

low in sulphur have been made for sheep (Kahlon *et al.*, 1973), beef cattle (Chalupa *et al.*, 1973) and dairy cattle (Chalupa *et al.*, 1971; Leibholz and Kang, 1973). It is suspected that the reduced feed intake is a result of the reduction in microbial population and the subsequent reduction in the rate of digestion of feed components in the rumen (Elam, 1975). A lower rate of digestion would be expected to reduce rate of passage of feed through the digestive tract and thereby cause a reduction in feed intake by the animal. Whanger and Matrone (1965) found that during eight weeks in which sheep were fed purified diets with and without sulphur, the control sheep lost only 1.4 kg, whereas the animal fed the deficient diet lost 11.0 kg. The sulphur-supplemented animal consumed the allotted daily ration of 910 g of feed, but the deficient animal would eat only about 341 g of feed daily. The general appearance of the control sheep was excellent, whereas emaciation, dullness, poor appetite and loss of wool were signs observed in the deficient animals.

Since the tissue ratio of nitrogen to sulphur is 15:1 (Whanger, 1972), Loosli (1952) suggested that this should be the approximate ratio in the diet in order to avoid sulphur deficiency. Moir *et al.* (1967-1968) reported that narrowing the mean dietary nitrogen:sulphur ratio from 12:1 to 9.5:1 improved nitrogen retention in sheep from 28.8% to 36.0%. Bray (1969c) suggested that the utilisation of recycled urea nitrogen via the saliva may be severely limited in sheep fed low-sulphur diets. Since the nitrogen:sulphur ratios of some roughages are wider than 10:1 (Garrigus, 1970), there are clearly circumstances under which the animal cannot profit from the nitrogen recycling mechanism because of a concomitant sulphur deficiency. If the amount of sulphur is already limiting, further dietary supplements of non-protein nitrogen without additional sulphur are valueless, and this could explain the failure of some attempts to improve poor quality roughages by additions of urea alone (Whanger, 1968-1969).

Numerous blood characteristics have been reported altered in a sulphur deficiency. Some of these are reduced blood volume (Chalupa *et al.*, 1971), reduced serum sulphate (Weir and Rendig, 1952; Bray and Hemsley, 1969), increased plasma urea (Chalupa *et al.*, 1971; Leibholz and Kang, 1973), increased blood lactate and blood sugar (Whanger and Matrone, 1967) and changes in plasma amino acid levels (Chalupa *et al.*, 1971). Studies were conducted by Chalupa *et al.* (1971) with Holstein bull calves on the effects

of sulphur in the diet on plasma amino acid levels. Elemental sulphur was added to a semipurified diet that contained 0.04% S. Urea supplied all of the dietary nitrogen. Plasma free methionine increased linearly with increased dietary sulphur. However, cystine, serine, histidine, citrulline and alanine were higher in the plasma of S-deficient calves. Plasma concentration of citrulline was extremely high in these studies and similar results have been reported in other studies with S-deficient sheep (Goodrich *et al.*, 1967). In a second calf study (Chalupa *et al.*, 1973) S-deficient calves were found to have higher plasma concentrations of proline, serine, glycine and citrulline, but lower levels of valine, methionine, leucine and phenylalanine than calves fed sulphur as sodium sulphate or elemental sulphur. Serum sulphate levels were reduced in sulphur deficient sheep from normal values of 2.0-4.0 mg/100 mℓ to below 1.0 mg/100 mℓ (Weir and Rendig, 1954). The levels were returned to normal by sulphate as well as by addition of methionine to the ration. Bray and Hemsley (1969) observed decreased residual nitrogen and sulphur in the protein fraction of the parotid saliva due to sulphur supplementation of a diet composed of oaten hulls, urea and minerals. Whanger (1965) reported that the sulphate urinary levels were 3-6 times lower, and the urinary lactate levels were significantly elevated from S-deficient sheep. Thus, blood and urine levels of sulphate may be indicative of the sulphur status of ruminants. Various anatomical differences were also observed by Chalupa *et al.* (1971). When expressed on the basis of percentage of bodyweight the livers, spleens and testes of sulphur deficient calves were smaller and the adrenals and brains were larger than for calves fed supplemental sulphur.

Although some pure strains of rumen micro-organisms have been shown to have a preference for certain forms of sulphur, due to the symbiotic relationship of a mixed culture of rumen micro-organisms, the form of sulphur does not appear to have a significant influence on their activity (Whanger, 1972). Influence of certain forms of sulphur on pure strains of rumen micro-organisms, however, have been reported. Emery *et al.* (1957b) found that a culture of *L. multiparus*, isolated from the rumen, synthesised cysteine and methionine from inorganic sulphate, and that cysteine appeared to stimulate the synthesis of glutathione. This organism preferred cysteine to inorganic sulphate for the synthesis of glutathione. Cysteine and methionine have been shown to be required by some strains of *B. ruminicola*

(Pittman and Bryant, 1964).

The sulphur-containing vitamins, thiamine and biotin, have been shown by Buziassy and Tribe (1960a, b) and Briggs *et al.* (1964), respectively, to be synthesised by the rumen microbes. The levels of B vitamins were higher in rumen contents of animals fed diets with urea as the source of nitrogen than those fed diets with preformed protein as the source of nitrogen (Briggs *et al.*, 1964). This is in agreement with the work of Buziassy and Tribe (1960a) who found that when the intake of the B vitamins was high, little microbial synthesis took place in the rumen, but when the diet was deficient in these vitamins, as would probably be the case with diets containing urea as the sole source of nitrogen, the levels of synthesis were high. Hunt *et al.* (1954) showed that sodium sulphate and methionine stimulated the synthesis of riboflavin and vitamin B_{12} by rumen micro-organisms to a greater degree than when the source of sulphur was cystine or elemental sulphur. Their data suggested that pantothenic acid was synthesised by different micro-organisms than those synthesising riboflavin and vitamin B_{12}, and the former microbes appeared to have a lower sulphur requirement than those synthesising the other two vitamins. Deficiencies of thiamine in ruminants under field conditions have not been thought to exist, but reports from England and the United States note clinical symptoms of thiamine deficiency in young cattle and sheep, cured by thiamine therapy (Nutrition Foundation, Inc. 1969).

Further evidence of the critical nature of sulphur in the rumen is the finding by various workers that the digestibility of certain ration constituents is reduced when sulphur is limiting in the diet. Starch digestion by rumen microbes was increased by the addition of various forms of sulphur (Kennedy *et al.*, 1968). Thus, when sodium sulphate, sodium thiosulphate, calcium sulphate, ammonium sulphate, magnesium sulphate, cysteine, and methionine were tested against a sulphur-deficient medium containing rumen micro-organisms, each sulphur source resulted in significant stimulation of starch digestion. Cysteine (29.4% increase) was slightly less stimulatory than each of the inorganic sources (32.5% increase), but the addition of methionine resulted in significantly less stimulation (22% increase) of starch digestion than did the other sulphur sources tested. Martin *et al.* (1964) reported that cellulose digestion

was significantly depressed (from 68% to approximately 20%) in steers fed purified rations without adequate levels of sulphur. Sulphate additions to a diet deficient in sulphur for sheep increased both crude fibre digestion as well as increased the nitrogen and sulphur retention (Bray and Hemsley, 1969). Barton *et al.* (1971) used *in vitro* techniques to show the marked influence of sulphur concentrations in the substrate on cellulose digestion by rumen microbes. Additional *in vitro* studies by Bull and Vandersall (1973) indicated that the addition of sodium sulphate, calcium sulphate, DL-methionine, and methionine analog were equal at equal sulphur content in promoting cellulose digestion. The optimum sulphur level was 0.16% to 0.24%.

Of the 10 essential amino acids tested, only methionine resulted in an increase in total lipid synthesis by an *in vitro* fermentation of rumen microorganisms (Patton *et al.*, 1968). Further work by Patton *et al.* (1970a) revealed that methionine increased the polar lipids from 31% to 45% of the total lipids and this increase was mostly associated with the rumen protozoa (81% increase) rather than with the rumen bacteria (18% increase). Methionine supplementation has been shown to increase the rumen microbial numbers (Williams and Moir, 1951), and the methionine hydroxy analog was reported to increase the concentration of rumen protozoa (Patton *et al.*, 1970b). Since methionine did not affect the fatty acid composition of the lipids (Patton *et al.*, 1970a), it would appear that the apparent increase of lipid synthesis may have been the result of increased microbial numbers and not necessarily due to stimulation of fatty acid synthesis (Whanger, 1972).

After oral administration of labelled sulphate to sheep (Kulwick *et al.*, 1957; Hansard and Mohammed, 1968) or cattle (Hansard and Mohammed, 1969), the liver, kidney, adrenals, thyroid, spleen, skin, intestinal tissues and cartilage showed the greatest uptake of radioactive sulphur. The labelled sulphur in liver, spleen and skin was present in the form of cystine and methionine (Kulwick *et al.*, 1957). Johnson *et al.* (1970b) reported that after oral administration of ^{35}S-labelled elemental sulphur, sodium sulphate or L-methionine via gelatin capsule to lambs, greater amounts of the retained ^{35}S from elemental sulphur and methionine were incorporated into muscle tissue than that from sodium sulphate. Greater amounts of sulphur from sulphate, however, were incorporated into cartilage.

Anderson (1956) observed that when tissue slices of liver and kidney were incubated with 50 µg sulphide in Ringer's buffer, aerobically at 37°C, nearly all of the sulphide was taken up by the liver within 30 minutes, but a much slower rate of uptake was observed with kidney slices. It was calculated that a whole intact liver in mature sheep could take up 0.6 g sulphide/h, which is sufficient to handle the amount of sulphide absorbed from the rumen under normal feeding regimens. Sulphate sulphur can be transferred across the placenta of gravid ewes (Hansard and Mohammed, 1968) and heifers (Hansard and Mohammed, 1969). Absorbed radio-sulphur traversed the placenta freely at all 3 trimesters of the ewe and cow and the maternal-fetal tissue concentration patterns were similar. Seven days after isotope administration to third-trimester ewes, 55.7% of that which was absorbed and retained was deposited in maternal tissues and 44.3% was transferred to the total fetus and products of conception. Of that transferred to the fetus and products of conception, 77% was found in the fetus, 16% in the placenta and 7% in the placental fluids (Hansard and Mohammed, 1968). The amount of sulphur transferred to the fetus of cows was slightly higher than in ewes (Hansard and Mohammed, 1969). Maternal tissue sulphur concentration was unaffected by pregnancy in either species of animals. Paduceva *et al.* (1966) reported an increased number of first-class Karakul pelts from lambs born to ewes that were fed a diet with supplemental sodium sulphate.

Ruminant products which deviate widely from the N:S ratio of about 15:1 are those high in keratins, namely wool and hair. Wool normally contains c. 13% cystine and 0.4% methionine (Hale and Garrigus, 1953; Reis, 1965a). However, the S content of wool varies over a wide range; for example; Reis (1965a) found values from 2.7% to 4.2% S (9.8% to 15.3% cystine) on a clean dry basis. These variations are due to altered proportions of certain components of the high-sulphur proteins which are extremely rich in cystine (Gillespie and Reis, 1966; Gillespie *et al.*, 1969). The N:S ratio in wool is low (4-5:1) compared with the ratios for other animal proteins, ruminal microbes, and most forages (Langlands *et al.*, 1973; Moir *et al.*, 1967-1968; Walker and Nader, 1968). Since wool has a high sulphur amino acid content, it would be expected that sulphur deficiency would affect wool both quantitatively and qualitatively. It has been shown by several research workers (Thomas *et al.*, 1951; Starks *et al.*, 1953; Broad

et al., 1970) that a deficiency of this element results in poor wool growth, loss of wool and loss in wool crimp. By using ^{35}S-labelled elemental sulphur, sulphate (Hale and Garrigus, 1953), or cysteine (Garrigus, 1970) sulphur from these sources was shown to be utilised in the production of wool proteins.

Reis (1965b) observed variations of up to 0.6% sulphur in wool growth by the same sheep under two different nutritional regimens. Different sheep under the same nutritional regimen produced wool varying by as much as 0.9% in sulphur content. Wool appears to be less homeostatic in content of sulphur than is muscle tissue (Garrigus, 1970). Reis *et al.* (1967) and Reis and Tunks (1968) in subsequent studies reported that the sulphur content of wools varied inversely with the rate of wool production. "High wool producers" grew wool with a mean sulphur content of 3.13%, whereas "low wool producers" grew wool with a mean sulphur content of 3.55%. Observations in these studies suggest genetic differences in the ability of sheep to incorporate sulphur into wool. Detailed studies of the wool fibre have been made by Downes *et al.* (1966), Frater (1966), Gillespie and Reis (1966), and Fraser (1969) showing that the non-microfibrillar low-sulphur protein contains submicrofibrillar proteins that may polymerize to form the three-dimensional helical structure of the microfibrillar proteins within cells. The incorporation of matrix proteins into protein-synthesising cells may be the means by which higher sulphur diets influence the total sulphur content of wools since these matrix proteins may contain 8.3% sulphur versus about 3.5% for total wool fibre. Dietary supplements of S-amino acids are usually ineffective in increasing the quantity of S-amino acid absorbed unless the diet is deficient in sulphur (see Downes *et al.*, 1975) because S-amino acids are degraded by rumen microbes. Thus L-cystine (Marston, 1932; Du Toit *et al.*, 1935) and DL-methionine (Colebrook *et al.*, 1968) did not stimulate wool growth when added to the diet. Although Bird (1972a) found that methionine was degraded relatively slowly in the rumen, Bird and Moir (1972) observed only a small response in wool growth to ruminal infusions of methionine.

Marston and Robertson (1928) suggested that cystine was likely to be the primary nutrient limiting wool growth under pastoral conditions. The first direct evidence of the importance of cystine was provided by Marston (1935) who recorded a response in wool growth in a sheep given L-cysteine sub-

cutaneously. Subsequently Reis and Schinckel (1963, 1964) found that the abomasal administration of either L-cysteine or DL-methionine caused a substantial increase in the rate of wool growth, and the effects of S-amino acids have now been investigated in some detail.

Sheep with a high genetic potential for wool growth respond to S-amino acid supplements to a greater extent than those with a low genetic potential (Williams *et al.*, 1972). Diet may also be important. Most published experiments in which abomasal supplements of S-amino acids have stimulated wool growth, have been with penned sheep receiving roughage diets or with grazing sheep (Downes *et al.*, 1975). Some recent experiments have indicated that the response to methionine is dependent on diet. Hemsley and Ferguson (unpublished data, cited by Downes *et al.*, 1975) observed that intraperitoneal infusions of DL-methionine were ineffective in stimulating wool growth in sheep receiving a diet of wheat grain and wheaten hay (3:1). Further, abomasal infusions of methionine as a supplement for a diet consisting solely of wheat caused the production of very weak fibres with a reduced diameter (Reis, unpublished data cited by Downes *et al.*, 1975). Reis and Schinckel (1963) demonstrated that abomasal infusion of casein substantially increased wool growth and its sulphur content.

The amount of additional cyst(e)ine or methionine required for the maximal stimulation of wool growth has been investigated for sheep receiving 800 g/day of a diet of lucerne and wheaten hays (1:1). With this diet, 1.0 to 1.5 g/day S-amino acids would be available for absorption from the intestines (Reis, 1967). The optimal amount of supplementary methionine on this diet was 1 to 2 g/day when given as an abomasal infusion (Reis, 1967; Reis *et al.*, 1973). Smaller amounts of methionine (0.5 to 0.6 g/day) provided sub-maximal responses (Reis, 1967; Reis *et al.*, 1973), whereas greater quantities (6 to 10 g/day) had an adverse effect and sometimes depressed wool growth (Reis, 1967 and 1970; Reis *et al.*, 1973). Reis and Schinckel (1964) and Gillespie and Reis (1966) noted that relatively small amounts of L-cystine infused into the abomasum were optimum for stimulating wool growth (123% to 181%) while 3.0 g per day resulted in less stimulation of wool growth than did a 1.5 g level per day. The sulphur content of the wool increased 9% to 19% with casein administration but did not increase with gelatin administration. Reis (1967) administered abomasally various

amounts of amino acids by aqueous drips. Small equimolar amounts 0.5 to 2.0 g per day of either L-cysteine or DL-methionine increased wool growth as much as 100%. With relatively high levels of 6 to 8 g per day of L-cysteine, rate of wool growth was reduced slightly below the maximum response (see also Reis *et al.*, 1973). Reis (1967); Reis *et al.* (1973) suggest that at low levels of supplementation (1 to 2 g/day) cystine, cysteine and methionine produce quantitatively similar responses in wool growth. A direct comparison (Williams *et al.*, 1972) of abomasal supplements of equimolar amounts of L-cystine (2.0 g) or DL-methionine (2.5 g) indicated a slightly greater response to methionine. However, a detailed study of the dose-response curve for each amino acid would be needed to evaluate this result. The basis for the effectiveness of methionine has not been established. It could be due solely to its conversion to cysteine either in organs such as the liver (Meister, 1965) or in the skin or wool follicles (Downes *et al.*, 1964), or it could have specific effects as suggested by Reis (1967).

Methionine hydroxy analog is also effective as an abomasal supplement for wool growth (Reis 1967 and 1970) but no direct comparisons have been made with methionine. It is not known whether the S of methionine hydroxy analog is converted directly to cysteine-S as distinct from supplying methionine to the sheep. Wright (1969) using either intraperitoneal injections of 1.5, 3.0, or 4.5 g methionine or dietary addition to a 0.15% sulphur basal diet found that sulphur-bearing amino acids were limiting for maximum gain in weight on an 8% C.P. diet and for wool production on both the 8% and a 12% crude protein diet. Direct comparisons with L-cyst(e)ine and L-methionine have demonstrated that the abomasal, duodenal or intravenous routes are equally effective for supplying S-amino acids for wool growth (Barger *et al.*, 1973; Reis *et al.*, 1973). Recently, Doyle and Bird (1975) found a supplement of 3.8 g/day DL-methionine increased wool growth by 1.15 g/day which represented an 18% increase in the total wool production. Barry *et al.* (1973) found that wool growth rate and fibre diameter were increased by both formaldehyde treatment of silage and by intraperitoneal supplementation with DL-methionine. The effect of formaldehyde treatment could have been due to its effect of increasing intake and/or its probable effect of increasing the amount of protein digested in the intestines (Barry and Fennessy, 1973). S-amino acid supplements have usually been given as in-

fusions for 6 to 24 hours each day. However, single daily injections of methionine, or even less frequent dosing, also stimulate wool growth (Langlands, 1970; Wickham, 1970; Barry, 1971; Robards, 1971).

The presence of radioactivity in the milk of cows (Block and Stekol, 1950; Conrad *et al.*, 1967, 1967a; Pereira *et al.*, 1966, 1968) and goats (Block *et al.*, 1951) after administration of ^{35}S-labelled compounds indicates that sulphur is also eliminated in the milk. Block *et al.* (1951) found that the distribution of ^{35}S in milk from administration of ^{35}S-labelled sulphate was about 80% in the protein fractions and 20% in the trichloroacetic acid filtrates. After administration of ^{35}S-labelled sulphate (Block and Stekol, 1950; Block *et al.*, 1951; Pereira *et al.*, 1966, 1968), sulphide (Pereira *et al.*, 1966, 1968; Conrad *et al.*, 1967, 1967a) or methionine (Pereira *et al.*, 1966, 1968), radioactivity has been found in methionine and cystine of the milk proteins. The form of dietary sulphur, however, does have an influence on the type of sulphur compounds in the milk. Thus, preferential labelling of milk proteins was obtained with ^{35}S-methionine or ^{35}S-sulphide administration whereas preferential labelling of non-protein sulphur was obtained with ^{35}S-sulphate (Pereira *et al.*, 1966). Pereira *et al.* (1968) reported that more hydrogen sulphide and volatile mercaptans and less volatile organic sulphides and derivatives of 2,4-dinitrophenylhydrazine were respectively present in milk from cows administered with ^{35}S-labelled sulphide than those administered with ^{35}S-labelled sulphate or methionine. Dunham *et al.* (1968) showed that the level of dimethyl sulphide in milk was increased when methionine was included in the diet. Various sulphur compounds have been implicated in connection with milk flavours (Pereira *et al.*, 1966, 1968; Dunham *et al.*, 1968). Jacobson *et al.* (1967) found that voluntary feed intake and milk production were significantly higher for lactating cows fed a basal concentrate mixture (0.1% sulphur) with supplemented sodium sulphate to make it 0.18% with respect to sulphur than those fed the unsupplemented basal diet. Since the sulphur-supplemented cows did not perform as expected, the inorganic sulphur was postulated to only partially meet the dietary sulphur requirement of the cow (Jacobson *et al.*, 1969). Palfij *et al.* (1962) and Palfij (1963) increased milk production in cows fed silage containing 2 to 3 kg sodium sulphate per ton. Although the inclusion of sulphite in the diet was shown to affect the molar percentage of the ruminal fatty acids, no effect on

percent milk fat, solids-not-fat, milk protein, or total milk production was observed (Alhassan *et al.*, 1969).

Downes *et al.* (1975) suggest that growth and milk production differ from wool growth in two important respects in relation to responses to S-amino acids. Tissue and milk proteins are not rich in S-amino acids, and there is a high energy requirement associated with their synthesis. Growth and milk production are usually restricted by energy intake and the proportion of calories supplied as protein rather than by a deficiency of S-amino acids.

In ruminants producing large amounts of milk the output of S-amino acids in milk proteins is relatively greater than the output of cystine in wool by wool-producing sheep (Downes *et al.*, 1975). Thus, a high-producing sheep may produce 15 g clean dry wool/day, which would contain 1.5 to 2.3 g cystine, whereas a high-producing goat (similar in size to a sheep) could be expected to secrete milk containing 3 to 4 g/day S-amino acids. However, while there is some evidence that methionine may be the first limiting amino acid for milk protein synthesis in dairy cows (Brown, 1969), the supply of this amino acid to the udder is usually adequate (Fisher, 1969; Teichman *et al.*, 1969).

Section 6: SULPHUR TOXICITY

High levels of calcium sulphate (gypsum) are known to reduce feed intake of ruminants (Christensen *et al.*, 1947). This principle has been used as an effective means for regulating intake of pasture supplements for livestock. Barrentine and Ruffin (1958) reported on the use of gypsum in this manner, but found that cattle became sick after the consumption of high levels of sulphate. Albert *et al.* (1956) reported that high levels of sulphur (above 0.4%) as elemental sulphur or sodium sulphate reduced the performance of lambs. On the other hand, some researchers have observed no harmful effects from feeding relatively high levels of sulphur. For example, Chalupa *et al.* (1971) fed elemental sulphur at levels up to 1.72% in a purified diet for Holstein calves with no observable ill effects. In later studies (Chalupa *et al.*, 1973), either sodium sulphate or elemental sulphur was added to the diet of Angus steers to provide levels of sulphur up to 0.62% and 0.56% sulphur, respectively, with no deleterious effects observed in the cattle.

Johnson *et al.* (1968) reported the addition of 0.5% sulphur as calcium sulphate reduced the daily gains of lambs. They found that an equal amount of sodium sulphate did not reduce gains as much as calcium sulphate. In addition, Bouchard and Conrad (1973a) reported reduced feed intake by dairy cows when calcium sulphate was used to increase dietary sulphur to 0.3% or more. They found excessive retention of sulphur by cows for all sources (calcium sulphate, sodium sulphate, potassium and magnesium sulphate) of sulphate tested at levels above 0.3% dietary sulphur. Thompson *et al.* (1972) reported reduced feedlot gain for steers supplemented with elemental sulphur to produce a 5:1 nitrogen:sulphur ratio. Lower dry matter intakes were recorded in dairy cows when the level of sulphur was 0.35% or above (Bouchard and Conrad, 1974). No overt toxicity was reported in heifers allowed access to drinking water with up to 2500 ppm sulphate (Digesti and Weeth, 1973).

Dougherty *et al.* (1965) suggest that the generation of large quantities of hydrogen sulphide in the rumen depresses rumen motility, and causes severe nervous and respiratory distress if it is absorbed through the lungs during eructation. Bird (1972c) has shown that single ruminal infusions of sulphide (0.94 g sulphur) in solution (Na_2S-H_2S) resulted in temporary respiratory distress and collapse of sheep; rumen motility was temporarily abolished,

but smaller doses resulted in a moderate transitory depression of motility. In the same work (Bird, 1972c), continuous ruminal infusions of sulphide (2.93 g sulphur/day) solution (sodium sulphide in water) resulted in a significant decrease in dry matter intake. Ruminatory activity was decreased, but independently of the reduced feed intake; rumen motility was also decreased, but this was at least partly as a result of the decreased intake. Bird (1972c) also found that ruminal infusion of 6 g sulphur as sodium sulphate per day resulted in complete inappetence; 4 g sulphate sulphur per day infused intraruminally or up to 6 g given duodenally did not adversely affect the sheep. Bray (1969a) found that in one experiment, where 1.01 g of sulphur as sodium sulphide (supplied as 50 mℓ of aqueous solution) was given into rumen, hydrogen sulphide could be smelt on the animal's breath and one sheep became unsteady on its feet and fell to its knees 17 minutes after dosing, but 10 minutes later it had regained its feet and had fully recovered; in another experiment, where 0.4 g of sulphur as sodium sulphide was given, there were no observable effects of sulphide on the sheep. No toxic effects of sulphur were reported by Gawthorne and Nader (1976) where 10 g of sodium sulphate per day were infused continuously into the rumen of sheep. Bird (1972c) states that "intake by sheep of not more than 4 g sulphur/day, or the addition to ruminant diets of 0.2% sulphate sulphur or S-amino acid sulphur, should satisfy microbial and tissue sulphur requirements without adversely affecting feed intake".

Sulphur toxicity was reported in a group of 20 yearling heifers which had consumed S mixed with corn (Julian and Harrison, 1975). The animals showed respiratory disease and abdominal pain. Vasculites and necrosis of the rumen and abomasal wall were recorded upon examination of one of the animals. Lower feed and water consumption, weight loss and diuresis were reported in growing heifers offered water containing 5000 ppm sulphate (Weeth and Hunter, 1971). Water intake was not affected by adding 1462 or 2814 ppm sulphate to the drinking water, but hay intake was depressed at the higher level (Weeth and Capps, 1972) and rate of gain was decreased by both sulphate levels. In a later study Digesti and Weeth (1976) concluded that 2500 ppm sulphate in drinking water represents a safe tolerance concentration.

Sulphide toxicity has, however, apparently not been associated with the feeding to ruminants of supplemental sulphate (e.g. Boyazoglu *et al.*, 1967)

or with high levels of sulphates in drinking waters (Peirce, 1960). During an investigation into the fate of sulphate infused ruminally or duodenally (Bird and Moir, 1971) sheep receiving a continuous ruminal infusion of 5-6 g/day of sulphate sulphur eventually refused to eat or drink. Bird (1972c) suggests that since duodenal infusions of sulphate did not affect intake, and in view of the presence of dissimilatory sulphate-reducing bacteria in the rumen (Huisingh *et al.*, 1974) and their ability to rapidly adapt to sulphate infusions (e.g. Lewis, 1954; Bird and Hume, 1971; Bird and Moir, 1971), the adverse effects of sulphate infusion noted by Bird and Moir (1971) were attributed to sulphide toxicity.

Hubbert *et al.* (1958) reported that cellulose digestion by ruminal micro-organisms *in vitro* was depressed slightly by 1000 µg sulphate sulphur per millilitre of medium. However, in later studies Kennedy *et al.* (1971), found no depression of starch digestion by ruminal micro-organisms *in vitro* when sulphur up to 11000 µg/mℓ was added (as sodium sulphate) and no diminution of growth *in vitro* by ruminal microbes was observed in studies by Bird (1972c) when sulphide levels up to 1500 µg/mℓ were used.

Toxicity symptoms have been reported with the use of elemental sulphur in cattle (Coghlin, 1944) and ewes (White, 1964). Thus, Goghlin (1944) reported that the symptoms in the early stages of hydrogen sulphide poisoning in cattle included twitching of certain groups of muscles especially those of the jaws, eyelids and ears. There was evidence of pain by lying down and getting up, switching and uneasiness, and a staggery gait was also noticed. During the morning there was no evidence of diarrhoea but by evening most of the affected cattle were scouring badly. As the cases advanced they became unable to rise, acted as if blind, struggled a great deal, with grunting and fast laboured breathing. Finally they became more or less comatose with their heads lying on the side which was followed by death. During a post-mortem examination the lungs were very dark in colour, congested and oedematous; the liver was very light presenting a boiled appearance; the spleen was normal in colour except for some light spots on its surface; the stomachs showed little evidence of inflammation but the intestines were very acutely inflamed; some of the muscles over the loins and back were almost black in colour. Likewise, in a similar report of sulphur poisoning in ewes by White (1964), those ewes which were ill were

very depressed, being unwilling to stand, breathing very heavily and in some cases having a temperature up to 105°F. The breath smelt of H_2S and they had a black diarrhoea with the passage of some blood from the rectum which in some ewes was followed by death. Post-mortem examinations revealed an intense inflammation of the abomasum and small intestine with a large quantity of peritoneal effusion, the fluid after death containing quite large fibrinous clots; the kidneys were acutely inflamed and appeared nearly black in some cases; petechial haemorrhages were very marked throughout the carcase, in the heart, lungs, pleura and even in the musculature on the inside of the scapula when the shoulder was removed.

In the ruminant the toxic effect of sulphide on the nervous system is apparently mediated via eructation of hydrogen sulphide, with other gases, from the rumen and absorption through the lungs (Bird, 1972c). Most of the eructated ruminal gases enter the lungs and infusion of hydrogen sulphide into the rumen of sheep with blocked trachea produced no symptoms, whereas sheep with open trachea collapsed after several eructations (Dougherty *et al.*, 1965). Sulphide absorbed from the rumen may be detoxified by oxygenated haemoglobin in the blood; *in vivo* the reduction of oxyhaemoglobin is reversible (Evans, 1967). The liver itself, through the action of a sulphide oxidase system, is also clearly capable of detoxifying sulphide (see Anderson, 1956), hence it is unlikely that much free sulphide would reach the brain after being absorbed from the rumen into the portal system (Bird, 1972c). Bird (1972c) states that "the direct and shorter route to the heart and brain afforded by the inspiration of hydrogen sulphide and transfer into the pulmonary vein effectively by-passes the liver and enables hydrogen sulphide to exert its toxic effect on the respiratory-circulatory systems". Evans (1967) concluded that spinal vasomotor centres were affected in the same manner by hydrogen sulphide as were medullary centres.

The minimum lethal dose of sodium metabisulphite in sheep was 2.25 g/kg bodyweight (Nikolaev and Dzhidzheva, 1973); signs of toxicity were restlessness, feed refusal, ruminal atony, rapid pulse and respiration, cyanosis and death. Kaemmerer *et al.* (1972) reported no harmful effects in sheep fed dried beet pulp containing 1% SO_2 as sodium disulphite in a ration of 500 g hay, 500 g dried beet pulp and 200 g ground oats. A detailed review of amino acid toxicities including physiological, metabolic and biochemical

effects associated with the toxicity has been made by Harper *et al.* (1970).

Excesses of methionine or its hydroxy analog adversely affect feed consumption by ruminants. Dietary supplements, ruminal infusions and abomasal infusions of these compounds can decrease dry matter intake by ruminants. Thus, in cattle, intraruminal infusion of DL-methionine, 2.5% or more of dietary dry matter intake, resulted in a toxic effect on feed intake (Satter *et al.*, 1975). Papas *et al.* (1974) reported that feed consumption by lambs was decreased from 1000 g to 786 g/day when methionine hydroxy analog was included in the diet at a level of 1.2%. Further abomasal infusion of DL-methionine (0, 2.7, 5.4 and 8.1 g/day) resulted in a linear reduction in feed intake from initial values, while methionine hydroxy analog had no effect. The differences between DL-methionine and methionine hydroxy analog may be due on the one hand to greater resistance of methionine hydroxy analog to microbial destruction in the rumen (see Belasco, 1972) and on the other hand to a low replacement value of methionine hydroxy analog for methionine in the tissues (see Papas *et al.*, 1974).

Feed intake in cattle was decreased with abomasal infusion of 0.6% DL-methionine (Satter *et al.*, 1975) and also in sheep with 0.3, 0.5 and 0.9% (Papas *et al.*, 1974). Abomasal infusions of 7.4 to 9.8 g/day of DL-methionine caused a substantial reduction in wool growth rate in sheep when compared to infusions of 0.6 to 2.0 g/day (Reis, 1967). Langlands (1970) found that a single intraperitoneal injection of 28 g of methionine hydroxy analog depressed live weight and resulted in the death of three of the five sheep injected.

Excessive amounts of methionine fed to monogastric animals (Snyderman *et al.*, 1968) or infused into the abomasum of sheep (Reis *et al.*, 1973a) substantially increased plasma methionine concentrations and altered the plasma amino acid pattern. Neither oral supplements of 12.3 g/day methionine hydroxy analog (Papas *et al.*, 1974) nor 10.0 g/day DL-methionine (Reis *et al.*, 1973a) given to sheep influenced plasma methionine concentrations or plasma amino acid patterns.

Tao *et al.* (1974) found that adult sheep receiving total nutriment by dual infusions (VFA infused intraruminally, and glucose, vitamins and amino

acids via jugular cannula) and supplement of L-methionine in excess of 3.6 g/day had elevated plasma methionine concentrations; plasma levels of valine, isoleucine, leucine and threonine were decreased by the L-methionine supplement, but non-essential amino acids including glycine and serine were increased. Previous work has shown that toxic amounts of methionine have caused lower plasma concentrations of glycine and serine in rats (Benevenga and Harper, 1967) and sheep (Schelling *et al.*, 1973). Tao *et al.* (1974) suggest that their experimental design did not allow adaptation to the particular treatments, and thus resulted in divergent results.

Recently, Doyle and Adams (1980) investigated the toxic effects of large amounts of DL-methionine infused into the rumen of sheep. The sheep, fed *ad libitum* on a roughage diet comprised of equal parts of chopped lucerne hay and oaten chaff, received continuous infusions of DL-methionine into the rumen; the daily dose increasing from 0 g (control treatment) up to 30 g in 3 g amounts at weekly intervals. They found that dry matter intake fell below the control level when 24 g/day or more of the amino acid was infused, while plasma free methionine levels increased substantially when 30 g/day was given; there was no effect of DL-methionine supplementation on the bodyweight of the animals; however, when 30 g/day of DL-methionine was infused one animal died and the acute condition of two others necessitated their slaughter; significant lesions included acute nephrosis and haemolytic anaemia, with milder changes in the liver and pancreas.

No reports of tissue damage in ruminants associated with large amounts of methionine given orally or via routes making the supplement available to the host animal's tissues are available in the literature.

Section 7: EXCRETION AND RETENTION OF SULPHUR

(a) Urinary Excretion

Sulphur can be excreted in the urine as both organic and inorganic compounds. Urinary excretion of SO_4^{2-}, the major end product of S-amino acid oxidation and inorganic sulphur metabolism in mammalian tissue, represents a major source of sulphur loss from the body. Generally, only trace amounts of amino acids appear in urine. Ruminants, like monogastrics (see Dziewiatkowski, 1970), excrete ester sulphates in the urine. Phenolic sulphates form the major portion of the ester sulphates (Bostrom *et al.*, 1963), but steroids and other metabolites also yield sulphated products (Pasqualini *et al.*, 1963; Bostrom and Wengle, 1964).

The kidney is regarded as being solely responsible for regulating the concentration of most anions found in the blood, with the possible exception of phosphate (McClean, 1960). Plasma inorganic SO_4^{2-} is completely filtrable by the glomerular membrane (e.g. Goudsmit *et al.*, 1939). Thus the major factors controlling the extent and rate of renal excretion of SO_4^{2-} are plasma inorganic SO_4^{2-} concentration (Bourdillon and Lavietes, 1936); the tubular maximum reabsorptive capacity (see Lotspeich, 1947a, b), which is influenced by electrolytes, glucose and certain amino acids (Berglund and Lotspeich, 1956a, b; Walser and Mudge, 1960); and by the glomerular filtration rate (see Berglund and Lotspeich, 1956b; Walser and Mudge, 1960). Recently, Bishara and Bray (1978b) observed that molybdate can inhibit reabsorption of sulphate. Inorganic SO_4^{2-} in blood in excess of the tubular reabsorptive capacity is rapidly excreted.

Variations in sulphur intake affect urinary sulphur excretion (Bray and Hemsley, 1969; Bird, 1971). Walker and Faichney (1964) found that the urinary ester sulphate sulphur output in the young lamb was influenced by diet, and Bray and Hemsley (1969) that the urinary neutral sulphur output by mature sheep was also affected by the intake of sulphur. Bird (1971) found that infusions of sodium sulphate (0-6 g S/day) into the rumen or duodenum significantly increased the excretion of total sulphur, ester sulphate, and inorganic sulphate in urine. On the basal treatment 15% - 20% of the urinary sulphur was inorganic sulphate sulphur, but the proportion was 80% - 90% when sulphate was infused. In the same work, Bird (1971) compared the effect of ruminal or duodenal infusions of sodium

sulphate on the excretion of the various forms of sulphur. Thus, the mean urinary neutral sulphur outputs were increased from 70 up to 353 mg S/day by intraruminal infusions of sodium sulphate, compared with from 51 up to 176 mg S/day by the corresponding intraduodenal infusions; similarly, the mean urinary ester sulphate sulphur outputs were increased from 134 up to 392 mg S/day by the intraduodenal infusions, compared with 136-205 mg S/day by the intraduodenal route. Bird (1972) reported that when sheep were fed a basal ration supplemented with sodium sulphate containing 494 mg S/day the urinary excretion of organic sulphur and ester sulphur was increased, but the excretion of inorganic sulphate did not change, indicating a limiting supply of sulphate ions for sulphation reactions at these intakes of sulphur.

Bird (1971) suggests that the incorporation of sulphur recycled to the rumen into microbial protein influences the excretion of inorganic sulphur in ruminants. Microbial activity in the hindgut may also affect the urinary excretion of inorganic sulphate. Bird and Thornton (1972) found that infusion of glucose into the hindgut decreased urinary sulphur excretion by reducing the output of sulphate. These results for sulphur correspond with those for nitrogen (Thornton *et al.*, 1970), where infusion of glucose decreaded urinary total nitrogen and urea nitrogen; the mechanism proposed, being that the supply of extra energy to this region led to an increase in microbial protein synthesis and its excretion in the faeces, i.e. faecal excretion is a *pre-emptive* pathway.

In sheep, ^{35}S from oral or intravenous doses of ^{35}S-Na_2SO_4 is rapidly absorbed and excreted in the urine. Bray (1969b) found that 27%-29% in 6 hours and 69%-78% in 24 hours of the dose was excreted in the urine after intravenous administration of ^{35}S-Na_2SO_4 while 23% in 6 hours and 34%-51% in 24 hours of the dose was excreted in the urine after intraruminal administration of ^{35}S-Na_2SO_4. Duodenal administration of ^{35}S-Na_2SO_4 resulted in urinary excretion of 18% - 20% in 6 hours and 38%-39% in 24 hours. When ^{35}S from taurine was administered by a single intraduodenal infusion in sheep, 41% - 51% was recovered in bile and pancreatic secretions and less than 4% in the urine in 3 days (Bird, 1972b); on the other hand, when labelled sodium sulphate was administered, 63% - 76% of the ^{35}S was recovered in urine in 4-5 days. The results showed that taurine is conserved, contribu-

ting to the sulphur economy of the animal.

Recently, Oldham *et al.* (1975) found that when ^{35}S-Na_2SO_4 was injected intravenously into mature sheep 55% - 80% of the dose was excreted in the urine within 106 hours; 99% of the recovered label was collected in 84 hours. Hansard and Mohammed have studied the excretion of sodium sulphate administered intravenously or orally to sheep (1968) and cattle (1969). The results with sheep show that about 75% of the dose was excreted via urine within a period of 168 hours. Data presented on cattle were similar except that a slightly larger percentage was excreted via urine. Route of administration had little effect on route of excretion. Older data published by Thomas *et al.* (1951) on sheep fed purified diets showed that 66% - 93% of S was excreted via urine, the percentage increasing as the sulphur content in the diet decreased. There have been some reports indicating that the N:S ratio in urine is relatively constant (Barrow and Lambourne, 1962), however, the results of Thomas *et al.* (1951) do not show this, since N:S ratios were 5.6:1 on adequate diets and 3:1 on deficient diets; neither do the results of Jacobson *et al.* (1967, 1969) show a constant N:S ratio in urinary sulphur.

A comparison of ruminal and abomasal infusions of 2.0 g DL-methionine/day given to sheep receiving a basal diet supplying 1.45 g S/day revealed greater excretion of total sulphur and inorganic sulphate sulphur in the urine by sheep receiving the ruminal infusion (Bird and Moir, 1972). The route of administration had no influence on the excretion of urinary ester sulphate sulphur and urinary neutral sulphur. The greater urinary excretion of inorganic sulphate sulphur with ruminal administration was due to degradation of methionine in the rumen, absorption of the sulphide produced, its oxidation to inorganic sulphate in blood or liver and eventually its excretion. Bird and Moir (1972) found that of the ^{35}S-L-methionine infused into the rumen 39.0% of the dose was excreted in urine, 37.1% as reducible ^{35}S, whereas abomasal infusion of the isotope resulted in 20.0% of the ^{35}S excreted in the urine, 18.4% as reducible ^{35}S.

Age may also be an important factor, since greater utilisation of inorganic sulphate sulphur by the tissues may result in animals in which rapid growth of bone or cartilage is occurring. Johnson *et al.* (1970a) found that only 20% of ^{35}S from oral doses of ^{35}S-Na_2SO_4 was lost in 8 days in the urine of ruminant lambs, 58% of the dose was retained, compared with about 9% in

mature cattle and sheep (Hansard and Mohammed, 1968, 1969) and 0% - 49% in mature sheep (Bray, 1969b). Although these findings require confirmation, they do suggest that in some cases supplements of inorganic sulphate sulphur in the diet of young ruminants could be beneficial by reducing the catobolism of sulphur amino acids.

The organic sulphates normally found in urine consist of compounds such as phenol and indole conjugated with sulphate. Compounds such as phenol and indole are quite toxic; they most probably originate from bacterial action in the gut and conjugation with sulphate is a mechanism utilised to render such compounds less toxic (White *et al.*, 1973).

(b) Faecal Excretion

The major portion of the faecal sulphur is organic (Bird, 1971; Bird and Hume, 1971) and is presumably derived from bacterial portein, since these residues are the main sources of protein in sheep faeces and may account for up to 78% of the faecal nitrogen excreted (Mason, 1969). Since the N:S ratio of bacterial protein is likely to be 11 or greater (see Bray and Till, 1975) other organic sulphur compounds must be excreted to give the narrower N:S ratio found for the faeces, e.g. 6.4 - 9.2 (Bird, 1971). Faecal ester sulphates may arise from the sulphation of bilirubin (Isselbacher and McCarthy, 1959), triiodothyronine (Roche *et al.*, 1959), cholesterol (Drayer *et al.*, 1964; Moser *et al.*, 1966), steroid hormones (Erickson *et al.*, 1969), plant and animal sterols (Gustafsson *et al.*, 1968), and intestinal mucins (Dziewiatkowski, 1962). Inorganic SO_4^{2-}, passing from the blood to the intestinal lumen by diffusion, may be excreted in the faeces.

The faecal excretion of total sulphur increases with sulphur intake (Moir *et al.*, 1967-1968; Bray and Hemsley, 1969; Bird 1971; Kennedy, 1974). Bray and Hemsley (1969), working with sheep, found that faecal sulphur increased as dietary sulphur levels increased and the faecal N:S ratio decreased. Excretion of faecal sulphur was also shown to be related to intake of sulphur, organic matter and digestible organic matter (Kennedy, 1974). However, this is not the case with monogastrics (Wright *et al.*, 1960) and the difference between species appears to be due to the intervention of the rumen and secretion of microbial protein or its degradation products.

Faecal neutral sulphur excretion by sheep may be increased when oral or

ruminal supplements of sodium sulphate are given (Bird, 1971, 1972; Bird and Hume, 1971). Thus, Bird (1971) found that infusions of sodium sulphate (0-6 g S/day) into the rumen or duodenum significantly increased the excretion of total sulphur in faeces. Inorganic sulphate excretion in faeces was increased significantly only by intraduodenal infusions, and the excretion of neutral sulphur in faeces only by intraruminal infusions. When sodium sulphate was infused intraruminally 87% - 94% of the faecal sulphur was in the neutral sulphur fraction, 4.1% - 5.4% was ester sulphate sulphur, and 0.5% - 4.0% was inorganic sulphate sulphur. Bird (1971) suggested that the intake of sulphur and the supply of digestible energy to the fermentative rumen and hindgut regions primarily determine the amount of organic sulphur excreted in the faeces by affecting the synthesis of bacterial sulphur. Bird and Hume (1971) found that the output of ester sulphates in the faeces was increased by both cystine and sulphate dietary supplementation. This increase was probably caused by an increase in blood sulphate levels and the subsequent incorporation of the sulphate into mucin by the intestinal mucose (Dziewiatkowski, 1962). Bird (1972) reported that when sheep were fed a basal ration supplemented with sodium sulphate containing 494 mg S/day the output of organic sulphur in faeces was increased. Organic sulphur compounds comprised 95% - 97% of the faecal sulphur compared with 87% - 94% found by Bird (1971) when up to 5 g of sulphate sulphur was infused daily into the rumen, or 92% - 95% found by Bird and Hume (1971). Faecal excretion of ester sulphate sulphur or inorganic sulphur was small and unaffected by change in sulphur intake. In Bird's (1972) experiment, and in others (Bird, 1971; 1972d; Bird and Hume, 1971) sulphur deficiency resulted in smaller outputs of faecal neutral sulphur, despite greater outputs of faecal dry matter which could possibly increase gut mucosal shedding or merely produce a greater loss of undigested plant protein sulphur. Increased bacterial growth associated with a greater digestibility of the diet and an increased faecal loss of bacterial sulphur due to the incomplete digestion of bacteria (see Bird, 1972e) probably accounts for much of the greater excretion of faecal neutral sulphur found at high intakes of sulphur.

Bird and Moir (1972) found that ruminal infusion of 2.0 g DL-methionine per day given to sheep receiving a lucerne-oaten chaff based diet supplying 1.45 g S/day resulted in greater excretion of faecal sulphur than similar infusions per abomasum.

Kennedy *et al.* (1976) found that more organically bound ^{35}S left the distal

end of the small intestine than passed from the stomach and that even more organically bound ^{35}S was excreted in the faeces and suggested that appreciable quantities of ^{35}S originally present in the plasma as sulphate were transported across the wall of both the small and large intestines and were found in the intestines in non-sulphate forms. Expressed as a proportion of the dose infused intravenously (as ^{35}S-Na_2SO_4), Kennedy *et al.* (1976) found that, approximately 7.7% of the ^{35}S passed in digesta from the stomach and the amounts of ^{35}S that passed from the ileum and in the faeces were both equivalent to approximately 26% of the dose, with most (88.2% and 94.2% respectively) of the ^{35}S present as organic sulphur.

Bray (1969b) reported the faecal recovery of 12% and 19% of ^{35}S from intravenous doses of ^{35}S-Na_2SO_4 and suggested that intravenously infused ^{35}S-SO_4^{2-} was secreted primarily into the post-ruminal tract in the form of sulphate esters or, inorganic sulphate, and excreted as such in the faeces, but Bird and Thornton's (1972) results show that these reducible sulphur or ^{35}S fractions in the faeces were of minor significance. Bird and Thornton found that the recovery of ^{35}S in faeces after a single intravenous infusion of ^{35}S-Na_2SO_4 was: Faecal ^{35}S (% of dose, 0-96 hr) = 11.5-22.7 with a composition (% of total) organic ^{35}S = 88.8-93.0 and reducible ^{35}S = 7.0-11.2. Bray's (1969b) conclusion was that little of the ^{35}S excreted could have been derived from the cycling of ^{35}S-SO_4^{2-} into the rumen and incorporation into microbial protein, whilst Bird and Thornton (1972) suggest that the protein ^{35}S that reached the ileum appears to be largely derived from ^{35}S cycled through the rumen.

Bird and Thornton (1972) state that "the transfer of sulphate from the blood to the fermentative areas of the alimentary tract is apparently the *pre-emptive* pathway of excretion in ruminants". The above authors found that there was a net transformation of reducible ^{35}S to organic ^{35}S forms in the hindgut when glucose was infused into the distal ileum and more organic sulphur and ^{35}S was excreted in the faeces than passed the terminal ileum. Under the same circumstances more nitrogen was excreted in the faeces than passed the terminal ileum (see Thornton *et al.*, 1970). These effects were attributed to cycling of endogenous sulphur and nitrogen to the hindgut (caecum and colon) and their incorporation into microbial protein which was

largely excreted in the faeces.

Bird and Moir (1972) after ruminal and abomasal infusions of ^{35}S-labelled methionine found a higher output of ^{35}S in the faeces with ruminal infusions (30.2% of dose) than with abomasal infusions (10.7% of dose). This was also found in the experiment of Downes *et al.* (1970) where c.17% of the ^{35}S given as ^{35}S-L-methionine in the diet, compared with c.9% of the abomasally infused ^{35}S, was excreted in the faeces.

The amount of sulphur ingested, the extent of fermentation in the rumen or hindgut or both, and the age of the animal will affect the retention of infused ^{35}S-SO_4^{2-} and the pathway of ^{35}S excretion (Bird and Thornton, 1972). In immature animals (Johnson *et al.*, 1970a), absorbed inorganic ^{35}S may be extensively esterified and incorporated into mucopolysaccharides of cartilage and bone and other structural tissues.

(c) Nitrogen and Sulphur Retention

The ratio of N retention to S retention is determined by the amounts of nitrogen and sulphur deposited in body tissue. The N:S ratio of body tissue is about 15:1 (Garrigus, 1970) and it could be argued that the ratio of N retention to S retention should be of the same order as the ratio in body tissue. Such an argument appears valid for cattle, but not for sheep where the large requirement of sulphur for wool production is frequently ignored. Consequently, the overall N:S ratio for retention should be narrower for sheep than cattle, and related to the amounts used for wool and tissue production. Kennedy (1974) found the mean ratio of nitrogen to sulphur retained in cattle fed tropical roughage was 13.8:1, this was similar to the ratio of 10.9-13.6:1 in calves (Liebholz and Naylor, 1971) and to that of 13.5:1 for sheep (Bird, 1972), but was wider than the 10:1 ratio observed by Moir *et al.* (1967-1968), and 8.8:1 ratio reported by Coombe *et al.* (1971). Bird's (1972) ratio of nitrogen to sulphur stored by sheep tissues is wider than the ratios found by other workers (Moir *et al.*, 1967-1968; Coombe *et al.*, 1971). One explanation for this difference may be a lesser incorporation of sulphur into wool in Bird's experiment. Assuming (Bray and Till, 1975) N:S ratios for tissue and wool to be 15:1 and 5:1 respectively, it can be calculated that for an overall N:S retention of 13.5:1 (e.g. Bird, 1972), 15% of the S was retained in wool and 85% in tissue.

Generally, diets supplying more sulphur tend to result in higher retention of both nitrogen and sulphur, and oral supplements of sulphur given to sheep receiving diets low in sulphur also result in increased percentages of nitrogen and sulphur retained (Bray and Hemsley, 1969; Hume and Bird, 1970). Large doses of sulphur, as DL-methionine (Doyle and Bird, 1975) or Na_2SO_4 (Bird, 1971), may tend to reduce nitrogen retention below that obtained at optimal levels of sulphur intake. Post-ruminal infusion of sulphur supplements result in more sulphur retained than with ruminal infusions (Bird, 1971; Bird and Moir, 1972).

Under conditions of dietary sulphur deficiency, where sulphur limits ruminal microbial utilisation of dietary or recycled nitrogen and there is insufficient intestinal absorption of sulphur amino acids, the appropriate sulphur supplement, in terms of form of the sulphur and route of administration, can improve nitrogen and sulphur retention.

Published results show very large variations in the ratio of N:S retained, and for some studies, particularly those with cattle, the reliability of excreta collection and/or analytical techniques appears questionable. Recently, Bray and Till (1975) used most of the published data for sheep to find a positive linear relationship ($N=10.37S - 0.038$, $r=+0.952$) between N-retention (g/day) and S-retention (g/day) in sheep (Figure 29), which shows the close relationship between nitrogen and sulphur metabolism. The individual groups of data used in obtaining the equation had intercepts ranging from 0.45 to 0.39 and slopes from 8 to 12. Bray and Till (1975) assumed this linear equation also applied to N and S excretion (see Figure 29). However, only limited data (Bray and Hemsley, 1969; Coombe *et al.*, 1971; Bird, 1972) were available for the negative (excretion) region of the curve and it is evident that more negative balance trial studies are required to ascertain the accuracy of one equation describing both retention and excretion of N and S.

Mucha (1980) found in sheep that the N:S ratios were different betwen negative and positive balance and that this could be explained by the relative deposition of N and S in tissue and wool in the two states. In positive balance N and S were being deposited in tissue and wool whereas in negative balance N and S were released from catabolised tissue (N:S = 15:1) but N and S were still being deposited in wool even though at a less-

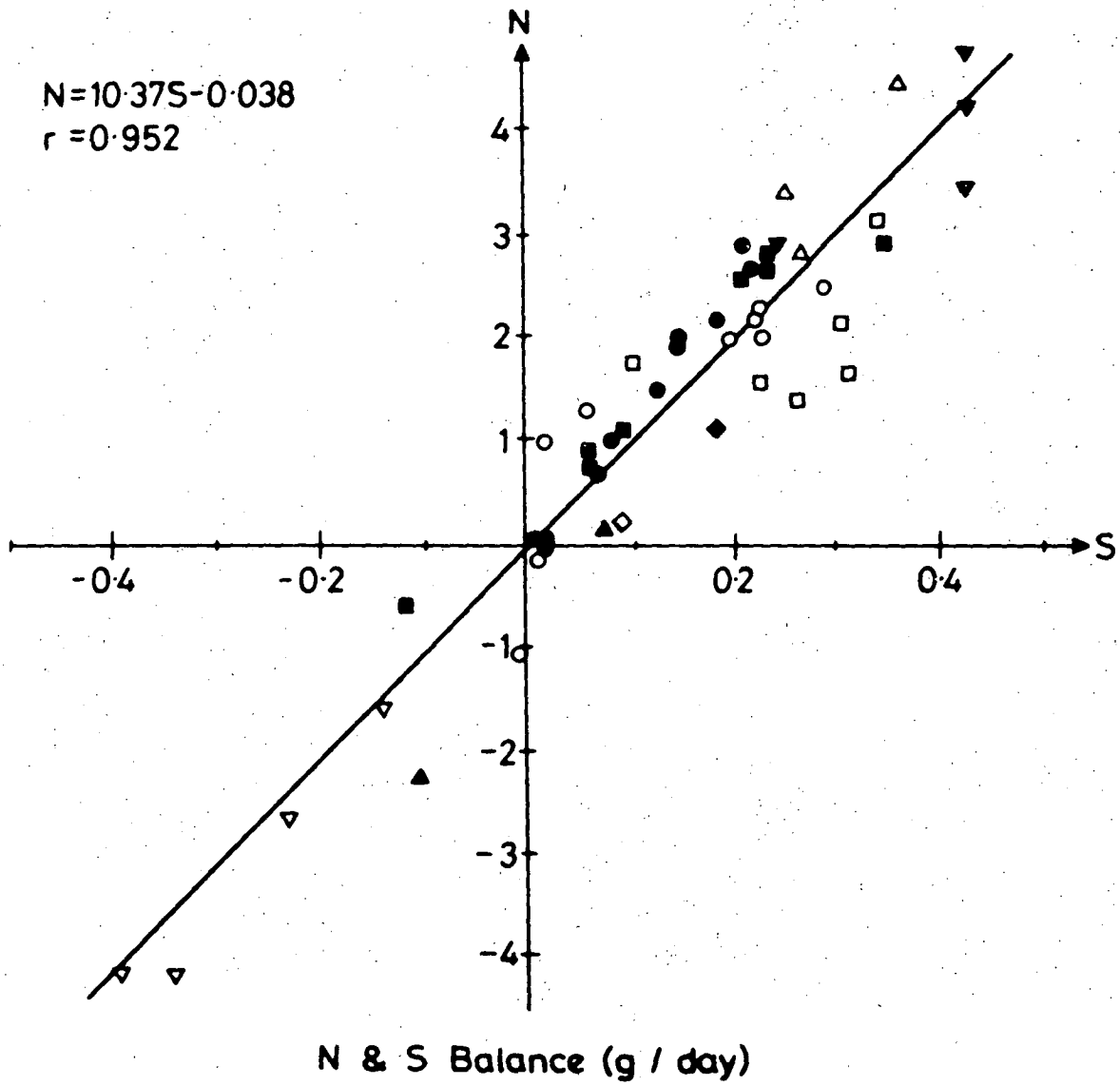


FIGURE 29. The relationship between N retention (g/day) and S retention (g/day) in sheep (Bray and Till, 1975)

er rate. This led to a preferential retention of sulphur due to the much narrower N:S ratio of wool, so that the N:S excretion ratio became much wider than that previously suggested by Bray and Till (1975).

SECTION 8: THE PROPOSED MODEL OF SULPHUR METABOLISM IN THE RUMEN OF SHEEP

A simple model-steady state system-of sulphur metabolism in the rumen of sheep was proposed and is shown in Figure 30. Sheep are fed their rations at one hourly intervals with inorganic sulphate as the sole source of sulphur. A constant rumen sulphide concentration is maintained under these conditions and pH and fermentation rate are constant. In this situation one can then make various estimates of exchange rates between sulphur pools in the rumen and between the rumen and tissues.

(a) Model Construction

The steps involved in construction of the model were:

(1) identification of the essential elements-various sulphur pools - that should be represented in the model;

(2) determination of pool sizes and fluxes (amount of material flow per unit time);

(3) calculations of rate constants and factors influencing flux;

(4) representation of the above as rate equations of the form :
flux = (rate constant) (pool size),
change in pool size = sum of fluxes; and

(5) validation of the model by comparison with experimental data, modification of the model as required for improvement of behaviour.

(b) Structuring the System

A compartment model of sulphur metabolism in the rumen of sheep is presented in Figure 31. It is viewed abstractly as a series of compartments with material flow between compartments as indicated by arrows.

The system is considered to be in a dynamic equilibrium such that the input per unit time into each compartment equals the loss from it. Microorganisms in rumen digesta are capable of producing relatively large quantities of sulphide by the reduction of inorganic sulphate. Some of this sulphide passes from the digesta across the rumen wall, a small amount flows down the gastrointestinal tract to the abomasum, and the remainder is used for *de novo* synthesis of S amino acids incorporated

FIGURE 30. A proposed simple model of sulphur metabolism in the rumen of sheep.

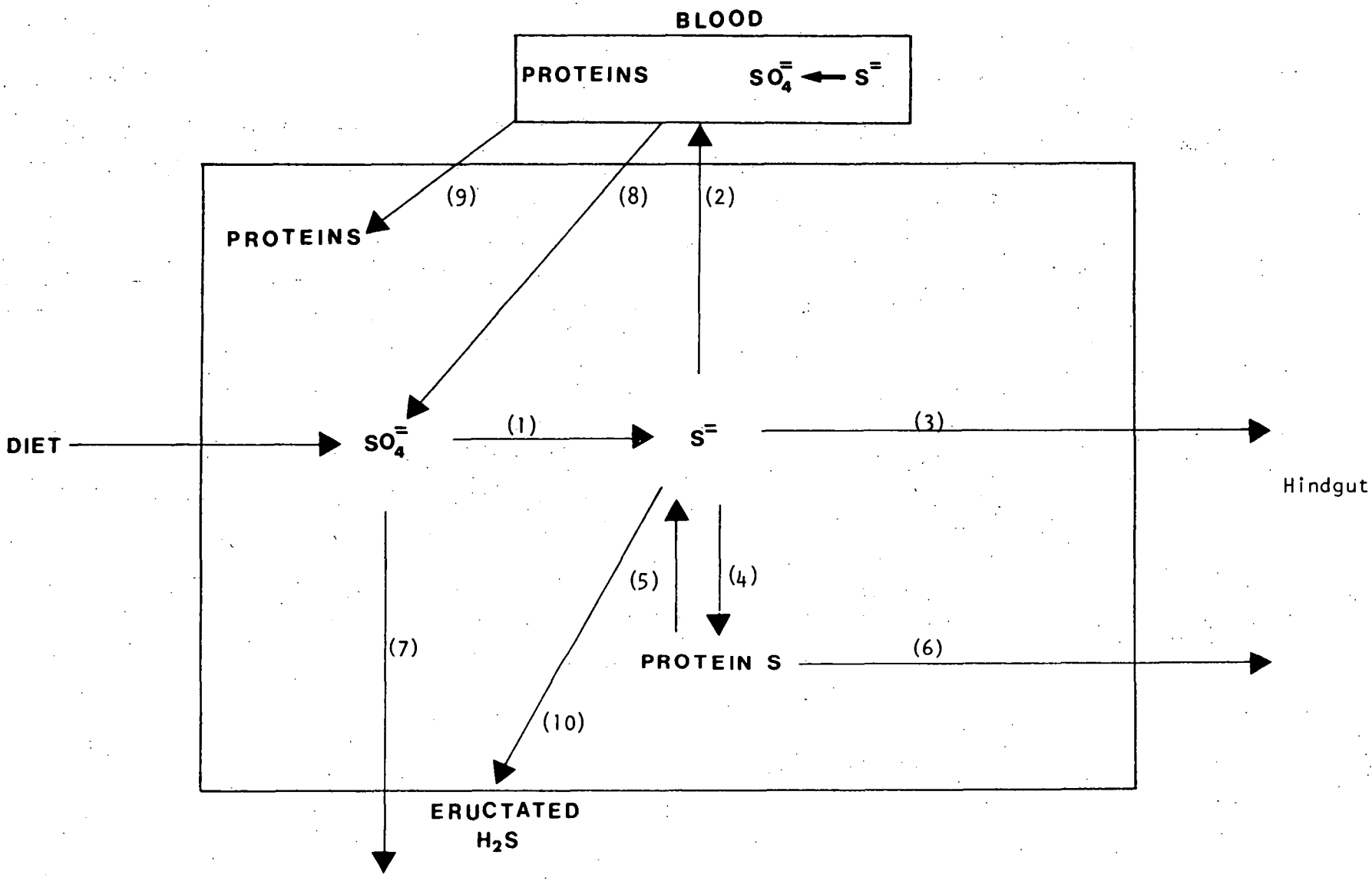
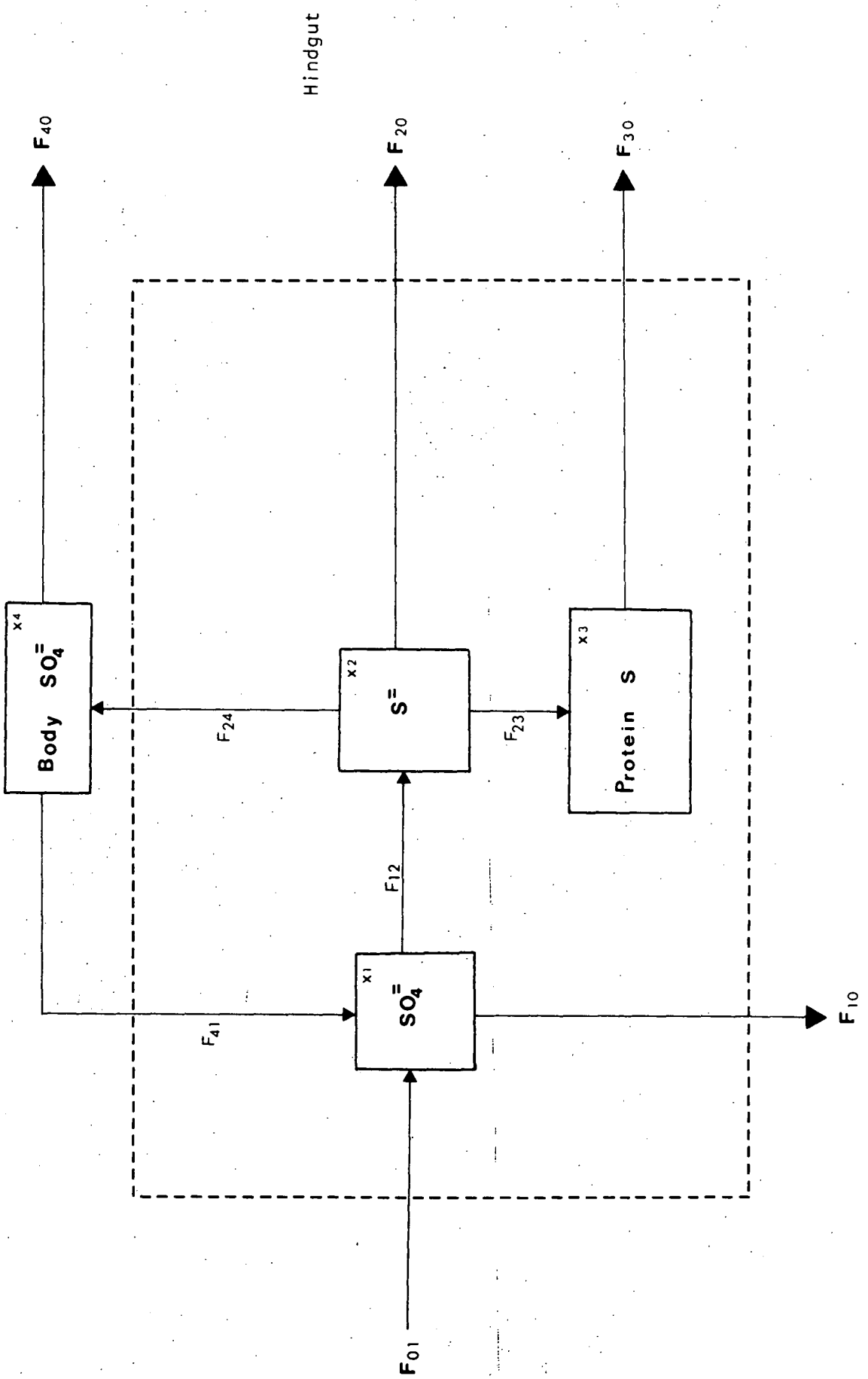

BLOOD
PROTEINS
$SO_4^=$ ← $S^=$
PROTEINS
(9)
(8)
(2)
DIET
$SO_4^=$
(1)
$S^=$
(3)
Hindgut
(5)
(4)
(7)
PROTEIN S
(6)
(10)
ERUCTATED
H_2S

FIGURE 31. Block diagram of the simple model of sulphur metabolism in the rumen of sheep. Rectangular boxes = pools (state variables); solid lines = flow of material; dotted line represents the rumen system.


Hindgut
F40
F20
F30
x4
Body SO4=
x2
S=
x3
Protein S
F24
F23
F12
x1
SO4=
F41
F10
F01

into microbial protein.

Step (10) of Figure 30 was omitted since we found that the losses of volatile sulphur from the rumen of sheep were less than 0.5% of the dose following intraruminal administration of ^{35}S-sodium sulphate. Steps (4) and (5) were taken as the net flow rate. Body SO_4^{2-} was treated as a compartment and therefore an excretion rate was added to that compartment.

(c) General System Equations

This model can be expressed mathematically by its system equations, differential equations describing change with time in the values of the compartments. The rate of change in each compartment, x_j, was expressed by a differential equation. By applying the principle of conservation of mass, the rate of change in the compartment was expressed as the sum of the input rates minus the sum of the output rates:

$$\frac{dx_j}{dt} = \sum_j F_{ji} - \sum_i F_{ij} \qquad (18)$$

where

dx_j/dt is the instantaneous rate of change in the jth compartment, F_{ji} signifies the rate of transfer of material from the ith to the jth compartment. Each of the flux rates, F_{ji}, was expressed as a constant fraction, ϕ_{ji}, of the material in a "donor" compartment, x_i, which was transferred to the "recipient" compartment, x_j, over a unit of time:

$$F_{ji} = \phi_{ji} x_i \qquad (19)$$

We use the convention that flow into a compartment is positive and flow out of a compartment is negative. Thus, referring to Figure 31, a suitable system of general equations is:

$$\begin{aligned} dx_1/dt &= F_{01} + F_{41} - F_{12} - F_{10} \\ dx_2/dt &= F_{12} - F_{24} - F_{23} - F_{20} \\ dx_3/dt &= F_{23} - F_{30} \\ dx_4/dt &= F_{24} - F_{41} - F_{40} \end{aligned}$$

where x_1 = inorganic sulphate-S, x_2 = sulphide-S, x_3 = microbial protein-S and x_4 = body sulphate-S compartment.

All feeding fluxes indicated in the block diagram of Figure 31 were consid-

ered to be linear, i.e. the assumed form of these fluxes was a constant fraction of the donor compartment in each case. This pattern was followed for each model.

Dietary S input to the rumen is represented by the system forcing function and is a constant input of the form $F_{01} = k$. Several different types of forcings were employed on each model to represent S outputs from the rumen and to observe their effects on system behaviour.

The second type of forcings used were of the form $F_{10} = [x_1]$ x flow rate, where $[x_1]$ = the concentration of compartment 1.

In the linear model daily flows were measured and take the mathematical form of equation (19). The values of x_i and x_j also are known (pool sizes), and thus the rate constant component of each flow can be determined from the relation

$$\phi_{ji} = \frac{F_{ji}}{x_i}$$

Thus, referring to Figure 31, the set of differential equations describing the linear system is:

$$\begin{aligned} dx_1/dt &= F_{01} + \phi_{41}x_4 - \phi_{12}x_1 - F_{10} \\ dx_2/dt &= \phi_{12}x_1 - \phi_{24}x_2 - \phi_{23}x_2 - F_{20} \\ dx_3/dt &= \phi_{23}x_2 - F_{30} \\ dx_4/dt &= \phi_{24}x_2 - \phi_{41}x_4 - F_{40} \end{aligned}$$

The system behaviour is obtained by solving the equations simultaneously. We have described here the development of a simple model of S metabolism in the rumen of sheep. It originates from currently accepted and defensible concepts and the numerical values used are based upon experimental data.

(d) Evaluation of the Model

Simulation models may be validated against experimental data (e.g. Arnold and Campbell, 1972; Vickery and Hedges, 1972). This involves an assessment of the adequacy of the model to mimic the behaviour of the simuland, i.e. the system being modelled (Anderson, 1974). A model is an approximation of reality (White, 1975). The design and level of detail incorporated in the model must be evaluated relative to the purpose for which it was constructed, given existing knowledge about the system being modelled.

Attention should be focused primarily on the location of variables within the model, on the relationships between these variables, and on whether major variables have been omitted. In general, in validation we should seek consistency with experience - the model should produce results consistent with one's previous knowledge that led to making specific observations. The behaviour of the model was examined by comparison of results predicted by the model with data from the literature and found to be consistent with published information. Also, the composition of sulphur in rumen fluid was examined; all the sulphur in rumen fluid was accounted for, thus indicating that all important pathways were considered.

PART II

GENERAL MATERIALS AND METHODS

Section 1: EXPERIMENTAL DIETS

A. Composition

The composition of experimental diets will be presented in the corresponding descriptions of each experiment. The diet, composed mainly of oat hulls, was a low protein diet with urea and inorganic sulphate providing the major sources of nitrogen and sulphur respectively (up to 85% of the sulphur intake was in the form of inorganic sulphate-S).

B. Diet Preparation

The diet was fed in the form of pellets. Urea and minerals were sprayed onto hammermilled (6mm screen) oat hulls, and thoroughly mixed in a rotary mixer. The mixture was dried, and then pelleted in a Lister 1500 pelleter fitted with a 16mm die.

C. Feed Presentation

In the volatile sulphur loss experiment, the diet was offered once daily at 0900 hours, supplying 800 g pellets per day. In the low sulphur, high sulphur, 15% starch and 30% starch intake experiments, the sheep were given 800 g pellets per day in approximately equal amounts at hourly intervals. An automatic feeder (Nicol and Corbett, 1971) fixed above the metabolism crates was used to deliver the previously weighed feed into bins in front of the sheep. The machine was refilled at 0900 hours every day. Tap water was available to the sheep *ad libitum* in stainless steel troughs; the daily intake was measured during the collection periods of the low sulphur, high sulphur, 15% starch and 30% starch intake experiments.

Section 2: QUANTITATIVE MEASUREMENTS

A. Live-weight

The wethers were weighed at the beginning of each experiment prior to feeding. The scales of the weighing crate (Gascoigne) were calibrated at each weighing against a standard weight.

B. Measurement of Extracellular Fluid Volume (Radiosulphate Space)

The extracellular fluid volume was estimated by dilution of intravenously

injected ^{35}S-Na_2SO_4. The volume of distribution was calculated as:

$$\frac{\text{Radioactivity (cpm) injected}}{\text{Radioactivity (cpm/mℓ) of plasma at zero time}}$$

where cpm = counts per minute.

The count-rate (cpm) of the two samples taken 15 and 30 minutes after the ^{35}S-Na_2SO_4 injection was plotted against time and the line extrapolated to zero time in order to obtain an estimate of the count-rate at the time of injection (Bray, 1969c).

C. Feeds, Faeces and Urine

(a) Feeds

Any residues from the previous 24 hours were collected just prior to the 0900 hour feeding. Dry matter determinations of feed and feed residues were made by drying subsamples for 24 hours in a hot-air oven at 100°C and dry matter intake was calculated. For each animal, an air dried subsample was bulked for each week and ground through a 1mm screen and a subsample was stored in an air-tight, pre-washed glass container for later analysis.

(b) Faeces

Faeces were collected quantitatively in a canvas faeces bag attached by a harness to the sheep. Faeces were removed from the bags each day prior to feeding. After weighing, the dry matter content was determined on a 10% aliquot by drying for 24 hours in an oven at 100°C, and dried samples from each sheep were later bulked separately for analysis. A further 10% aliquot from each animal's daily output was stored individually in Polythene beakers at -20°C for daily isotope analysis. At the end of each collection period all dried samples were ground through a 1mm screen, and a subsample stored in an air-tight Polythene beaker for subsequent analyses. For isotope determination, the samples of faeces were mixed with water, 1 part moist faeces:5 parts water and 5 g portions dispensed into the counting tubes. Cab-O-Sil, a thixotropic gel powder was added to facilitate the formation of a stable uniform suspension.

(c) Urine

Urine from each animal was collected in a 4ℓ Polythene beaker containing 10 mℓs of concentrated HCl (5N) for total sulphur determination and to enable any correction to be made for absorption of ^{51}Cr-EDTA and ^{103}Ru-phen.

The volume of urine was measured in a measuring cylinder and made up to 2ℓ with tap water. This was thoroughly mixed and 50 mℓs of diluted urine was taken and frozen for isotope analysis. Another 50 mℓs were used for total-sulphur analysis. If the volume of urine exceeded 2ℓ, the excess was measured, diluted to 2ℓ, and treated as before.

Section 3: MANAGEMENT AND PREPARATION OF SHEEP

Merino x Polwarth wethers aged about three years were used in all experiments. Sheep were selected from a flock on the basis of uniformity of weight, condition and conformation. All animals were fitted with permanent ruminal fistulae (Jarrett, 1948). They were kept permanently in galvanised iron metabolism crates in an animal house. For about two months they were allowed to acclimatise to the animal house conditions and human handling, and during this adaptation period sheep were weighed weekly to ensure that the diet was adequate for maintenance. To eliminate diurnal variations in light intensity the sheep were kept in a room (5.5 m x 5.0 m) continually lit by four 40W fluorescent tubes. Temperature was maintained at 20°C. No attempt was made to eliminate variations in the relative humidity. An intramuscular injection of vitamins A, D and E was given to each sheep during the adaptation period.

Section 4: COLLECTION AND TREATMENT OF RUMEN FLUID SAMPLES

Samples of rumen fluid were taken at 2 hourly intervals during the sampling period. Rumen fluid was sampled from a Polyethelene tube 8 mm in diameter that passed through the closed-rumen cannula into the mid-ventral sac of the rumen. The fluid passed through nylon square mesh of aperture 1 mm in diameter and was then drawn through the tube with a plastic syringe. The Polyethelene tube was fitted externally with a stopcock. Two 10 mℓ samples were withdrawn with syringes to flush the system which was then resealed with the stopcock. A third sample was then immediately drawn into a 50 mℓ syringe which was analysed immediately for sulphide-sulphur. The pH was also measured immediately after the sampling. The first two fluid samples were returned to the rumen as soon as the third sample had been taken. All samples were strained through surgical gauze (muslin) and the remaining samples were stored at -20°C for subsequent analyses.

Section 5: RUMINAL FLUID VOLUMES AND RATES OF FLOW OF DIGESTA

^{103}Ru-Tris (1, 10-Phenanthroline) Ruthenium (II) Chloride (^{103}Ru-phen) and ^{51}Cr-Ethylenediamine tetraacetic acid (^{51}Cr-EDTA)

^{103}Ru-phen and ^{51}Cr-EDTA (obtained from The Radiochemical Centre Pty. Ltd., Surry Hills, New South Wales) markers were used in the low sulphur intake experiment and ^{51}Cr-EDTA was used in high sulphur, 15% starch and 30% starch intake experiments. ^{103}Ru-phen was prepared from $^{103}RuCl_3$ by the method of Tan *et al.* (1971). ^{51}Cr-EDTA solution was used to mark the water phase of digesta and ^{103}Ru-phen solution to mark the particulate matter of digesta as this isotope is readily and strongly absorbed on to food particles.

In the low sulphur intake experiment, a priming dose (520 mℓ) of ^{51}Cr-EDTA (100 µCi) and ^{103}Ru-phen (20 µCi) was injected into the rumen, followed by a continuous infusion (520 mℓ/day containing 100 µCi ^{51}Cr-EDTA and 20 µCi ^{103}Ru-phen) for three days. Rumen fluid samples were centrifuged at 2500 g (4000 R.P.M.) for 30 minutes and 3 mℓ portions of the supernatant fraction were counted. The sediment from each of the centrifuged rumen samples was weighed and then counted. The samples were assayed for ^{51}Cr and ^{103}Ru simultaneously in duplicate vials using an Auto-Gamma spectometer (Packard Instrument Co., model 5215). Corrections were made for the contribution of ^{103}Ru to the counts recorded for ^{51}Cr. There was no contribution from ^{51}Cr to the ^{103}Ru counts recorded. The counts were used to reconstitute "true digesta" by the mathematical method of Faichney (1975).

In the high sulphur, 15% starch and 30% starch intake experiments ^{51}Cr-EDTA was used to estimate ruminal fluid volumes and rates of flow of fluid out of the rumen by the method of Downes and McDonald (1964).

Section 6: ANALYTICAL METHODS

(i) Feeds, Faeces and Urine

(a) Dry Matter

Dry matter determinations of feed and faeces were made daily by drying for 24 hours in an oven at 100°C during the experimental period.

(b) Ash

Samples were ignited in an electric furnace at 550°C for five hours.

(c) Organic Matter

The organic matter content of feed and faeces was estimated as the difference between dry matter and ash.

(d) Total Nitrogen

Total nitrogen concentration was determined in feed by a modified method of the standard Kjeldahl method (Fleck and Munro 1965; Official Methods of Analysis of A.O.A.C., 1970). Triplicate samples of feed were analysed by a semi-micro-Kjeldahl technique.

(e) Sulphur

The concentration of total sulphur was estimated in the feed, faeces and urine. Samples were prepared for total sulphur analysis by oxidation as described by Bird and Fountain (1970). The oxidised samples were then analysed for total sulphur by Johnson-Nishita's method (1952) as modified by Dean (1966). The total sulphate sulphur and ester sulphate sulphur in feed were determined using the preparation methods of Bird and Fountain (1970). Inorganic sulphate sulphur and neutral sulphur values were obtained by difference.

(ii) Rumen Fluid

(a) pH

The pH of digesta samples was measured immediately after collection using a Phillips Model 290 pH meter.

(b) Protein Nitrogen

Protein nitrogen in rumen fluid was precipitated with tungstic acid (Winter *et al.*, 1964). In this method 2 mℓ of 1.07 N HCl and 2 mℓ of 10% sodium tungstate were added to 10 mℓ of rumen fluid in a 20 mℓ centrifuge tube and mixed thoroughly. After standing 2 hours the tubes were centrifuged at 2000 x g for 20 minutes. Nitrogen in the sediments obtained by tungstic acid precipitations was determined by the Kjeldahl technique, using selenium as a catalyst. Sediment samples were digested with 3 mℓ concentrated sulphuric acid and 1.5 g catalyst mixture consisting of 100 parts potassium sulphate and 1 part powdered selenium.

(c) Sulphur

Rumen fluid sulphide was determined by the method of Bray (1969b) using the colour reaction of Dean (1966). Total sulphur, total sulphate sulphur and ester sulphate sulphur were determined using the preparation methods of Bird and Fountain (1970). However, the total sulphate sulphur was determined after extraction of the sulphur from rumen liquor with 2N HCl and the ester sulphate sulphur by precipitating the inorganic sulphate sulphur

with 40% barium chloride solution. The recovery of ^{35}S-sodium sulphate added to rumen liquor was 97.6% using this method. Determinations of sulphate sulphur in rumen fluid and dried rumen fluid showed no difference in the inorganic sulphate sulphur content of rumen fluid. Inorganic sulphate sulphur and neutral sulphur values were obtained by difference. The protein sulphur content of tungstic acid precipitates (see above (ii)(b)) was obtained by total sulphur determination of the wet precipitate using the preparation methods of Bird and Fountain (1970). Soluble organic sulphur was determined as total sulphur content of supernatant from tungstic acid precipitation (Bird and Hume, 1971) using the preparation methods of Bird and Fountain (1970). However, in the final step sulphide was determined by the colour reaction of Dean (1966).

(iii) Blood

Blood samples were collected in citrated evacuated tubes from the jugular vein at 2 hourly intervals during the sampling period. Plasma was obtained by centrifuging whole glood at 6000 R.P.M. for 10 minutes immediately after collection. Plasma inorganic sulphate sulphur concentration was determined as described by Bray (1969b), using the trapping solution and colour reaction of Dean (1966).

(iv) Radioactivity

Radioactivity was measured with a liquid scintillation counter (Packard Prias PL), using the Dimilume scintillant (inhibiting chemiluminescence). Duplicate samples were counted for 5 minutes and quenching was corrected by taking the external standard ratio and estimating the counting efficiency. The minimum amount of radioactivity to be used was calculated (from the nomogram of Davidon, 1953) so that a collection of 1% of ^{35}S administered would be significantly different from background at 5% standard error.

Section 7: STATISTICAL ANALYSIS

The paired t-test was carried out in the low sulphur, high sulphur, 15% starch and 30% starch intake experiments according to the methods of Steel and Torrie (1960). Coefficients of variation of individual measurements in the above experiments were also calculated by the methods of Steel and Torrie (1960).

PART III

THE LOSS OF VOLATILE SULPHUR FROM SHEEP

A. Introduction

In designing a model of rumen sulphur metabolism all pathways of sulphur loss and gain from the rumen must be considered. Little is known about the loss of sulphur as volatile sulphur compounds through eructation. Bray (1969a) observed the presence of ^{35}S-sulphur on a sheep's breath during rumen sulphide absorption experiments. However, there has been no research into the quantities of sulphur lost in this manner.

These experiments were initiated to quantify volatile sulphur loss through eructation. Dougherty *et al.* (1965) have shown that H_2S is eructated and, along with methane and other gases, passes into the lungs. Since sulphide diffuses rapidly, low concentrations of H_2S in the eructated gas probably do not result in much respiratory S^{2-} loss, most of the H_2S is probably absorbed into the blood from the lungs. Doyle (1977) suggests that it is unlikely that respiratory loss of sulphur is a major source of error in sulphur balance work.

B. Materials and Methods

(a) The Preparation of the Dose

A sodium ^{35}S-sulphide solution was prepared by reducing 0.55 g of Na_2SO_4 together with 2 mc of carrier free ^{35}S-Na_2SO_4 in the acid reducing mixture of Johnson and Nishita (1952). The released H_2S was trapped in 200 mℓ of N NaOH.

(b) The Estimation of Volatile Sulphur Loss

The method proposed was to enclose the sheep in a transparent gas tight box. Then air was drawn through the box and discharged into an alkiline absorbing solution thus any volatile sulphur compounds could be trapped. Rumen volatile sulphur compounds were labelled with ^{35}S by infusing ^{35}S-sodium sulphate into the rumen.

Preliminary experiments indicated that the volume of the box and the rate of air flow were the two critical factors affecting the recovery of ^{35}S-sodium sulphide released in the box. After these experiments the following technique was used:

(1) the box had a wooden frame covered with 0.3 cm perspex

with 1 x 1 x 1 m dimensions to give an internal volume of 1 m^3;

(2) a pump (model Kevatron 201, Labair, see Figure 33) was used to draw air through the box and discharge through six air diffusion bubblers (made of scintered glass, porosity 4) into 18 litres of normal sodium hydroxide;

(3) a small fan (Hitachi 15W, miniature) was placed in the box to circulate the air and thus avoid any dead space.

C. Recovery

The efficiency of collection of volatile sulphur was measured by releasing a known amount of ^{35}S-H_2S in the box and measuring the total radioactivity in the absorbing solution. This was done by placing 50 mℓ of 1N NaOH containing ^{35}S-H_2S in the box and then releasing the trapped gas by acidifying with 10 mℓ of 6N HCl (see Figure 33). The best recoveries were obtained using air flow rates of 15 litres/minute over a collection period of eight hours (see Table 7). Following this the effect of the quantity of sulphide released on the percentage recovery was studied. As it can be seen in Figure 34, the efficiency of recovery increased from 50% when 0.5 mg of sulphide was released to 80% when 20 mg of sulphide was released. Although these recoveries were far from ideal it was decided that a volatile sulphur loss of less than 1% of the dose that was infused into the rumen could be detected and if the losses observed were less than this then volatile sulphur loss would be negligible and could be safely ignored. However, if the losses appeared to be significant then a more efficient method would have to be devised.

D. Sheep Treatments

Four cross Merino x Polwarth wethers (about 35 kg body weight) fitted with permanent rumen fistulae were used in these experiments. The sheep were kept in metabolism crates for the duration of the experimental period and were fed on a diet containing approximately 0.4% sulphur and 2% nitrogen (dry matter basis). In all experiments distilled water was offered *ad libitum*.

The diet was mainly oat hulls, pollard and urea with sodium sulphate the main source of sulphur. The composition of the diet is shown in

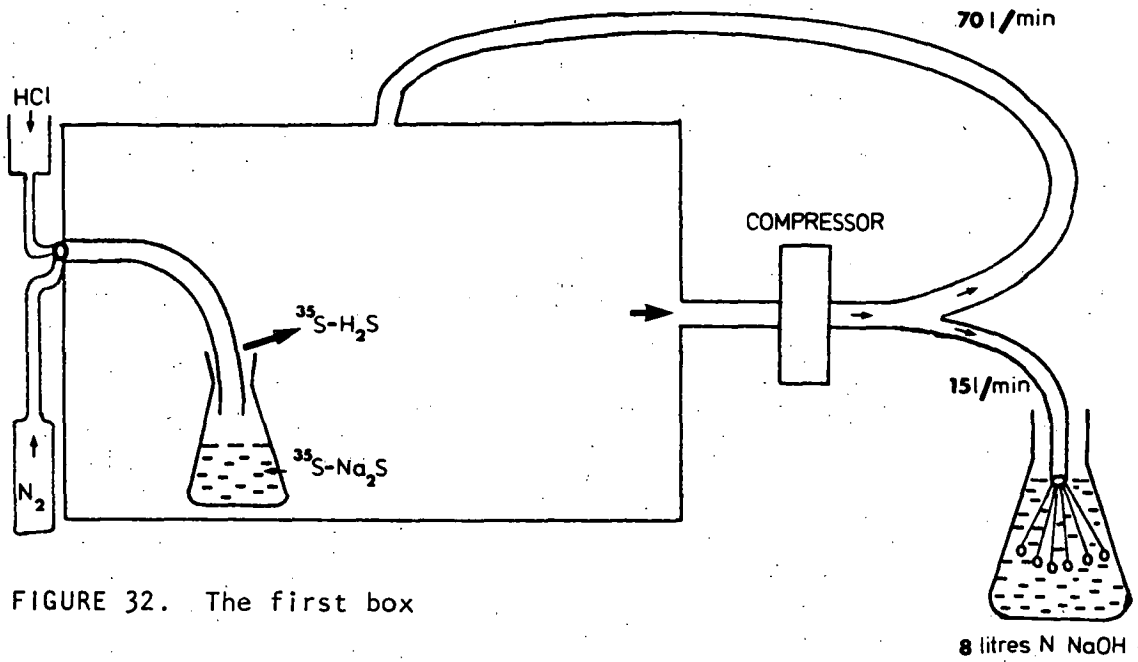


FIGURE 32. The first box

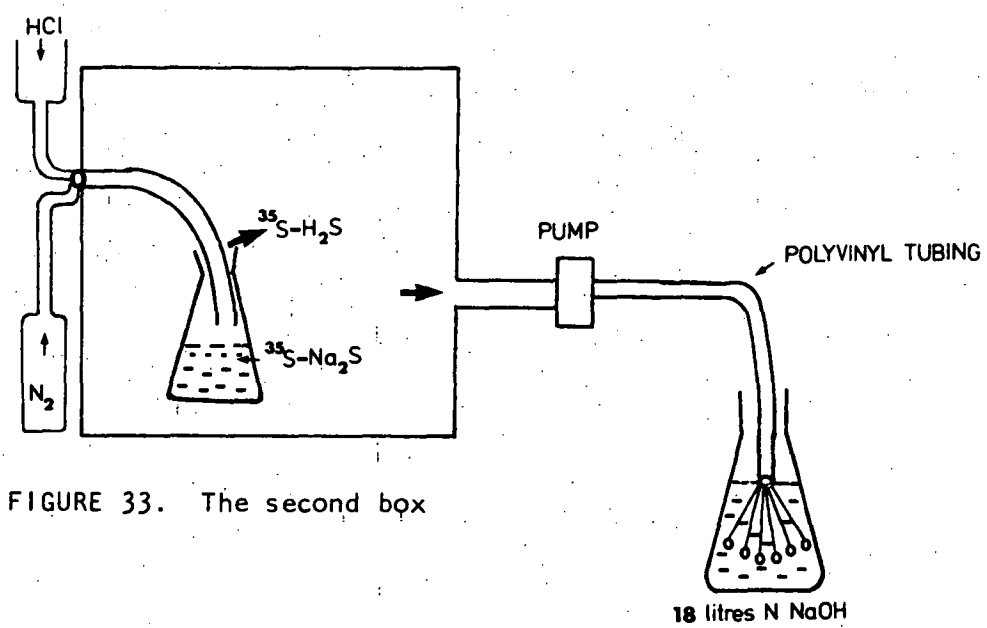


FIGURE 33. The second box

TABLE 7

The effect of gas flow rate and collection period on the percentage recovery. The amount of sulphide used was 2.2 mg S.

Efficiency of collection (% of Dose)					
Time (h) / Flow Rate (ℓ/min)	4	6	8	12	24
10	41	49	53	54	54
15	45	54	60	60	60
20	24	34	38	38	38
30	10	16	21	22	22

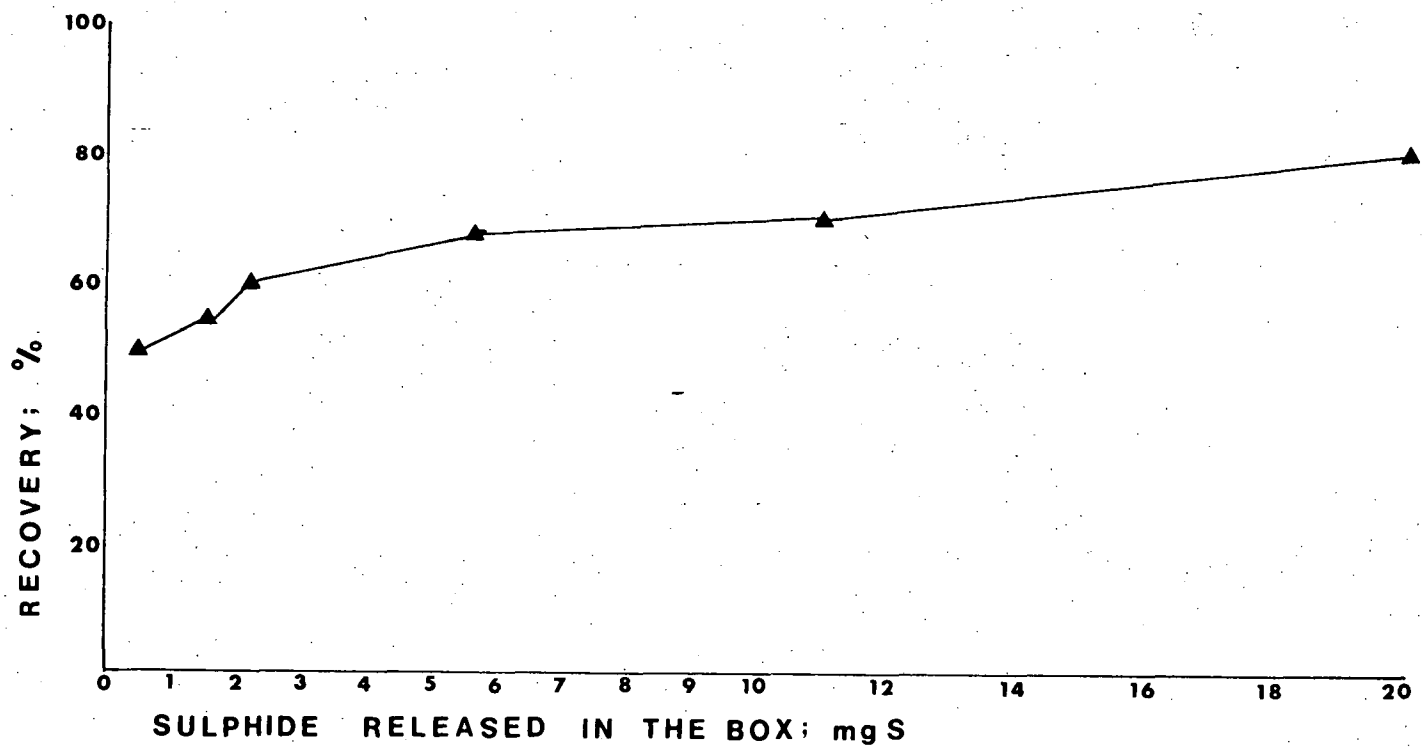


FIGURE 34. Effect of amount released S^{2-} and percentage recovery

Appendix 1. The ration was designed to provide excess levels of sulphur. At 0900 daily the sheep were offered 800 g of the ration in all experiments (see Part II, Section 1). However, some feed refusals occurred and feed residues were collected and measured in each experiment before the sheep were placed in the metabolism crate. This regime commenced some three weeks prior to the experimental period.

E. Experimental Programme and Treatments

Details of each experiment are presented in Table 8. Samples of rumen fluid and trapping solution were taken at various time intervals during the six to eight hours of the experimental period following the administration of ^{35}S-sulphate. A sample of rumen fluid was also taken just prior to feeding. A 30 mℓ quantity of rumen liquor was taken into a flask with 1 pellet of N NaOH each time in order to minimise any losses of sulphide by diffusion. Sulphide analyses were carried out within 5 to 10 minutes of sampling.

F. Results and Discussion

^{35}S was detected in the trapping solution of N NaOH in all experiments. ^{35}S was also detected in the rumen fluid. That sulphate is reduced to sulphide within the rumen is well established. The concentration of sulphide in the rumen liquor rose to a peak within three to six hours of feeding during the experiments 1, 2 and 3 and eight hours during the experiments 4, 5 and 6 (see Figure 35). The high sulphide levels on these treatments should be due to the reduction of dietary and infused sulphate. Radioactivity was demonstrated in the rumen sulphide fraction through the six to eight hours of the experimental period after the addition of sulphur-35-sulphate to the rumen (see Figure 36). It is probable that by this time the sulphur-35 remaining in the rumen had not been absorbed by micro-organisms. The mixing of radioactive S and stable S in the rumen S pool was considered to be homogeneous. In all the experiments the ^{35}S-Na_2SO_4 had been added to the rumen after feeding the normal ration. The distribution of sulphur-35 may have been different if the ^{35}S-Na_2SO_4 had been added at a prior stage.

The results from these experiments (see Table 9) indicate that volatile sulphur loss by eructation is negligible in the overall sulphur balance of the sheep. On this basis it was decided to ignore sulphur loss by eructation in the sheep model.

TABLE 8

Summary of experimental treatments

Expt.	Sheep	Site of infusion	Sulphur Dosage			Time of Dosing	Time of experimental period
			Form of Sulphur given	S^{35} in Dose ($\overline{mC}$)	Total sulphur (g) [S in ration + carrier]		
1	S_4	rumen	Na_2SO_4 carrier free	1.43	2.90 + 0 = 2.90	10.00 am, 1 hr after feeding	6 hr
2	S_3	rumen	Na_2SO_4 carrier free	1.49	2.83 + 0 = 2.83	10.00 am, 1 hr after feeding	6 hr
3	S_1	rumen	Na_2SO_4 + carrier	1.65	2.43 + 2.09 = 4.52	11.00 am, 2 hr after feeding	6 hr
4	S_2	rumen	Na_2SO_4 + carrier	1.60	1.34 + 2.09 = 3.43	11.00 am, 2 hr after feeding	6 hr
5	S_4	rumen	Na_2SO_4 + carrier	1.36	2.14 + 5.0 = 7.14	10.30 am, 1.5 hr after feeding	8 hr
6	S_3	rumen	Na_2SO_4 + carrier	1.44	2.36 + 5.0 = 7.36	10.30 am, 1.5 hr after feeding	8 hr

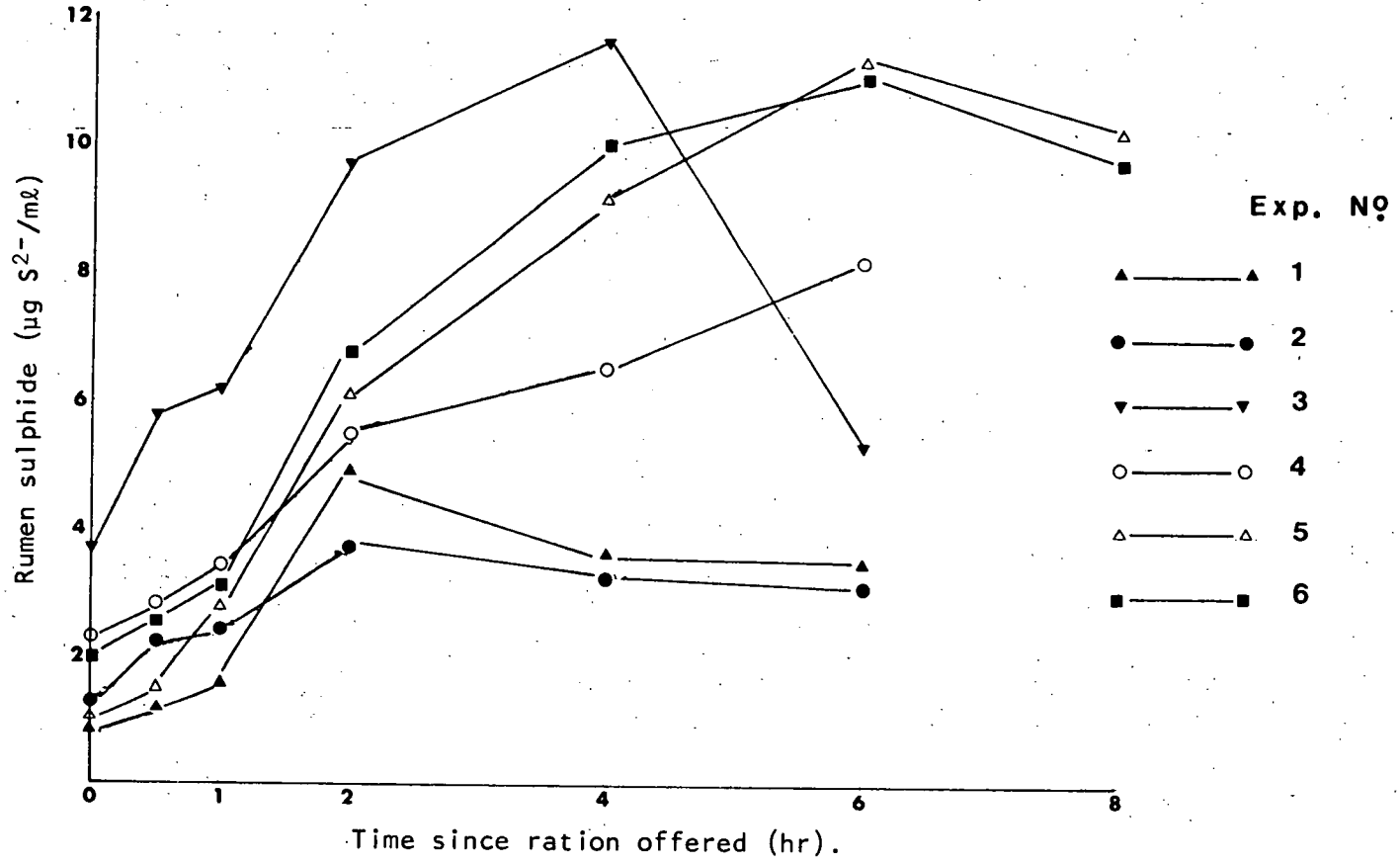


FIGURE 35. Rumen S^{2-} levels

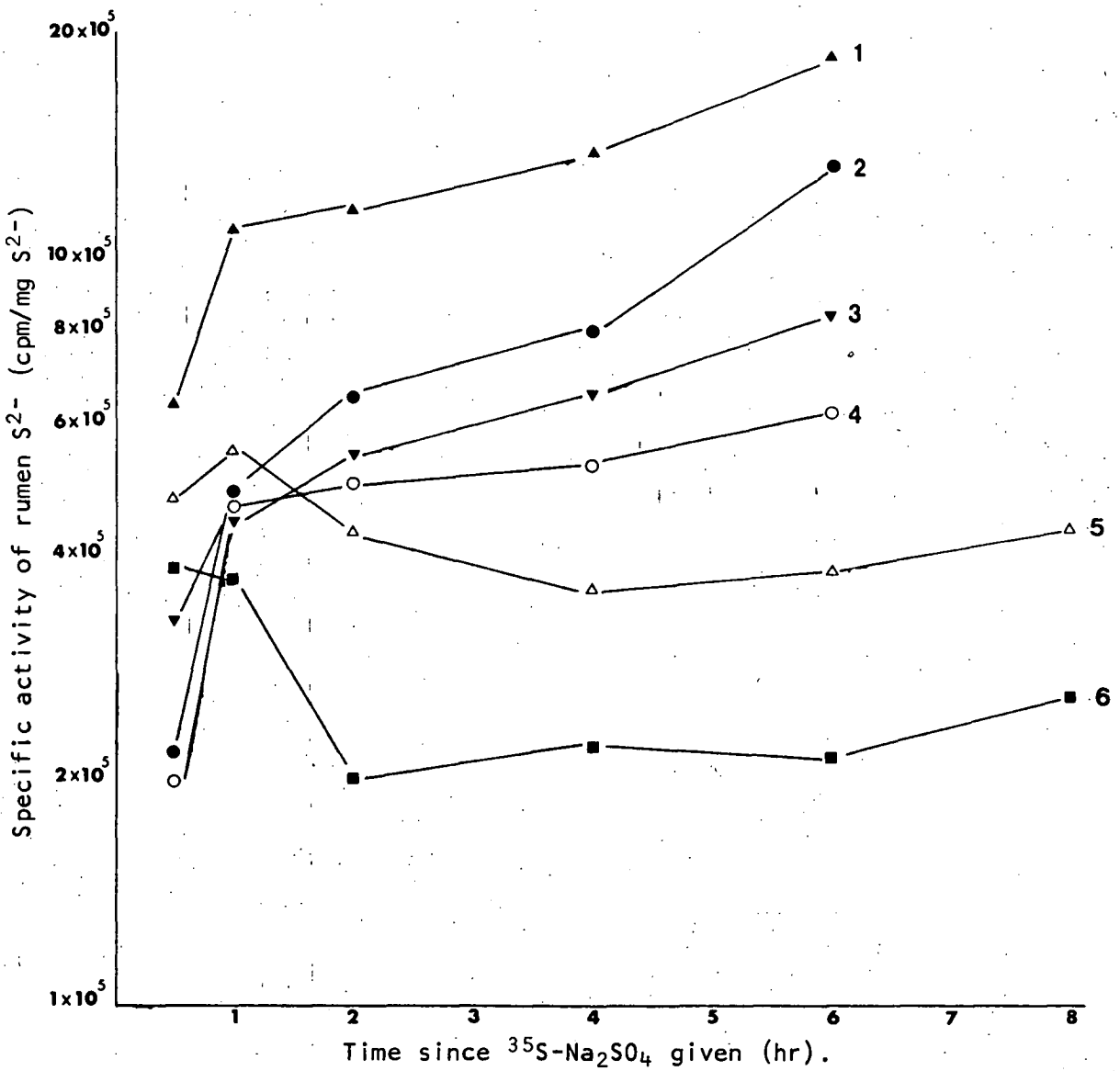


FIGURE 36. Specific activity of rumen sulphide (1, 2, 3, 4, 5 and 6 refer to experiment numbers)

TABLE 9

Recovery of ^{35}S from the rumen (% dose given)

Expt. / Time	1	2	3	4	5	6
T30 Min.	0	0	0.01-0.02	0.004-0.006	0.10-0.16	0
T1h	0	0	0	0	0.07-0.10	0.008-0.01
T2h	0	0	0	0.06 -0.09	0.07-0.10	0.06 -0.10
T4h	0.04-0.07	0	0.02-0.04	0.06 -0.09	0.10-0.15	0.10 -0.15
T6h	0	0	0.02-0.04	0.10 -0.14	0.15-0.24	0.15 -0.24
T8h	-	-	-	-	0.10-0.16	0.22 -0.35

PART IV

THE EFFECT OF LOW DIETARY SULPHUR INTAKE ON RUMEN SULPHUR DYNAMICS

(a) Introduction

The experiments reported in this part are the first of a series in which the proposed model is compared with observed sulphur dynamics. At this stage the important basis of the model demands steady state conditions so, data recorded will be concerned with sulphur dynamics and data which can be used to judge if steady state conditions have been achieved.

The quantities of sulphide and other sulphur-containing fractions passing out of the rumen in the digesta flow will be determined in part by the balance between assimilatory and dissimilatory processes in the rumen. Dissimilatory sulphate reduction (Peck, 1962) may produce hydrogen sulphide in excess of assimilatory requirements, and may therefore result in losses of sulphate by absorption of S^{2-} from the rumen. Such losses of sulphide are suggested to be greater at more acid pH values (Spais *et al.*, 1968; Bray, 1969a). Other factors such as the direct incorporation of sulphur amino acids into bacterial protein (Pittman and Bryant, 1964) and protozoal protein (Muller and Krampitz, 1955), and the supply of energy and nitrogen which may limit microbial growth at the time of maximal sulphide release may also be important.

Spais *et al.* (1968) demonstrated a positive relationship between the intake of inorganic sulphate and the concentrations of sulphide, sulphur amino acids, or protein sulphur in rumen fluid. However, there are few quantitative data on the extent of degradation of dietary sulphur compounds in the rumen, nor on the nature or low rate of sulphur-containing compounds from the rumen. The present study deals with various estimates of the exchange rates between sulphur pools in the rumen and the excretion of sulphur in the urine and faeces.

(b) Materials and Methods

(1) Animals and Diet

Three wethers fed a maintenance ration of 800 g pellets/day containing 10.3 g total nitrogen and 1.16 g total sulphur with a N/S ratio of 8.9/1. The diet, composed mainly of oat hulls, was a low protein diet with urea

and inorganic sulphate providing the major sources of nitrogen and sulphur respectively.

(2) Experimental Procedures

The experimental timetable and the time of rumen fluid collections is presented in Table 10.

(2.a) Estimation of Rumen Volumes and Flow Rates from the Rumen

Most of the criteria of an ideal marker substance have been enumerated by Faichney (1975). Kotb and Luckey (1972) suggested that before a substance qualifies as an effective nutritional marker it should: (1) be inert with no toxic physiological or psychological effects; (2) be neither absorbed nor metabolised within the gastrointestinal (GI) tract; (3) have no appreciable bulk; (4) mix intimately with and remain uniformly distributed in the digesta; (5) have no influence on GI secretion, digestion, absorption, or normal motility; (6) have no influence on the microflora of the GI tract; (7) have physico-chemical properties, readily discernible throughout the GI tract, which allow ready, precise, quantitative measurement. However McRae (1974); Engelhardt (1974) state that none of the available markers satisfy all these criteria and it is left to the judgement of an experimenter to select the material which most nearly meets his requirements. Consequently selection of a marker must be made with due consideration given to the errors that might arise.

Faichney (1975) suggests that digesta may be considered to consist of two phases, a liquid phase and a particulate or solids phase. When sampling through a cannula, it is difficult to obtain samples containing not only particulate matter, but also dissolved substances in the same proportions as are present in the digesta flowing past the cannula (Hogan, 1964a; Hogan and Weston, 1967a). Similarly, any single marker may not be present in a sample in the same concentration as in the digesta flowing past the cannula. Hogan and Weston (1967a) suggest that this problem can be overcome by the use of two markers, one of which remains in solution while the other is intimately associated with particulate matter. However, the flow of each phase can be accurately estimated only if the markers associate exclusively with and are distributed uniformly throughout one or other phase. ^{103}Ru-phen is not distributed uniformly throughout or associated exclusively with the particulate phase (see Tan *et al.*, 1971). Therefore

TABLE 10

Timetable of the experimental period of the low sulphur intake experiment with details of marker infusions and digesta collections

Days 1 - 14	:	Experimental prefeeding period
Day 15	:	Priming dose of ^{103}Ru and ^{51}Cr was injected into the rumen and continuous infusion of ^{103}Ru and ^{51}Cr commenced at 0900h. Digestibility trial commenced at 0930h.
Day 16	:	Rumen fluid samples collected at 0900, 1100, 1300, 1500 and 1700h.
Day 17	:	Rumen fluid samples collected at 0900, 1100, 1300, 1500 and 1700h.
Day 18	:	Rumen fluid samples collected at 0900, 1300 and 1700h.
Day 19	:	Infusions of ^{103}Ru and ^{51}Cr terminated at 0900h. Rumen fluid samples collected at 0900, 1000, 1100, 1300, 1500 and 1700h.
Day 22	:	Digestibility trial ended at 0930h.
Day 24	:	Collection period of faeces and urine for markers recovery ended.

this marker cannot be used to estimate particulate flows or particulate volume. This difficulty of separating the flow of the two phases of digesta can be avoided if the true composition of digesta passing the sampling point is determined from the marker concentrations (Faichney, 1975), a method that does not require that each marker associate exclusively with one phase.

The rumen volumes and flow rates of digesta from the rumen were estimated by reference to the two markers ^{51}Cr-EDTA and ^{103}Ru-phen or the ^{51}Cr-EDTA alone. Samples taken from the rumen every four hours during the continuous infusion experimental period were used to determine the concentrations of the two markers in the rumen.

In the present experiment the reconstitution of true digesta was carried out by the method of Faichney (1975), using the following formulae. If x is a quantity of digesta (D), y is a quantity of fluid (F) which when added to or removed from x reconstitutes true digesta (TD), S_D, S_F and S_{TD} are concentrations of the solute marker, and P_D, P_F and P_{TD} are concentrations of the particulate marker, then

$$x\,S_D + y\,S_F = x\,P_D + y\,P_F\ ,$$

so that

$$\frac{y}{x} = \frac{P_D - S_D}{S_F - P_F} = R$$

where R is the reconstitution factor, i.e. the number of units of fluid that must be added to (or removed from) one unit of digesta to obtain true digesta. Then

$$\frac{S_D + R\,S_F}{1 + R} = S_{TD} = \frac{P_D + R\,P_F}{1 + R} = P_{TD}$$

and the flow of true digesta (TD) is given by

$$\text{flow of TD} = 1/S_{TD} = 1/P_{TD}.$$

A mathematical study of the movement of particles and solutes by Warner (1966) and a digesta flow study by Weston and Hogan (1967a) indicate that in a "steady state" system

$$F = \frac{0.693\ V}{T}\ ,$$

where F is the rate of fluid flow from the rumen, V is the volume of liquid in the rumen, and T is the time for the equivalent of half of the liquid in the rumen to be transferred to the omasum. When a water-soluble marker (^{51}Cr-EDTA) is infused continuously into the rumen it may be shown that

$$F = \frac{I}{C}$$

and

$$R = \frac{V}{F} = 1.44\ T$$

where I is the rate of infusion of marker into the rumen, C is the concentration of marker in the liquid leaving the rumen, and R is the mean retention time of a population of marker molecules in the rumen. The differences in rumen volumes and flow rates from the rumen calculated by using the combination of the two markers ^{51}Cr-EDTA and ^{103}Ru-phen or the water-soluble marker ^{51}Cr-EDTA alone were not significant.

(2.b) Estimation of Ruminal Fluid Sulphur Pools and Flow of Sulphur in Fluid to the Omasum

In the present work concentrations and volumes are based only on strained ruminal fluid. Samples taken from the rumen every two hours were used for pH measurement, sulphide-S, inorganic sulphate-S and total-S analysis; samples taken every four hours were used to determine the sulphur in microbial protein.

The pool sizes of sulphide-S, sulphate-S, microbial protein-S and total-S in the rumen were calculated by multiplying the rumen volumes by the respective concentrations in rumen fluid.

The body sulphate pool was estimated assuming an extracellular fluid volume of 25% of body weight and a plasma inorganic sulphate concentration of about 30 µg S/mℓ. Then the body sulphate pool was calculated by multiplying the extracellular fluid volume by the plasma inorganic sulphate concentration.

The flow of sulphide-S, inorganic sulphate-S and total-S from the rumen was calculated by multiplying the fluid flow rates by the respective concentrations in rumen fluid.

For microbial protein, when a protein-free diet is given under equilibrium

conditions the amount of protein flowing from the rumen daily may be equated with the daily net production of microbial protein in the rumen, because microbial protein is not absorbed from the rumen. The small amount of protein entering the rumen in the saliva (McDonald, 1948; Somers, 1961a) and by desquamation of the rumen epithelium (Phillipson, 1964) will introduce little error into this assumption. The amount of protein flowing from the rumen daily is then the product of the rate of flow of digesta and the concentration of protein in this digesta.

(2.c) Estimation of the Absorption Flow Rate and Endogenous Sulphur Recycling to the Rumen

Bray (1969a) added ^{35}S-Na_2S to a rumen in which the contents had been replaced with a buffer solution and showed that sulphide absorption was concentration-dependent and extremely rapid (half-lives of 10 to 15 minutes). Bray and Till (1975) suggest that at each value of pH studied, the concentration was related to time by the equation

$$\ln C_t = \ln Co + K_R\, t$$

where Co and C_t are the initial and resultant concentrations of sulphide, t is the elapsed time, and K_R is the absorption rate constant at a specific pH.

In solution the dissociation of hydrogen sulphide can be represented by

$$H_2S \underset{}{\overset{K_1}{\rightleftharpoons}} HS^- + H^+, \text{ and } HS^- \overset{K_2}{\rightleftharpoons} H^+ + S^{2-}$$

where K_1 and K_2 are the primary and secondary dissociation constants respectively. Except under very alkaline conditions (pH > 9) the sulphide ion concentration will be insignificant (< 0.1% of total sulphide). Consequently, for the rumen only the primary dissociation need be considered ($K_1 = 1.8 \times 10^{-7}$ at 37^oC) and the region of rapid transition between HS^- and H_2S lies mainly within the normal rumen pH range. Bray and Till (1975) suggested that the overall absorption rate constant K_R is a function of the absorption rate constants for hydrogen sulphide (K_{H_2S}) and for sulphydryl ion (K_{HS^-}), and the proportions of sulphur in these two forms, i.e.

$$K_R \times 100 = K_{HS^-} \times \% HS^- + K_{H_2S} \times \% H_2S$$

The experimentally determined values of K_R (see Bray and Till, 1975) were

linearly related (r = 0.995) to the calculated proportions of sulphide as H_2S and HS^- at the particular pH values studied. The intercepts K_{H_2S} = -0.0843 ± 0.0026 (SE) min^{-1} and K_{HS^-} = -0.0236 ± 0.0022 min^{-1} used in the present experiment for the above equation were taken from Bray and Till (1975). Thus, the absorption flow rate was estimated as a function of sulphide-S concentration in the rumen (the elapsed time between the initial and resultant concentrations of sulphide was taken as 1 second), absorption constant of rumen sulphide and rumen volume.

As the sheep were maintained under "steady state" conditions, the daily flow of sulphur out of the rumen plus the absorption of sulphur from the rumen must equal the daily sulphur inputs into the rumen. Sulphide-S lost "calculation" was made from the difference between the amounts entering and leaving the rumen. Sulphur recycled to the rumen was also calculated by difference, i.e. (sulphur out of the rumen + sulphur absorbed - sulphur in diet) or (sulphur "predicted" - sulphur "calculated").

(2.d) Estimation of Microbial Protein Synthesis in the Rumen

Thomas (1973) clearly differentiated between organic matter (OM) apparently digested in the rumen (i.e. Dietary OM intake - OM leaving stomach) and OM truly digested (i.e. OM apparently digested + OM incorporated into the microbes).

The amount of dietary OM "truly digested" in stomach is given by the equation:

$$OMTD = I + S - F - R \qquad (2.1)$$

where

OMTD = dietary OM "truly digested" in the rumen
I = dietary OM intake
S = endogenous OM added (saliva)
F = dietary OM leaving stomach
R = OM absorbed in the reticulo-rumen

Since endogenous OM added (saliva) S and OM absorbed in the reticulo-rumen R are both small and of similar magnitude (1% to 2%) of digestible OM, Czerkawski, 1978), they will cancel and equation (2.1) can be simplified to

$$OMTD = I - F \qquad (2.2)$$

Since $F = f - M$ (2.3)

where f = OM leaving stomach

M = microbial OM leaving stomach

then substituting (2.3) into (2.2)

$OMTD = I + M - f$ or

dietary OM "truly digested" in stomach = dietary OM intake + microbial OM leaving stomach - OM leaving stomach.

In the present work, microbial nitrogen is referred to nitrogen precipitated with tungstic acid. Hume (1969) has shown that at least 25% more nitrogen is precipitated by tungstic acid than by trichloroacetic acid. Winter *et al.* (1964) using mixed cultures of rumen bacteria grown on cellulose have shown that tungstic acid precipitated more protein from the *in vitro* rumen fermentation than did trichloroacetic acid. Protein nitrogen in fluid rumen samples was precipitated with tungstic acid and protein obtained by multiplying the figure for tungstic acid-precipitated nitrogen (TA-nitrogen) by 6.25.

In order to estimate the approximate amount of dietary OM leaving the rumen an estimate of the amount of OM present in the microbial fraction was made. Microbial OM was estimated on the basis that c. 10.5% of bacterial cells is nitrogen (Hungate, 1966).

(c) Results and Discussion

The results of these experiments are presented in Tables 2.1 to 2.21. Almost all of the ^{51}Cr and ^{103}Ru injected into the rumen was excreted in the faeces (see Table 2.2). Thus the recoveries of ^{103}Ru from the faeces were very high, at 99% to 100% of the amount introduced intraruminally; the quantities of ^{103}Ru recovered in the urine were very low at 0.1% to 0.3% of the dose given. Similarly, the recoveries of ^{51}Cr from the faeces were 97% to 98% of the dose and in the urine 2% to 3% of the dose given, thus less than 5% in urine, which is within the normal range. The retention times of ^{103}Ru-phen and ^{51}Cr-EDTA in the rumen were 14.8 and 8.7 h respectively, which confirms that particulate matter is retained longer in the ruminant stomach than is the liquid phase of digesta (Ellis and Huston, 1968; Grovum and Williams, 1973).

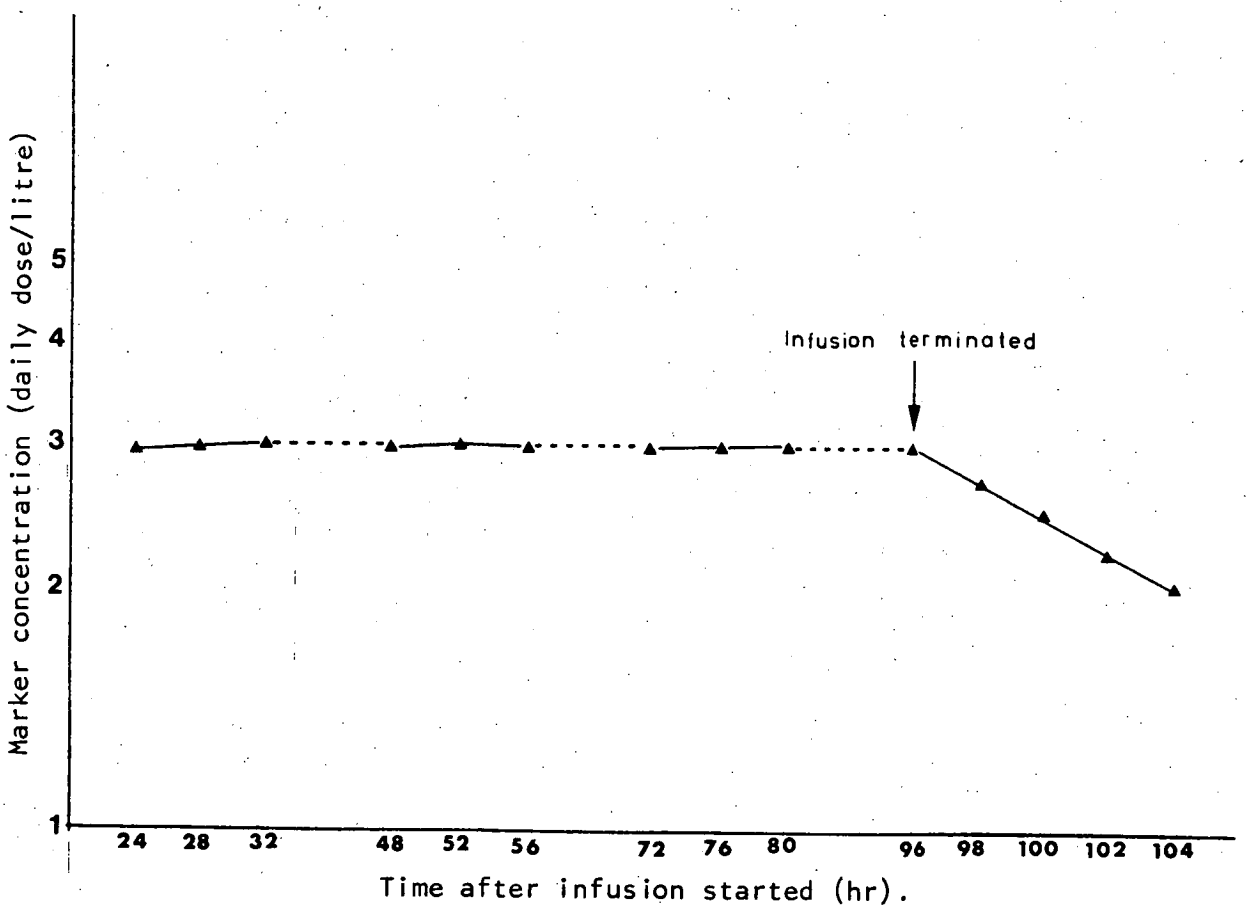


FIGURE 2.1 Concentration (as fraction of daily dose per litre) of ^{51}Cr-EDTA in rumen liquor during continuous infusion of markers (0-96 hr) into the rumen and after the infusion was terminated at 96 hr.

TABLE 2.1

Rumen fluid volumes and flow rates during the continuous infusion experimental period. The flow rates are given in litres per day.

Sheep No.	A		B		C	
Day	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d
1	2.890	7.861	2.874	7.959	2.890	8.004
2	2.918	7.937	2.900	7.970	2.901	8.124
3	2.898	7.914	2.905	8.107	2.924	8.145
Mean	2.902	7.904	2.893	8.012	2.905	8.091
CV*	0.4971	0.4932	0.5752	1.0294	0.5973	0.9402

* Coefficient of variation of individual measurements

TABLE 2.2

The cumulative recovery of ^{103}Ru and ^{51}Cr in excreta following intraruminal administration of ^{103}Ru-phen and ^{51}Cr-EDTA to sheep fed 800 g pellets daily. The collection period was 9 days.

Sheep No.	Recovery (% of dose)					
	Faeces		Urine		Total	
	^{103}Ru	^{51}Cr	^{103}Ru	^{51}Cr	^{103}Ru	^{51}Cr
A	99.9	97.9	0.1	2.8	100.1	100.7
B	100.0	98.1	0.2	2.9	100.2	101.0
C	99.8	98.3	0.3	1.9	100.1	100.1
Mean	99.9	98.1	0.2	2.5	100.1	100.6
± S.E. of the mean	0.07	0.11	0.05	0.34	0.05	0.27

TABLE 2.3

^{51}Cr-EDTA and ^{103}Ru-phen half-time in the rumen (h), ^{51}Cr-EDTA and ^{103}Ru-phen mean retention time in the rumen (h), average daily water intake during the markers experimental period and digestibility trial (mℓ) and apparent sulphur digestibility (%).

	Sheep				
	A	B	C	Mean	± S.E.
^{51}Cr-EDTA half-time in the rumen	6.1	6.0	5.9	6.0	0.04
^{103}Ru-phen half-time in the rumen	10.6	10.2	9.9	10.2	0.20
^{51}Cr-EDTA mean retention time in the rumen	8.8	8.7	8.6	8.7	0.06
^{103}Ru-phen mean retention time in the rumen	15.3	14.6	14.4	14.8	0.29
Average daily water intake during the markers experimental period	2010	1890	1880	1927	41.8
Average daily water intake during the digestibility trial	1930	1797	1770	1832	49.6
Apparent sulphur digestibility	71.5	69.4	67.1	69.3	1.3

TABLE 2.4

Intakes and Digestibility of Dry matter (DM) and Organic matter (OM)

	Sheep No.				
	A	B	C	Mean	± S.E.
DM					
Intake (g/day):	728	728	728	728	0
Faecal output (g/day):	330	326	332	329	1.7
Apparently digested (g/day):	398	402	396	399	1.7
Apparent digestibility (%):	54.6	55.2	54.5	54.8	0.23
OM					
Intake (g/day):	670	670	670	670	0
Faecal output (g/day):	277	284	293	285	4.6
Apparently digested (g/day):	393	386	377	385	4.6
Apparent digestibility (%):	58.7	57.6	56.3	57.5	0.69
Digestible OM intake (g/day/kg $BW^{0.75}$	28.4	28.7	26.1	27.7	0.83
BW = sheep wt (kg):	33.2	32.1	35.3	33.5	0.94

TABLE 2.5

Sulphur intake and excretion data

	Sheep No. A	B	C	Mean ± S.E.
Sulphur intake (mg/day):	1158	1158	1158	1158 0
Faecal sulphur (mg/day):	330	355	381	355 14.8
Urine sulphur (mg/day):	549	548	562	553 4.5
Sulphur retention:				
mg/day:	278	255	215	249 18.7
mg/100mg S intake:	24.0	22.0	18.5	21.5 1.62

TABLE 2.6

Sulphur lost from the rumen (mg S/day)

	Sheep No. A	B	C	Mean ± S.E.
Sulphur intake (1)	1158	1158	1158	1158 0
Daily flow of total S from rumen (2)	599	598	588	595 3.5
Sulphur lost from the rumen other than flow down the tract(S^{2-} lost "calculation") (1)-(2) = (3)	558	559	569	562 3.5
Faecal S	330	355	381	355 14.8
Urinary S	549	548	562	553 4.5
Intake S - Faecal S	828	803	777	802 14.8
Sulphide lost "prediction" (4)	627	629	586	614 14.1
Estimated recycled S (4) - (3)	69	70	17	52 17.5

TABLE 2.7

Digestion of organic matter (OM) in the rumen of sheep

	Sheep No. A	B	C	Mean ± S.E.
OM intake (g/day)	670	670	670	670 0
Faecal OM output (g/day)	277	284	293	285 4.6
OM apparently digested: Amount (g/day)	393	386	377	385 4.6
as % of intake	58.7	57.6	56.3	57.5 0.69
OM in digesta leaving rumen (%)	4.78	4.80	4.75	4.78 0.014
OM leaving rumen (g/day)	378	385	384	382 2.2
Apparent OM digested in rumen: Amount (g/day)	292	285	286	288 2.2
as % of intake	43.6	42.6	42.7	43.0 0.33
Microbial nitrogen leaving rumen (g/day)	7.45	7.30	7.10	7.28 0.101
Microbial OM leaving rumen (g/day)	71	70	68	69 1.0
Dietary OM leaving rumen (g/day)	307	315	316	313 3.0
Dietary OM "truly digested" in rumen: Amount (g/day)	363	355	354	357 3.0
as % of OM intake	54.2	53.0	52.8	53.3 0.45
as % of total OM digested	92.4	91.9	93.7	92.7 0.52

TABLE 2.8

Mean flow rate and protein production data

	Sheep No. A	B	C	Mean ± S.E.
Rumen volume (ℓ)	2.902	2.893	2.905	2.900 0.0036
Flow of digesta from rumen (ℓ/day)	7.904	8.012	8.091	8.002 0.0542
Flow of TA-nitrogen (g/day)	7.45	7.30	7.10	7.28 0.101
Flow of microbial protein (TA-nitrogenx6.25) (g/day)	46.5	45.6	44.4	45.5 0.63
Protein synthesised per 100 g OM "truly digested" in rumen (g)	12.8	12.8	12.6	12.7 0.09

TABLE 2.9

Rumen fluid pH sampled every two hours over an eight hour period

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	6.60	6.65	6.60	6.70	6.75	6.66	0.979	6.65	6.75	6.80	6.70	6.60	6.70	1.180
B	6.65	6.75	6.60	6.70	6.70	6.68	0.853	6.55	6.60	6.65	6.55	6.65	6.60	0.757
C	6.60	6.55	6.45	6.50	6.60	6.54	0.997	6.60	6.55	6.55	6.65	6.55	6.58	0.680

* Coefficient of variation of individual measurements

TABLE 2.10

The calculated absorption constants (K_R) for rumen sulphide at rumen pH values and the estimated sulphide absorption half-lives

Sheep No.	pH	% H_2S	% HS^-	K_R	Rumen sulphide half-life (min)
A	6.68	53.7	46.3	-0.0562	12.3
B	6.64	56.0	44.0	-0.0576	12.0
C	6.56	60.5	39.5	-0.0603	11.5
Mean	6.63	56.7	43.3	-0.0580	12.0
± S.E.	0.035	1.98	1.98	0.00120	0.25

TABLE 2.11

Concentration of Sulphur in rumen fluid (µg S/mℓ)

	Sheep No. A	B	C	Mean ± S.E.
Total S	76	75	73	74 0.9
Inorganic sulphate S	1.8	1.7	1.7	1.7 0.03
Sulphide S	2.8	2.8	2.6	2.8 0.06
Protein S	70	68	66	68 1.1

TABLE 2.12

The concentrations of total sulphur in the rumen fluid of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300hh	1500h	1700h	Mean	CV*
A	74	77	73	75	77	75	2.3	78	75	75	78	77	77	2.3
B	76	74	78	75	76	76	2.4	73	75	71	74	74	74	2.2
C	72	74	72	75	72	73	1.8	71	74	72	71	73	72	1.5

* Coefficient of variation of individual measurements

TABLE 2.13

The concentrations of fluid inorganic sulphate sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	1.76	1.82	1.84	1.79	1.84	1.81	1.914	1.64	1.74	1.68	1.73	1.66	1.69	2.579
B	1.64	1.73	1.65	1.67	1.71	1.68	2.305	1.75	1.80	1.74	1.82	1.79	1.78	1.905
C	1.63	1.56	1.64	1.63	1.59	1.61	2.106	1.66	1.68	1.73	1.64	1.74	1.69	2.579

* Coefficient of variation of individual measurements

TABLE 2.14

The concentrations of fluid sulphide sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	2.79	2.75	2.85	2.83	2.78	2.80	1.428	2.83	2.96	2.86	2.90	2.85	2.88	1.787
B	2.77	2.88	2.81	2.85	2.79	2.82	1.586	2.72	2.68	2.80	2.79	2.81	2.76	2.065
C	2.64	2.57	2.65	2.53	2.66	2.61	2.184	2.61	2.72	2.57	2.71	2.64	2.65	2.431

* Coefficient of variation of individual measurements

TABLE 2.15

The concentrations of fluid protein sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	70	68	71	70	1.9	69	72	70	71	1.6
B	68	68	69	68	1.1	68	67	67	67	1.1
C	66	67	67	67	0.7	66	65	67	66	1.4

* Coefficient of variation of individual measurements

TABLE 2.16

The nitrogen to sulphur ratio (N/S) in ruminal microbial protein fraction of sheep sampled at different times of the day

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	13.27	13.40	13.38	13.35	0.524	13.59	13.46	13.54	13.53	0.485
B	13.26	13.30	13.40	13.32	0.541	13.45	13.63	13.48	13.52	0.713
C	13.39	13.28	13.23	13.30	0.615	13.07	13.10	13.25	13.14	0.734

* Coefficient of variation of individual measurements

TABLE 2.17

The concentrations of microbial protein sulphur in the rumen of sheep sampled at different times of the day (mg S/100g wet precipitate)

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	34.5	35.4	35.2	35.0	1.39	36.4	35.7	36.3	36.1	1.10
B	33.5	33.8	34.5	33.9	1.48	34.8	35.6	35.0	35.1	1.19
C	34.8	34.4	33.7	34.3	1.61	32.9	33.2	33.7	33.3	1.27

* Coefficient of variation of individual measurements

TABLE 2.18

The concentrations of microbial protein nitrogen in the rumen of sheep sampled at different times of the day (% N in wet precipitate)

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	0.457	0.474	0.471	0.467	1.942	0.495	0.480	0.491	0.489	1.589
B	0.444	0.450	0.462	0.452	2.028	0.468	0.485	0.471	0.475	1.912
C	0.466	0.457	0.446	0.456	2.195	0.430	0.435	0.447	0.437	1.998

* Coefficient of variation of individual measurements

TABLE 2.19

Rumen sulphur pools (mg S)

	Sheep No. A	B	C	Mean ±	S.E.
Total S	220	216	211	216	2.6
Protein S	204	196	193	198	3.2
Inorganic sulphate S	5.1	5.0	4.8	5.0	0.09
Sulphide S	8.2	8.1	7.6	8.0	0.18

TABLE 2.20

Daily flow of sulphur from the rumen (mg S/day)

	Sheep No. A	B	C	Mean ±	S.E.
Total S	599	598	588	595	3.5
Protein S	554	544	537	545	5.1
Inorganic sulphate S	13.8	13.9	13.4	13.7	0.17
Sulphide S	22.5	22.4	21.3	22.0	0.37

TABLE 2.21

Daily excretion flow rates of inorganic sulphate (mg S/day)

Sheep No.	A	B	C	Mean ±	S.E.
	558	559	569	562	3.5

The mean inorganic sulphate concentration in strained rumen fluid was 1.61 to 1.81 µg S/mℓ (see Table 2.13) and c. 2.0% of the sulphate intake passed unchanged to the omasum.

The calculated absorption constants for rumen sulphide at the rumen pH values observed and the estimated sulphide absorption half-lives are presented in Table 2.10. In the present experiments only small fluctuations were noted in rumen pH (see Table 2.9) and sulphide-sulphur concentrations (see Table 2.14). The mean rumen pH was 6.54 to 6.70 and the sulphide-sulphur concentration 2.61 to 2.88 µg S/mℓ strained rumen fluid. Little sulphide passed from the rumen in the digesta which was approximately 1.8% of the total sulphur entering the rumen, which is similar to the values of 0.4% to 2.8% found by Bird and Hume (1971).

The absorption of sulphide from the rumen was of the order of 586 - 629 mg S/day, thus c. 48.4% to 52.0% of the total sulphur entering the rumen respectively was absorbed as sulphide. Bray and Till (1975) suggested that 2-5 mg S/day/kg BW is recycled to the reticulo-rumen. In these experiments it was estimated that only 17-70 mg S/day (total sulphur) was recycled to the rumen.

The nitrogen to sulphur ratio (N/S) found in microbial protein precipitated with tungstic acid was approximately 13.3/1, a value similar to that reported by Moir *et al.* (1967-68). Data pertaining to the digestion of organic matter (OM) are presented in Table 2.7. The apparent digestibility of OM in the whole tract was approximately 57.5% and the apparent OM digested in the reticulo-rumen was equivalent to approximately 43.0% of intake. Of the total dietary OM digested it was estimated that approximately 92.7% was digested in the rumen.

The microbial protein synthesised per 100 g OM "truly digested" in the rumen was approximately 12.7 g, a value somewhat lower than that generally accepted. The values with OM "truly digested" are considerably smaller than the values with OM apparently digested in the rumen. The protein sulphur passed to the omasum was equivalent to approximately 45.1% of the total sulphur entering the rumen.

Three models (named A, B and C) were constructed for these experiments,

each based on different assumptions, thus:

(1) Model A: (a) all the sulphur intake was in the form of inorganic sulphate and therefore passed through the inorganic sulphate pool, and (b) all of the sulphur incorporated into microbial protein first passed through the free H_2S pool (Walker and Nader, 1968; Nader and Walker, 1970).

(2) Model B: (a) approximately 82% - 85% of the sulphur in the diet and 25% of the recycled sulphur was in the inorganic sulphate form, and (b) all of the sulphur incorporated into microbial protein first passed through the free H_2S pool (Walker and Nader, 1968; Nader and Walker, 1970).

(3) Model C: (a) approximately 82% - 85% of the sulphur in the diet and 25% of the recycled sulphur was in the inorganic sulphate form and (b) approximately 55% of sulphur in microbial protein originated from the sulphide pool, and 45% from digested plant and salivary proteins (Gawthorne and Nader, 1976).

The block diagrams of the models A, B and C are shown in Figures 2.2, 2.3 and 2.4, respectively and the differential equations describing the linear system of each model are presented in Table. 2.22.

In order to have the body sulphate compartment in a steady state, an excretion rate has to be considered and a value must be given to it. The amount of sulphate excreted in the urine is the net result of three processes: (1) filtration in the glomeruli; (2) secretion by the tubules; and (3) reabsorption by the tubules. In these models, the excretion rate which would maintain a constant body sulphate compartment was approximately 390 µg S/min. The excretion of inorganic sulphate proposed by the model at a plasma level of inorganic sulphate concentration of 30 µg s/mℓ does not agree with the amounts that can be calculated using either the data of Kennedy and Milligan (1978) or Bishara and Bray (1978b). The urinary excretion of inorganic sulphate in relationship to plasma inorganic sulphate is fully discussed in part VIII.

FIGURE 2.2 Model A. The state variables (compartments) are:

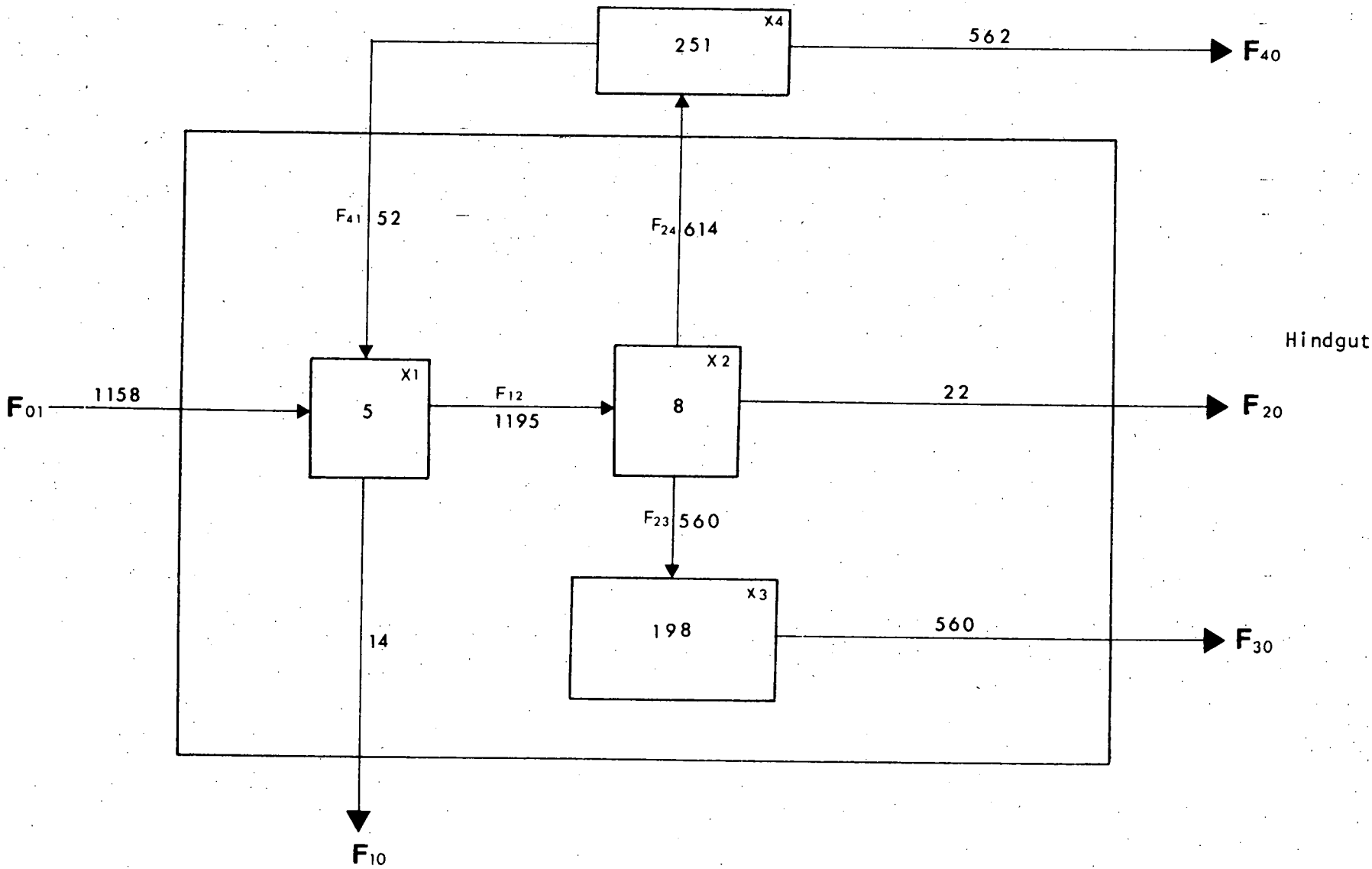
x_1 = inorganic sulphate

x_2 = sulphide

x_3 = protein sulphur

x_4 = body sulphate

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


X4
251
562
F_{40}
F_{41} 52
F_{24} 614
Hindgut
X1
5
X2
8
F_{01}
1158
F_{12}
1195
22
F_{20}
F_{23} 560
X3
198
560
F_{30}
14
F_{10}

200a

FIGURE 2.3 Model B. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

x_3 = protein sulphur

x_4 = body sulphate

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.

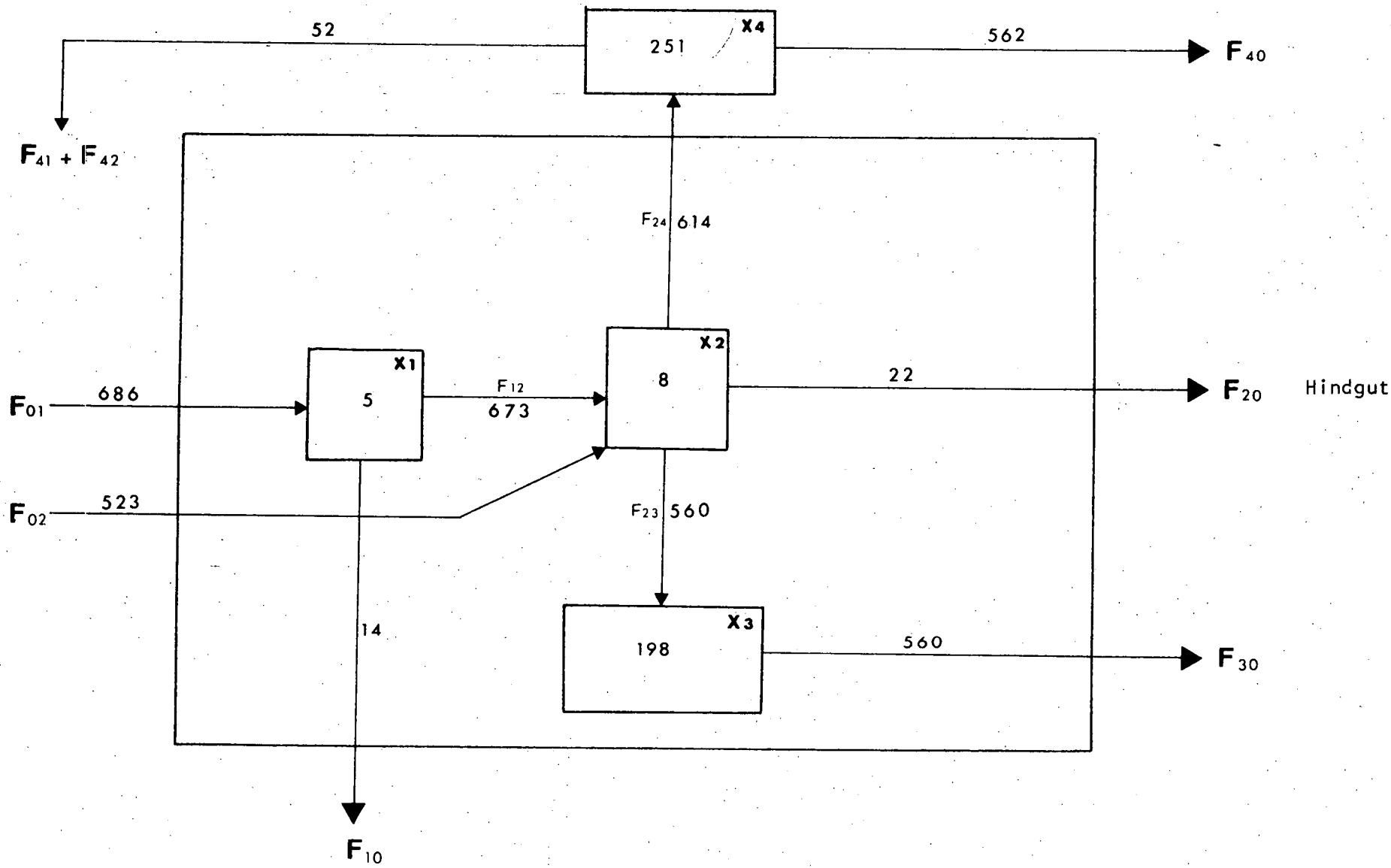

52
251
X4
562
F_{40}
$F_{41} + F_{42}$
F_{24} 614
X1
5
F_{12}
673
X2
8
22
F_{20}
Hindgut
F_{01}
686
F_{02}
523
F_{23} 560
14
X3
198
560
F_{30}
F_{10}

FIGURE 2.4 Model C. The state variables (compartments) are:

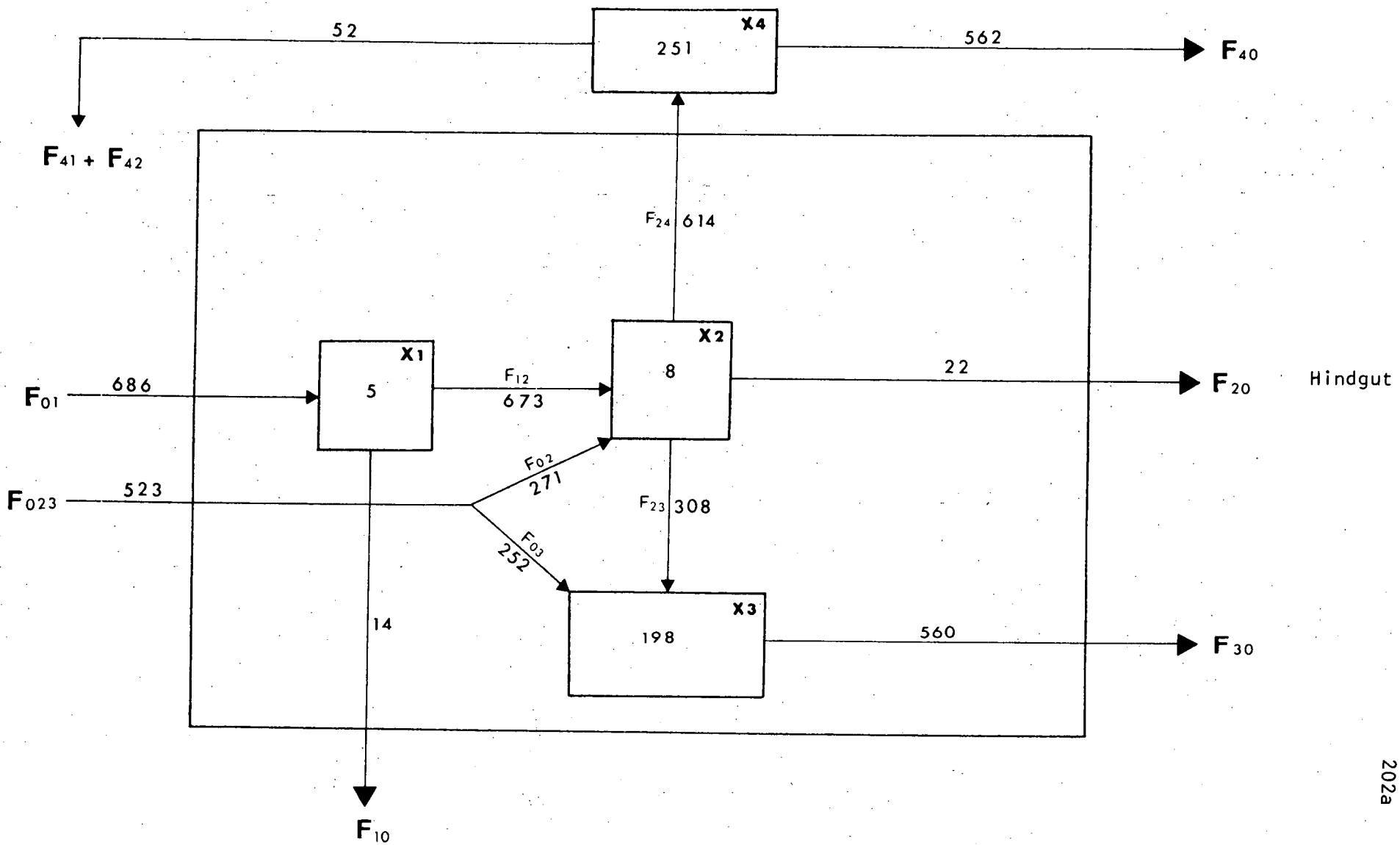
x_1 = inorganic sulphate

x_2 = sulphide

x_3 = protein sulphur

x_4 = body sulphate

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


52
X4
251
562
F_{40}
$F_{41} + F_{42}$
F_{24} 614
X2
8
22
F_{20}
Hindgut
X1
5
F_{12}
673
F_{01}
686
F_{02}
271
F_{023}
523
F_{23} 308
F_{03}
252
X3
198
560
F_{30}
14
F_{10}

202a

TABLE 2.22

The set of differential equations describing the linear system of each of the three models is:

Model A:

$$dX_1/dt = F_{01} + \phi_{41}X_4 - \phi_{12}X_1 - F_{10}$$

$$dX_2/dt = \phi_{12}X_1 - F_{20} - \phi_{24}X_2 - \phi_{23}X_2$$

$$dX_3/dt = \phi_{23}X_2 - F_{30}$$

$$dX_4/dt = \phi_{24}X_2 - \phi_{41}X_4 - F_{40}$$

Model B:

$$dX_1/dt = F_{01} + \phi_{41}X_4 - \phi_{12}X_1 - F_{10}$$

$$dX_2/dt = \phi_{12}X_1 + F_{02} - \phi_{24}X_2 - F_{20} - \phi_{23}X_2$$

$$dX_3/dt = \phi_{23}X_2 - F_{30}$$

$$dX_4/dt = \phi_{24}X_2 - \phi_{41}X_4 - F_{40} - F_{42}$$

Model C:

$$dX_1/dt = F_{01} + \phi_{41}X_4 - \phi_{12}X_1 - F_{10}$$

$$dX_2/dt = \phi_{12}X_1 + F_{02} - \phi_{24}X_2 - F_{20} - \phi_{23}X_2$$

$$dX_3/dt = \phi_{23}X_2 + F_{03} - F_{30}$$

$$dX_4/dt = \phi_{24}X_2 - \phi_{41}X_4 - F_{40} - F_{42}$$

PART V

THE EFFECT OF HIGH DIETARY SULPHUR INTAKE ON RUMEN SULPHUR DYNAMICS

(a) Introduction

The sulphur dynamics of the rumen are largely a reflection of microbial activity thus any factor which affects a specific area of microbial activity may in turn affect the transfer rate of sulphur from one pool to another. In this context the supply of sulphide may be a variable which affects microbial growth and thus limits the extent of incorporation of sulphur into microbial protein.

Hume *et al.* (1970) demonstrated a close relationship between the level of intake of nitrogen (up to 9 g/day) and the amount of protein produced daily by the rumen micro-organisms. There was no further increase when the nitrogen intake was raised to 16 g/day. On the evidence of Moir *et al.* (1967-68) it was suggested that further protein synthesis may have been limited by a deficiency of sulphur, since at the highest nitrogen intake the dietary nitrogen/sulphur ratio was 20:1. Hume and Bird (1970) found that when sheep were fed on a diet supplying 0.61 g sulphur per day, 82 g microbial protein was produced daily in the rumen. Raising the sulphur intake to 1.95 g/day increased protein production to 94 g/day ($P < 0.05$), but there was no further increase when the intake was raised to 3.42 g/day. Protein production was not influenced by the form of the supplementary sulphur (Hume and Bird, 1970).

The effect of the level of sulphur intake on the production of microbial protein in the rumen and the absorption of sulphur from the rumen was examined in these experiments.

(b) Materials and Methods

(1) Animals and Diet

Three wethers fed a high sulphur ration of 800 g pellets/day containing 13.2 g total nitrogen and 2.32 g total sulphur with a N/S ratio of 5.7/1. The diet, composed mainly of oat hulls, was low protein with urea and inorganic sulphate providing the major sources of nitrogen and sulphur respectively. The wethers were fed at hourly intervals from an automatic feeder. The composition of the diet is shown in Appendix 3.

(2) Experimental Procedures

The experimental procedures used in these experiments were the same as those described in the low sulphur intake experiment Part IV, except that, the rumen volumes and flow rates of digesta from the rumen were estimated by reference to the water-soluble marker ^{51}Cr-EDTA as described by Downes and McDonald (1964). Thus, the half-time of the marker was calculated from the slope of the curve given by plotting log concentration of marker against time. If instantaneous mixing of the marker with the rumen contents had occurred, extrapolation of the above curve should give the concentration of marker at zero time. From this value and the total amount of marker injected, the total volume of rumen liquor was calculated.

The extracellular fluid volume was estimated by dilution of intravenously injected carrier-free ^{35}S-Na_2SO_4 (approximately 300 µCi) in 5 mℓ of sterile physiological saline as described in Part II (Section 2).

(c) Results and Discussion

The results of these experiments are presented in Tables 3.1 to 3.26.

The recoveries of ^{51}Cr were similar to those reported in the previous section (Part IV) i.e. 95% - 97% of the ruminally infused ^{51}Cr-EDTA being recovered in the faeces and 4% - 5% being recovered in the urine.

Two additional pools were introduced to the rumen system, i.e. the ester sulphate pool and the soluble organic sulphur pool. The mean inorganic sulphate concentration in strained rumen fluid was 4.5 to 5.4 µg S/mℓ (see Table 3.16) and c. 2.1% of the inorganic sulphate intake passed unchanged to the omasum.

The calculated absorption constants for rumen sulphide at the rumen pH values observed and the estimated sulphide absorption half-lives are presented in Table 3.10. In these experiments only small fluctuations were noted in rumen pH (see Table 3.9) and sulphide-sulphur concentrations (see Table 3.17). The mean rumen pH was 6.65 to 6.84 and the sulphide-sulphur concentration 4.8 to 5.3 µg S/mℓ strained rumen fluid. Little sulphide passed from the rumen in the digesta which was approximately 1.7% of the total sulphur entering the rumen, which is similar to the

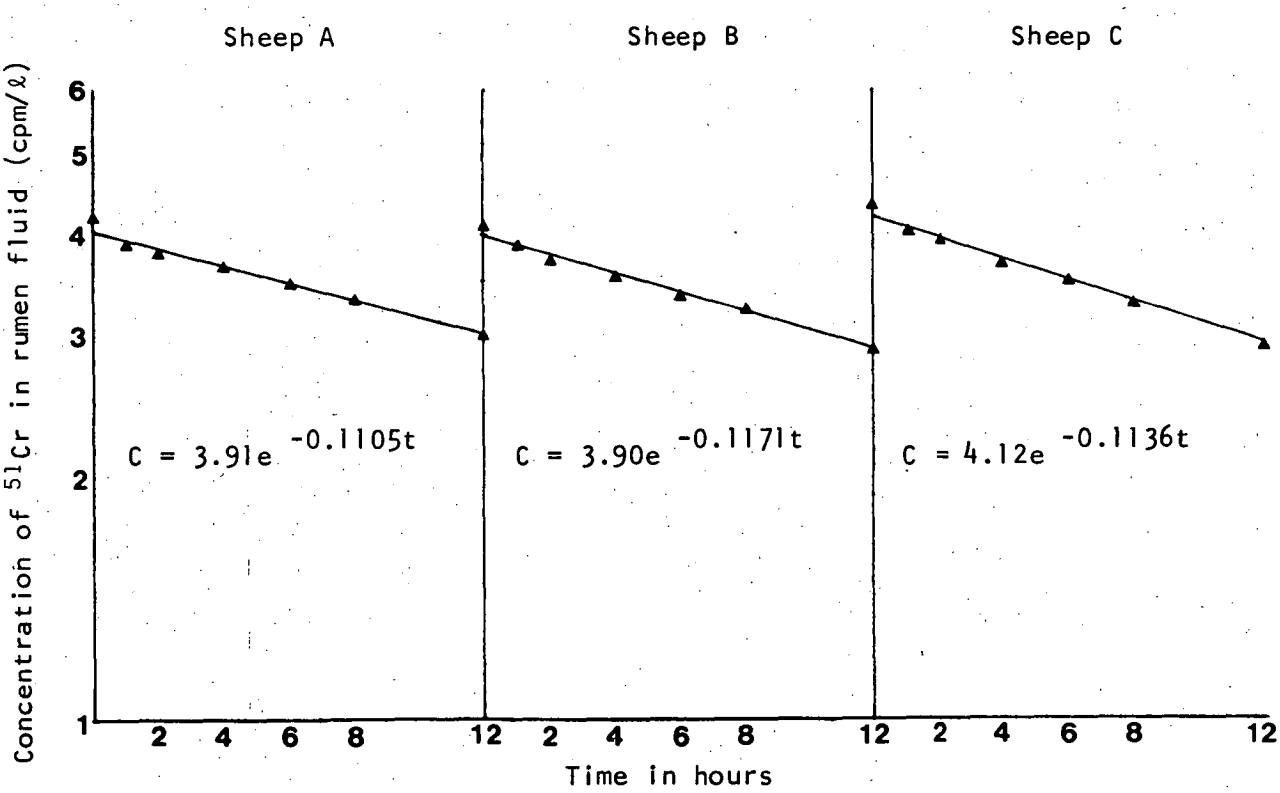


FIGURE 3.1 Changes in the rumen concentration of ^{51}Cr-EDTA following administration on successive days. Each curve represents the mean result of three successive days of the ^{51}Cr-EDTA infusion into the rumen.

TABLE 3.1

Rumen fluid volumes and flow rates. The volumes are calculated at 0, 24, 48 hours. The flow rates are given in litres per day.

Sheep No.	A		B		C	
Day	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d
1	3.079	8.152	2.901	8.166	2.965	8.109
2	3.102	8.254	2.933	8.217	2.982	8.130
3	3.089	8.179	2.911	8.199	3.014	8.196
Mean	3.090	8.195	2.915	8.194	2.987	8.145
CV*	0.3732	0.6449	0.5616	0.3157	0.8329	0.5574

* Coefficient of variation of individual measurements.

TABLE 3.2

The cumulative recovery of ^{51}Cr in excreta following intraruminal administration of ^{51}Cr-EDTA to sheep fed 800 g pellets daily

Sheep No.	Recovery (% of dose)*		
	Faeces	Urine	Total
A	95.6 (10)	4.7 (10)	100.3
B	94.9 (9)	5.3 (9)	100.2
C	97.1 (9)	4.7 (9)	101.8
Mean	95.8	4.9	100.8
± S.E. of the mean	0.63	0.19	0.52

* Figures in parentheses are the number of days during which collections were made.

TABLE 3.3

^{51}Cr-EDTA half-time in the rumen (h), ^{51}Cr-EDTA mean retention time in the rumen (h), average daily water intake during the markers experimental period and digestibility trial (mℓ) and apparent sulphur digestibility (%)

	Sheep No. A	B	C	Mean ±	S.E.
^{51}Cr-EDTA half-time in the rumen	6.3	5.9	6.1	6.1	0.10
^{51}Cr-EDTA mean retention time in the rumen	9.1	8.5	8.8	8.8	0.15
Average daily water intake during the markers experimental period	2134	2008	1986	2043	46.1
Average daily water intake during the digestibility trial	2086	2003	1963	2017	36.2
Apparent sulphur digestibility	71.2	74.5	73.1	72.9	0.96

TABLE 3.4

Intakes and Digestibility of Dry matter (DM) and Organic matter (OM)

	Sheep No. A	B	C	Mean ±	S.E.
DM					
Intake (g/day):	722	722	722	722	0
Faecal output (g/day):	251	240	230	240	6.1
Apparently digested (g/day):	471	482	492	482	6.1
Apparent digestibility (%):	65.2	66.8	68.1	66.7	0.84
OM					
Intake (g/day):	661	661	661	661	0
Faecal output (g/day):	222	211	203	212	5.4
Apparently digested (g/day):	439	450	458	449	5.4
Apparent digestibility (%):	66.4	68.1	69.2	67.9	0.81
Digestible OM intake (g/day/kg $BW^{0.75}$)	30.7	32.3	30.9	31.3	0.53
BW = sheep wt (kg):	34.8	33.5	36.4	34.9	0.84

TABLE 3.5

Sulphur intake and excretion data

	Sheep No. A	B	C	Mean ±	S.E.
Sulphur intake (mg/day):	2317	2317	2317	2317	0
Faecal sulphur (mg/day):	666	591	622	627	21.8
Urine sulphur (mg/day):	1263	1257	1269	1263	3.3
Sulphur retention:					
mg/day:	388	469	426	427	23.3
mg/100 mg S intake:	16.7	20.2	18.4	18.5	1.01

TABLE 3.6

Sulphur lost from the rumen (mg S/day)

	Sheep No. A	B	C	Mean ±	S.E.
Sulphur intake (1)	2317	2317	2317	2317	0
Daily flow of total S from rumen (2)	1330	1321	1344	1332	6.7
Sulphur lost from the rumen other than flow down the tract (S^{2-} lost "calculation") (1)-(2) = (3)	987	996	973	985	6.7
Faecal S	666	591	622	627	21.8
Urinary S	1263	1257	1269	1263	3.3
Intake S - Faecal S	1651	1726	1695	1690	21.8
Sulphide lost "prediction" (4)	1068	1091	1075	1078	6.9
Estimated recycled S (4) - (3)	81	95	102	93	6.3

TABLE 3.7

Digestion of organic matter (OM) in the rumen of sheep

	Sheep No. A	B	C	Mean ±	S.E.
OM intake (g/day)	661	661	661	661	0
Faecal OM output (g/day)	222	211	203	212	5.4
OM apparently digested: Amount (g/day)	439	450	458	449	5.4
as % of intake	66.4	68.1	69.2	67.9	0.81
OM in digesta leaving rumen (%)	4.39	4.38	4.27	4.35	0.038
OM leaving rumen (g/day)	360	356	348	354	3.5
Apparent OM digested in rumen: Amount (g/day)	301	305	313	307	3.5
as % of intake	45.6	46.2	47.4	46.4	0.53
Microbial nitrogen leaving rumen (g/day)	10.69	10.57	11.31	10.86	0.229
Microbial OM leaving rumen (g/day)	102	101	108	103	2.2
Dietary OM leaving rumen (g/day)	258	255	240	251	5.6
Dietary OM "truly digested" in rumen: Amount (g/day)	403	406	421	410	5.6
as % of OM intake	61.0	61.4	63.7	62.0	0.84
as % of total OM digested	91.8	90.2	92.0	91.3	0.58

TABLE 3.8

Mean flow rate and protein production data

	Sheep No. A	B	C	Mean ±	S.E.
Rumen volume (ℓ)	3.090	2.915	2.987	2.997	0.0508
Flow of digesta from rumen (ℓ/day)	8.195	8.194	8.145	8.178	0.0165
Flow of TA-nitrogen (g/day)	10.69	10.57	11.31	10.86	0.229
Flow of microbial protein (TA-nitrogenx6.25) (g/day)	66.8	66.1	70.7	67.9	1.44
Protein synthesised per 100 g OM "truly digested" in rumen (g)	16.6	16.3	16.8	16.5	0.15

TABLE 3.9

Rumen fluid pH sampled every two hours over an eight hour period

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	6.70	6.75	6.75	6.70	6.65	6.71	0.623	6.80	6.75	6.85	6.80	6.85	6.81	0.614
B	6.70	6.70	6.60	6.70	6.70	6.68	0.669	6.60	6.55	6.60	6.65	6.70	6.62	0.861
C	6.80	6.75	6.80	6.75	6.85	6.79	0.616	6.90	6.90	6.90	6.85	6.90	6.89	0.325

* Coefficient of variation of individual measurements

TABLE 3.10

The calculated absorption constants (K_R) for rumen sulphide at rumen pH values and the estimated sulphide absorption half-lives

Sheep No.	pH	% H_2S	% HS^-	K_R	Rumen sulphide half-life (min)
A	6.76	49.1	50.9	-0.0534	13.0
B	6.65	55.4	44.6	-0.0572	12.1
C	6.84	44.7	55.3	-0.0507	13.7
Mean	6.75	49.7	50.3	-0.0538	12.9
± S.E.	0.055	3.12	3.12	0.00189	0.45

TABLE 3.11

Concentration of sulphur in rumen fluid (µg S/mℓ)

	Sheep No. A	B	C	Mean ± S.E.
Total S	162	161	165	163 1.1
Total sulphate S	6.1	6.0	7.2	6.4 0.39
Ester sulphate S	1.6	1.3	1.8	1.6 0.14
Inorganic sulphate S	4.5	4.7	5.4	4.8 0.28
Sulphide S	4.9	4.8	5.3	5.0 0.12
Protein S	146	148	150	148 1.1
Soluble Organic S	1.0	0.8	1.5	1.1 0.20
Neutral S	156	155	158	156 0.8
Total Reducible S	6.1	6.0	7.2	6.4 0.39

TABLE 3.12

The concentrations of fluid total sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	157	165	163	159	158	161	2.2	163	164	161	168	162	164	1.7
B	156	163	163	159	154	159	2.5	162	167	165	163	160	163	1.8
C	165	164	169	166	173	168	2.0	160	160	168	164	161	163	2.0

* Coefficient of variation of individual measurements

TABLE 3.13

The concentrations of plasma inorganic sulphate in sheep taken at different times of the day (μg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	43	41	44	41	44	43	2.8	43	42	44	45	44	44	2.1
B	44	44	43	44	42	43	2.1	45	43	46	44	46	45	2.8
C	47	45	44	46	48	46	3.0	45	46	43	45	43	44	2.9

* Coefficient of variation of individual measurements

TABLE 3.14

The concentrations of fluid total sulphate sulphur in the rumen of sheep sampled at different times of the day (μg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	5.8	5.8	6.0	6.1	5.9	5.9	2.00	6.1	6.2	6.4	6.2	6.1	6.2	1.90
B	5.8	5.7	5.9	5.8	6.0	5.8	2.03	6.2	6.4	6.0	6.1	6.1	6.2	2.24
C	7.3	7.6	7.4	7.3	7.6	7.4	1.97	7.2	6.9	7.0	6.9	6.9	7.0	1.70

* Coefficient of variation of individual measurements

TABLE 3.15

The concentrations of fluid ester sulphate sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	1.3	1.3	1.4	1.4	1.4	1.4	4.56	1.8	1.8	1.9	1.8	1.7	1.8	4.19
B	1.1	1.1	1.2	1.2	1.3	1.2	5.97	1.5	1.6	1.4	1.4	1.5	1.5	5.58
C	1.9	2.1	2.0	1.8	2.1	2.0	6.07	1.8	1.7	1.7	1.5	1.6	1.7	7.48

* Coefficient of variation of individual measurements

TABLE 3.16

The concentrations of fluid inorganic sulphate sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	4.5	4.5	4.6	4.7	4.5	4.5	1.26	4.3	4.4	4.5	4.4	4.4	4.4	1.35
B	4.7	4.6	4.7	4.6	4.7	4.7	1.30	4.7	4.8	4.6	4.7	4.6	4.7	1.39
C	5.4	5.5	5.4	5.5	5.5	5.4	0.74	5.4	5.2	5.3	5.4	5.3	5.3	1.09

* Coefficient of variation of individual measurements

TABLE 3.17

The concentrations of fluid sulphide sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	4.9	5.0	4.9	5.1	4.8	4.9	1.76	4.9	4.9	4.8	4.7	4.9	4.9	1.59
B	4.8	4.9	4.8	5.0	5.0	4.9	2.07	4.9	4.9	4.7	4.9	4.8	4.8	1.70
C	5.1	5.3	5.3	5.1	5.1	5.2	2.11	5.3	5.2	5.4	5.3	5.4	5.3	2.01

* Coefficient of variation of individual measurements

TABLE 3.18

The concentrations of fluid protein sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	144	147	145	145	1.1	145	149	148	147	1.2
B	146	149	146	147	1.2	151	148	149	149	0.8
C	153	153	150	152	1.2	149	146	150	148	1.1

* Coefficient of variation of individual measurements

TABLE 3.19

The nitrogen to sulphur ratio (N/S) in ruminal microbial protein fraction of sheep sampled at different times of the day

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	8.87	8.94	8.89	8.90	0.405	8.88	8.94	9.00	8.94	0.671
B	8.70	8.73	8.67	8.70	0.345	8.77	8.68	8.71	8.72	0.526
C	9.29	9.34	9.21	9.28	0.707	9.24	9.19	9.29	9.24	0.541

* Coefficient of variation of individual measurements

TABLE 3.20

The concentrations of microbial protein sulphur in the rumen of sheep sampled at different times of the day (mg S/100 g wet precipitate)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	71	74	73	73	2.1	73	74	75	74	1.5
B	75	75	72	74	2.0	75	72	73	73	1.7
C	77	79	76	78	1.8	80	79	81	80	1.1

* Coefficient of variation of individual measurements

TABLE 3.21

The concentrations of microbial protein nitrogen in the rumen of sheep sampled at different times of the day (% N in wet precipitate)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	0.631	0.663	0.646	0.647	2.476	0.650	0.666	0.678	0.665	2.113
B	0.651	0.657	0.628	0.645	2.372	0.654	0.626	0.640	0.640	2.188
C	0.720	0.739	0.703	0.721	2.499	0.740	0.725	0.748	0.738	1.583

* Coefficient of variation of individual measurements

TABLE 3.22

The concentrations of fluid soluble organic sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	0.9	1.1	1.0	1.0	8.10	1.0	1.0	1.2	1.1	10.81
B	0.8	0.7	0.9	0.8	12.16	0.9	0.8	0.9	0.8	8.43
C	1.5	1.5	1.7	1.6	8.33	1.4	1.3	1.5	1.4	6.74

* Coefficient of variation of individual measurements

TABLE 3.23

Ruminal fluid sulphur pools (mg S)

	Sheep No. A	B	C	Mean ±	S.E.
Total S	502	470	493	488	9.4
Neutral S	483	453	471	469	8.8
Protein S	452	432	448	444	6.2
Total reducible S	19	17	22	19	1.2
Total sulphate S	19	17	22	19	1.2
Ester sulphate S	5	4	6	5	0.5
Inorganic sulphate S	14	13	16	14	0.8
Sulphide S	15	14	16	15	0.4
Soluble organic S	3.2	2.4	4.5	3.4	0.61

TABLE 3.24

Daily flow of sulphur from the rumen (mg S/day)

	Sheep No. A	B	C	Mean ±	S.E.
Total S	1330	1321	1344	1332	6.7
Neutral S	1280	1272	1286	1279	3.9
Protein S	1199	1214	1222	1212	6.7
Total reducible S	50	49	59	53	3.1
Total sulphate S	50	49	59	53	3.1
Ester sulphate S	13	11	15	13	1.2
Inorganic sulphate S	37	38	44	40	2.2
Sulphide S	40	40	43	41	0.9
Soluble organic S	8.4	6.7	12.2	9.1	1.62

TABLE 3.25

Sulphate space (Extracellular Fluid Volume, E.C.F.) of experimental sheep, estimated by dilution of intravenously injected sodium [^{35}S] sulphate. The E.C.F. as a percentage of body weight is presented in parenthesis.

Time	Extracellular Fluid Volume (mℓ)		
	A	B	C
T15	8666 (24.9)	8075 (24.1)	9595 (26.4)
T30	9218 (26.5)	8681 (25.9)	10349 (28.4)

TABLE 3.26

E.C.F. (% of body weight), plasma inorganic sulphate concentration (µg S/mℓ), body sulphate pools (mg S) and daily excretion flow rates of inorganic sulphate (mg S/day).

	Sheep No.				
	A	B	C	Mean ±	S.E.
E.C.F.	22.7	22.7	25.2	23.6	0.85
Plasma inorganic sulphate concentration	43	44	45	44	0.6
Body sulphate pools	342	335	415	364	25.4
Daily excretion flow rates of inorganic sulphate	987	996	973	985	6.7

values of 0.4% - 2.8% found by Bird and Hume (1971).

The absorption of sulphide from the rumen was of the order of 1068 - 1091 mg S/day, thus c. 44.3% - 45.3% of the total sulphur entering the rumen respectively was absorbed as sulphide. In the present experiments it was estimated that 81 - 102 mg S/day (total sulphur) was recycled to the rumen.

The nitrogen to sulphur ratio (N/S) found in microbial protein precipitated with tungstic acid was approximately 9.0/1, a value somewhat lower than that generally accepted.

Data pertaining to the digestion of organic matter (OM) are presented in Table 3.7. The apparent digestibility of OM in the whole tract was approximately 67.9% and the apparent OM digested in the reticulo-rumen was equivalent to approximately 46.4% of intake. Of the total dietary OM digested it was estimated that approximately 91.3% was digested in the rumen.

The microbial protein synthesised per 100 g OM "truly digested" in the rumen was approximately 16.5 g, a value similar to that generally accepted.

The protein sulphur passed to the omasum was equivalent to approximately 50.3% of the total sulphur entering the rumen.

The ester sulphate and soluble organic sulphur that left the rumen in digesta were equivalent to approximately 0.7% of the total sulphate intake and 0.4% of the total sulphur entering the rumen, respectively.

Three models (named A, B and C) were constructed for these experiments, each based on the same assumptions as those described in the low sulphur intake experiment. The block diagrams of the models A, B and C are shown in Figures 3.2, 3.3 and 3.4, respectively and the differential equations describing the linear system of each model are presented in Table 3.27.

As the sum of the flow rates F_{06}, F_{26} and F_{36} was calculated (by solving a system of n equations and n + 1 unknowns) to be equivalent to approximately 0.4% of the total flow rate from the rumen, each of the above flow rates were taken as zero for the convenience of calculation.

FIGURE 3.2 Model A. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

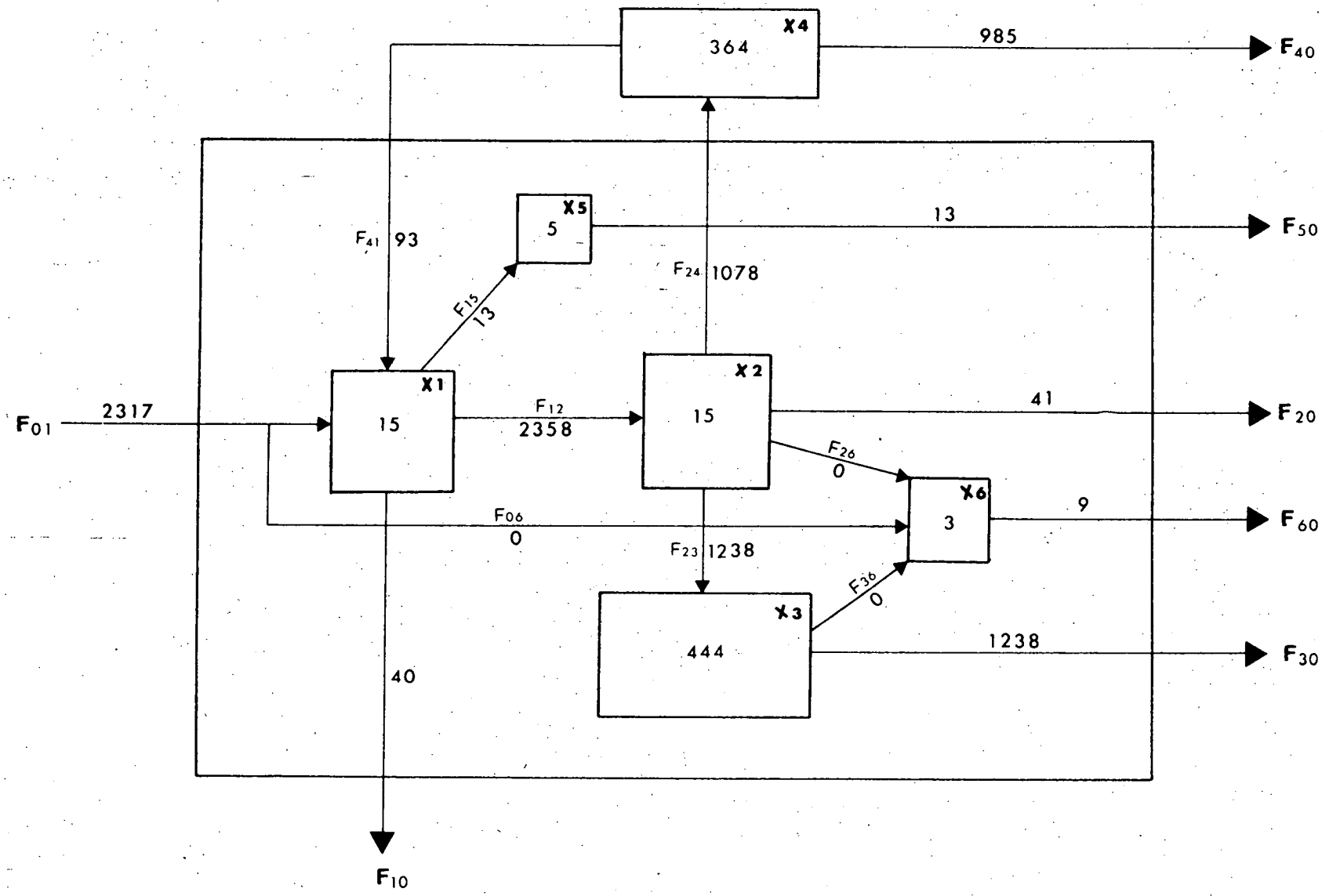
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


X4
364
985
F_{40}
X5
5
13
F_{50}
F_{41} 93
F_{15} 13
F_{24} 1078
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2317
X1
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F_{12}
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Hindgut
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3
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F_{60}
F_{06}
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F_{23} 1238
F_{36}
0
X3
444
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F_{30}
40
F_{10}

FIGURE 3.3 Model B. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

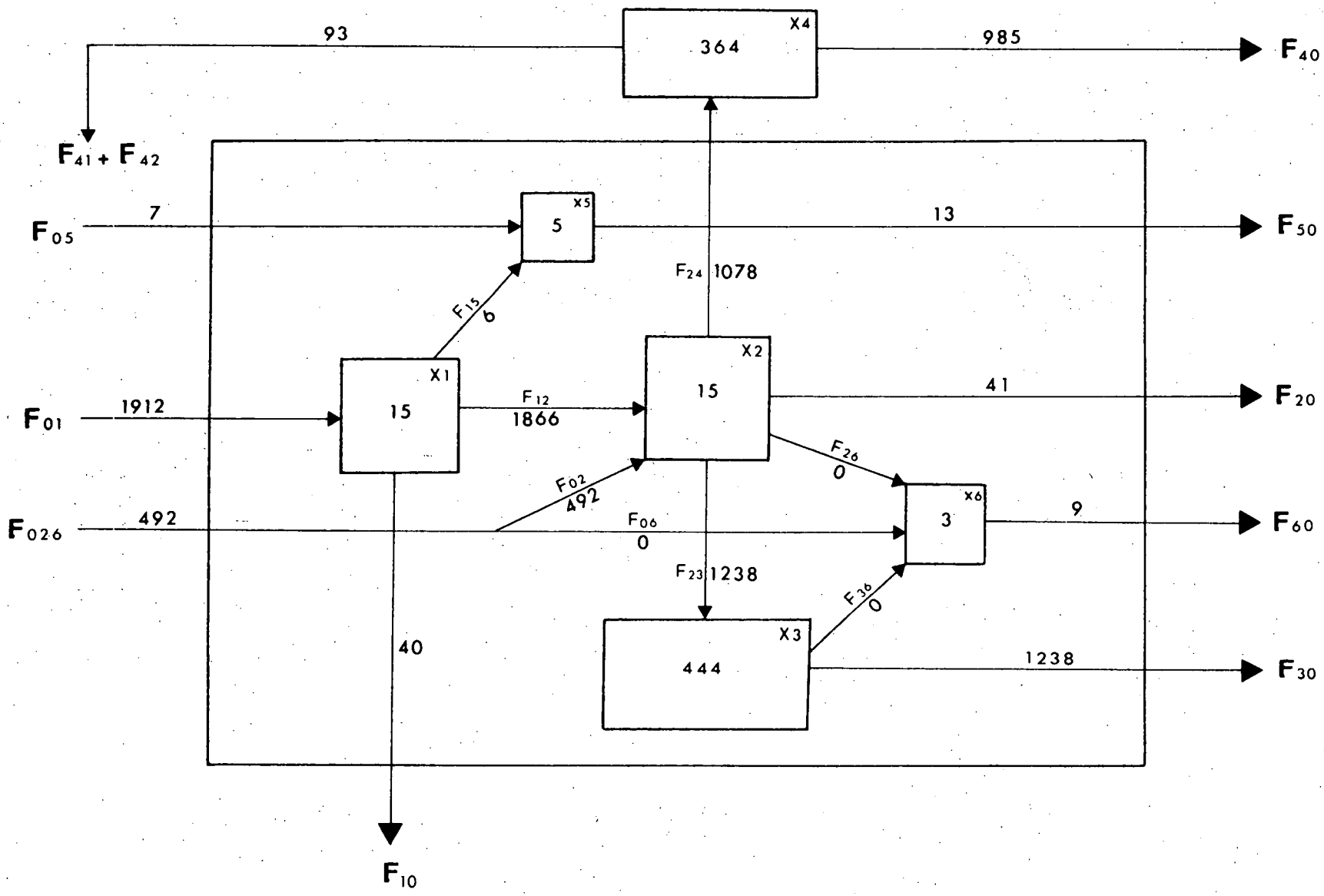
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.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X4
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F_{40}
$F_{41} + F_{42}$
F_{05}
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X5
5
13
F_{50}
F_{24} 1078
F_{13} 6
X2
15
41
F_{20}
Hindgut
F_{01}
1912
X1
15
F_{12}
1866
F_{26}
0
F_{02}
492
F_{026}
492
F_{06}
0
X6
3
9
F_{60}
F_{23} 1238
F_{36}
0
40
X3
444
1238
F_{30}
F_{10}

FIGURE 3.4 Model C. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

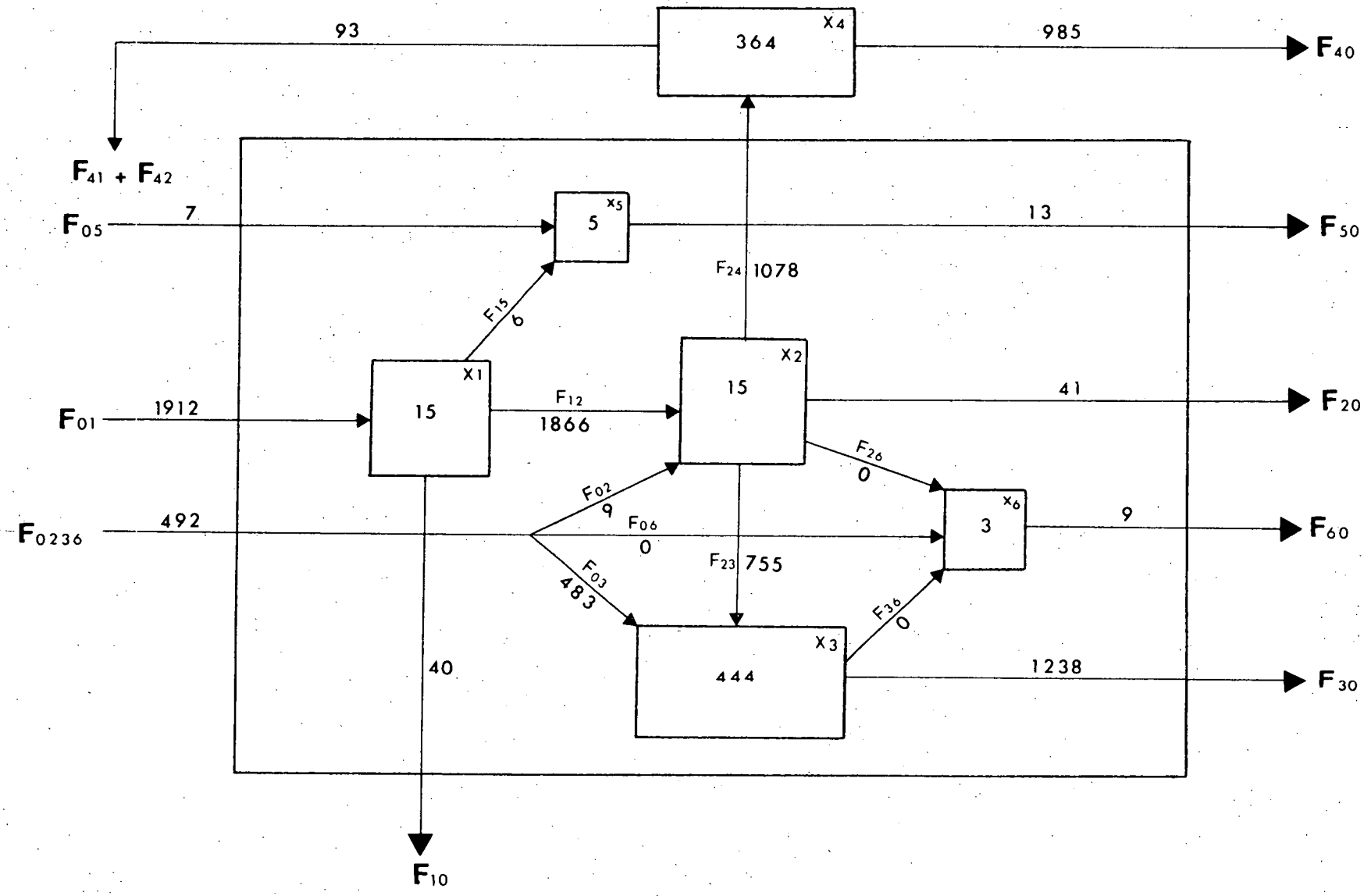
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


93
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F40
F41 + F42
F05
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X5
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F50
F24 1078
F15
6
F01
1912
15
X1
F12
1866
15
X2
41
F20
F26
0
F02
9
F0236
492
F06
0
3
X6
9
F60
F03
483
F23 755
F36
0
40
444
X3
1238
F30
F10

Hindgut

TABLE 3.27

The set of differential equations describing the linear system of each of the three models is:

Model A:

$$dX_1/dt = F_{01} + \phi_{41}X_4 - \phi_{12}X_1 - \phi_{15}X_1 - F_{10}$$
$$dX_2/dt = \phi_{12}X_1 - \phi_{24}X_2 - F_{20} - \phi_{23}X_2$$
$$dX_3/dt = \phi_{23}X_2 - F_{30}$$
$$dX_4/dt = \phi_{24}X_2 - \phi_{41}X_4 - F_{40}$$
$$dX_5/dt = \phi_{15}X_1 - F_{50}$$
$$dX_6/dt = F_{06} + F_{26} + F_{36} - F_{60}$$

Model B:

$$dX_1/dt = F_{01} + \phi_{41}X_4 - \phi_{12}X_1 - \phi_{15}X_1 - F_{10}$$
$$dX_2/dt = \phi_{12}X_1 + F_{02} - \phi_{24}X_2 - F_{20} - \phi_{23}X_2$$
$$dX_3/dt = \phi_{23}X_2 - F_{30}$$
$$dX_4/dt = \phi_{24}X_2 - \phi_{41}X_4 - F_{40} - F_{42}$$
$$dX_5/dt = F_{05} + \phi_{15}X_1 - F_{50}$$
$$dX_6/dt = F_{06} + F_{26} + F_{36} - F_{60}$$

Model C:

$$dX_1/dt = F_{01} + \phi_{41}X_4 - \phi_{12}X_1 - \phi_{15}X_1 - F_{10}$$
$$dX_2/dt = \phi_{12}X_1 + F_{02} - \phi_{24}X_2 - F_{20} - \phi_{23}X_2$$
$$dX_3/dt = \phi_{23}X_2 + F_{03} - F_{30}$$
$$dX_4/dt = \phi_{24}X_2 - \phi_{41}X_4 - F_{40} - F_{42}$$
$$dX_5/dt = F_{05} + \phi_{15}X_1 - F_{50}$$
$$dX_6/dt = F_{06} + F_{26} + F_{36} - F_{60}$$

In these models, the excretion rate which would maintain a constant body sulphate compartment was approximately 684 µg S/min, which is close to the maximum excretion rate of 650 µg S/min taken from results of Kennedy and Milligan (1978). In the present models, the urinary excretion of total sulphur was approximately 877 µg S/min, and since the excretion of inorganic sulphate was taken as 684 µg S/min, the difference of 193 µg S/min has to be accounted for. No attempt was made to differentiate between inorganic sulphate-S, ester sulphate-S and neutral S in urine and faeces. A mean value of 20 measurements of the composition of urinary sulphur taken from various experiments (Bird and Hume, 1971; Bird, 1971; Bird and Moir, 1972; Bird and Thornton, 1972) is (as % of total S (± S.E.)) : neutral-S = 10.9 (± 1.99), ester sulphate-S = 23.6 (± 4.07). From these values the difference of 193 µg S/min can be accounted for as excreted in urine either as ester sulphate-S or as ester sulphate-S and neutral-S.

The differences in rumen volumes and flow rates from the rumen between the low S intake and high S intake experiments were not significant.

Rumen pH in the high S intake was significantly higher ($P < 0.02$) than in the low S intake. There was no significant difference between the low S and high S intake experiments for the calculated absorption constant of rumen sulphide and the sulphide half-life in the rumen.

The N/S ratio in microbial protein was significantly reduced ($P < 0.01$) in the high S diet compared with the low S diet.

There was a high significant increase ($P < 0.001$) in urinary excretion of total sulphur and a significant increase ($P < 0.05$) in faecal excretion of total sulphur in comparing the high S intake with the low S intake experiment. The sulphur retention was greater ($P < 0.10$) with the high S intake experiment.

Both the sulphide lost "calculation" and sulphide lost "prediction" were significantly increased ($P < 0.01$) comparing the high S intake with the low S intake experiment.

The apparent dry matter (DM) digestibility was significantly greater ($P < 0.01$) and the apparent organic matter (OM) digestibility was significantly

greater ($P < 0.05$) when the high S diet was fed than when the low S diet was fed.

The apparent sulphur digestibility was significantly higher ($P < 0.02$) in the high S intake experiment. The apparent OM digested in the rumen was greater ($P < 0.10$) and the dietary OM "truly digested" in the rumen was significantly greater ($P < 0.05$) in the high S intake compared with the low S intake experiment.

The flow of microbial protein from the rumen was significantly increased ($P < 0.02$) and the protein synthesised per 100 g OM "truly digested" in the rumen was significantly increased ($P < 0.01$) in the high S intake compared with the low S intake experiment.

The concentration of total S in rumen liquor was highly significantly increased ($P < 0.001$) in the high S diet compared with the low S diet. Similarly, both the concentration of inorganic sulphate-S and the concentration of sulphide-S in rumen liquor were significantly increased ($P < 0.02$) and the concentration of protein S in rumen liquor was significantly increased ($P < 0.01$) in the high S intake compared with the low S intake experiment.

The daily flow of both total S and protein S from the rumen were highly significantly increased ($P < 0.001$) and the daily flows of both inorganic sulphate-S and sulphide-S from the rumen were significantly increased ($P < 0.02$) in the high S diet compared with the low S diet.

The daily flow of recycled sulphur was greater ($P < 0.10$) in the high S intake than in the low S intake experiment.

PART VI

THE EFFECTS OF VARIATION IN SOLUBLE CARBOHYDRATE CONTENT IN A RATION ON SULPHUR DYNAMICS IN THE RUMEN

Two groups of experiments were conducted (15% starch and 30% starch intake) to examine the effects of dietary energy levels on the synthesis of ruminal microbial protein and the absorption of sulphur from the rumen.

A 15% STARCH INTAKE

(a) Introduction

The extent of microbial growth and accordingly of protein synthesis in a complete medium under anaerobic conditions appears likely to be limited by the amount of biologically useful energy that the micro-organisms can obtain from the available energy source (Bauchop and Elsden, 1960) providing other nutrients are non limiting.

Bacterial growth in the reticulo-rumen requires the provision of ammonia, essential minerals, notably sulphur and phosphorus, and organic matter to provide both an energy source and structural units. One of the major factors influencing the utilisation of ruminal ammonia for microbial growth is the availability of carbohydrates to rumen organisms. While it is known that the nonstructural carbohydrates such as starch, promote utilisation of NH_3-N in the rumen (McDonald, 1952; Chalmers and Synge, 1954b; Lewis and McDonald, 1958; Phillipson *et al.*, 1962; Robertson and Hawke, 1965; Stern *et al.*, 1978) the optimum nonstructural carbohydrate to nitrogen ratio for microbial growth has not yet been determined. Stern *et al.* (1978) found that a major factor affecting the utilisation of degraded dietary nitrogen was the type and rate of availability of carbohydrates. It is apparent that the extent and rate of degradation in the rumen of both the nitrogen and carbohydrate sources are quite important in determining the efficiency of microbial growth.

Smith (1979) suggests that a controlled supply of energy is of paramount importance for encouraging efficient synthesis of microbial N compounds and that cooked starch appears to be more satisfactory for this purpose than soluble sugars, although chemical modification of the latter may improve their value. Manipulation of energy supply is likely to be more successful in improving non-protein nitrogen use than manipulation

of ammonia release.

(b) Materials and Methods

The animals, the feeding regime and the experimental procedures used in these experiments were the same as those described in the high sulphur intake experiment (Part V). The diet used was also the same, except that 15% of the oat hulls in the high sulphur intake diet were replaced by starch. The ration provided daily 12.9 g total nitrogen and 2.30 g total sulphur with a N/S ratio of 5.6/1. The composition of the diet is shown in Appendix 4.

(c) Results and Discussion

The results of these experiments are presented in Tables 4.1 to 4.25. Similar recoveries of ^{51}Cr were obtained as in previous experiments (see Table 4.2).

The mean inorganic sulphate concentration in strained rumen fluid was 3.8 to 4.4 µg S/mℓ (see Table 4.16) and c. 1.7% of the inorganic sulphate intake passed unchanged to the omasum.

The calculated absorption constants for rumen sulphide at the measured rumen pH values and the estimated sulphide absorption half-lives are presented in Table 4.10. In the present experiments only small fluctuations were noted in rumen pH (see Table 4.9) and sulphide-sulphur concentrations (see Table 4.17). The mean rumen pH was 6.03 to 6.49 and the sulphide-sulphur concentration 4.1 to 4.6 µg S/mℓ strained rumen fluid. Little sulphide passed from the rumen in the digesta which was approximately 1.3% of the total sulphur entering the rumen, which is similar to the values of 0.4% - 2.8% found by Bird and Hume (1971).

The absorption of sulphide from the rumen was of the order of 1165 to 1242 mg S/day, thus c. 43.9% to 46.8% of the total sulphur entering the rumen respectively was absorbed as sulphide. In these experiments it was found that 282 - 475 mg S/day (total sulphur) was recycled to the rumen (estimated by difference).

The nitrogen to sulphur ratio (N/S) found in microbial protein precipitated

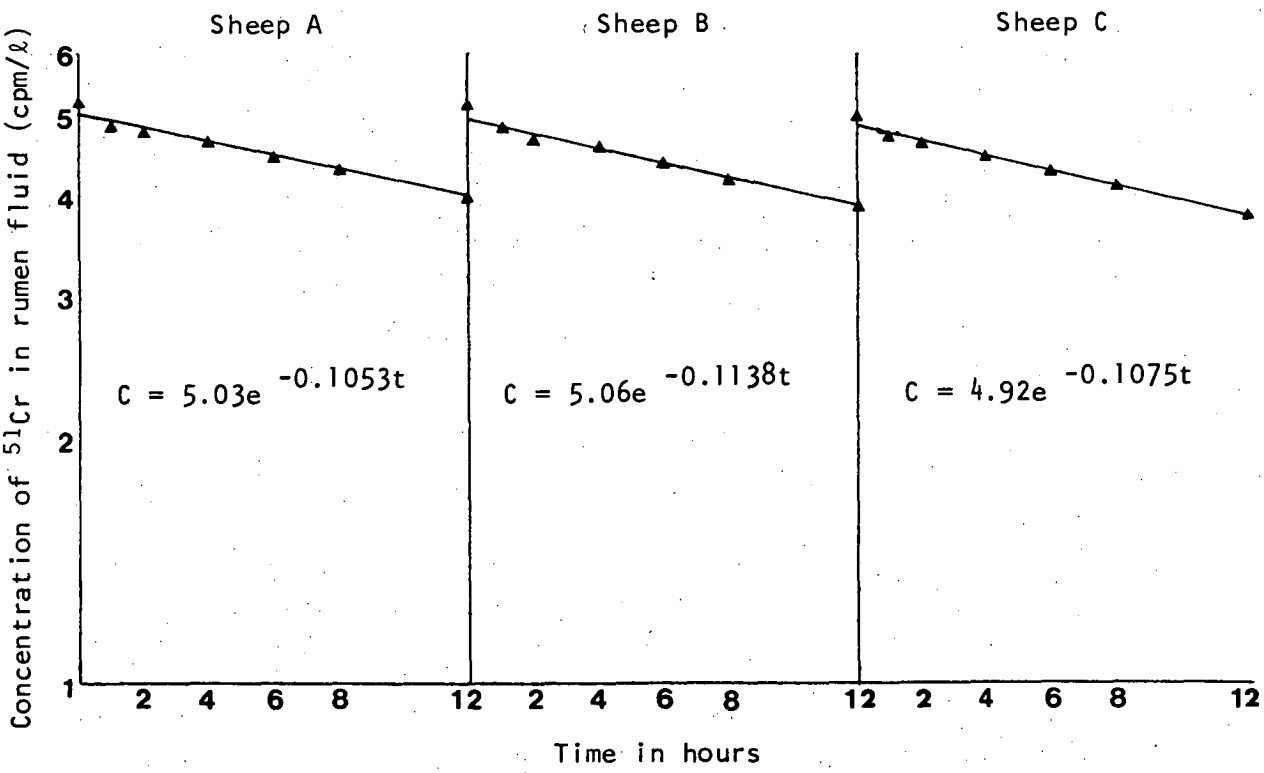


FIGURE 4.1 Changes in the rumen concentration of ^{51}Cr-EDTA following administration on successive days. Each curve represents the mean result of three successive days of the ^{51}Cr-EDTA infusion into the rumen.

TABLE 4.1

Rumen fluid volumes and flow rates. The volumes are calculated at 0, 24, 48 hours. The flow rates are given in litres per day

Sheep No.	A		B		C	
Day	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d
1	3.200	8.082	3.091	7.955	2.986	8.140
2	3.235	8.167	3.087	7.947	2.999	8.199
3	3.210	8.123	3.134	8.128	3.030	8.297
Mean	3.215	8.124	3.104	8.010	3.005	8.212
CV*	0.5612	0.5230	0.8393	1.2771	0.7522	0.9661

* Coefficient of variation of individual measurements

TABLE 4.2

The cumulative recovery of ^{51}Cr in excreta following intraruminal administration of ^{51}Cr-EDTA to sheep fed 800 g pellets daily. The collection period was 9 days.

Sheep No.	Recovery (% of dose)		
	Faeces	Urine	Total
A	99.0	2.0	101.0
B	97.9	2.4	100.3
C	96.8	3.2	100.0
Mean	97.9	2.5	100.5
± S.E. of the mean	0.62	0.36	0.29

TABLE 4.3

^{51}Cr-EDTA half-time in the rumen (h), ^{51}Cr-EDTA mean retention time in the rumen (h), average daily water intake during the markers experimental period and digestibility trial (mℓ) and apparent sulphur digestibility (%)

	Sheep No. A	B	C	Mean ±	S.E.
^{51}Cr-EDTA half-time in the rumen	6.6	6.4	6.1	6.4	0.15
^{51}Cr-EDTA mean retention time in the rumen	9.5	9.3	8.8	9.2	0.22
Average daily water intake during the markers experimental period	2190	2060	2104	2118	38.2
Average daily water intake during the digestibility trial	2290	2150	2027	2156	75.9
Apparent sulphur digestibility	72.8	79.3	79.2	77.1	2.15

TABLE 4.4

Intakes and Digestibility of Dry matter (DM) and Organic matter (OM)

	Sheep No. A	B	C	Mean ±	S.E.
DM					
Intake (g/day):	737	737	737	737	0
Faecal output (g/day):	220	228	229	226	2.7
Apparently digested (g/day):	517	509	508	511	2.7
Apparent digestibility (%):	70.1	69.1	68.9	69.3	0.36
OM					
Intake (g/day):	673	673	673	673	0
Faecal output (g/day):	187	194	203	195	4.8
Apparently digested (g/day):	486	479	470	478	4.8
Apparent digestibility (%):	72.3	71.2	69.8	71.1	0.72
Digestible OM intake (g/day/kg $BW^{0.75}$)	33.8	34.2	31.6	33.2	0.82
BW = sheep wt (kg):	35.0	33.8	36.6	35.1	0.81

TABLE 4.5

Sulphur intake and excretion data

	Sheep No. A	B	C	Mean	± S.E.
Sulphur intake (mg/day):	2298	2298	2298	2298	0
Faecal sulphur (mg/day):	625	475	478	526	49.6
Urine sulphur (mg/day):	1138	1282	1414	1278	79.7
Sulphur retention:					
mg/day:	535	541	406	494	44.0
mg/100 mg S intake:	23.3	23.6	17.7	21.5	1.91

TABLE 4.6

Sulphur lost from the rumen (mg S/day)

	Sheep No. A	B	C	Mean	± S.E.
Sulphur intake (1)	2298	2298	2298	2298	0
Daily flow of total S from rumen (2)	1531	1415	1398	1448	41.6
Sulphur lost from the rumen other than flow down the tract (S^{2-} lost "calculation") (1)-(2) = (3)	767	883	900	850	41.6
Faecal S	625	475	478	526	49.6
Urinary S	1138	1282	1414	1278	79.7
Intake S - Faecal S	1673	1823	1820	1772	49.6
Sulphide lost "prediction" (4)	1242	1165	1214	1207	22.5
Estimated recycled S (4) - (3)	475	282	314	357	59.4

TABLE 4.7

Digestion of organic matter (OM) in the rumen of sheep

	Sheep No. A	B	C	Mean ± S.E.
OM intake (g/day)	673	673	673	673 0
Faecal OM output (g/day)	187	194	203	195 4.8
OM apparently digested: Amount (g/day)	486	479	470	478 4.8
as % of intake	72.3	71.2	69.8	71.1 0.72
OM in digesta leaving rumen (%)	4.34	4.42	4.31	4.36 0.033
OM leaving rumen (g/day)	353	354	354	354 0.5
Apparent OM digested in rumen: Amount (g/day)	320	319	319	319 0.5
as % of intake	47.6	47.4	47.4	47.5 0.07
Microbial nitrogen leaving rumen (g/day)	13.21	12.78	12.51	12.83 0.204
Microbial OM leaving rumen (g/day)	126	122	119	122 1.9
Dietary OM leaving rumen (g/day)	227	232	235	232 2.4
Dietary OM "truly digested" in rumen: Amount (g/day)	446	441	438	441 2.4
as % of OM intake	66.3	65.5	65.1	65.7 0.35
as % of total OM digested	91.8	92.0	93.3	92.4 0.49

TABLE 4.8

Mean flow rate and protein production data

	Sheep No. A	B	C	Mean ± S.E.
Rumen volume (ℓ)	3.215	3.104	3.005	3.108 0.0607
Flow of digesta from rumen (ℓ/day)	8.124	8.010	8.212	8.115 0.0585
Flow of TA-nitrogen (g/day)	13.21	12.78	12.51	12.83 0.204
Flow of microbial protein (TA-nitrogenx6.25) (g/day)	82.6	79.9	78.2	80.2 1.27
Protein synthesised per 100 g OM "truly digested" in rumen (g)	18.5	18.1	17.8	18.1 0.19

TABLE 4.9

Rumen fluid pH sampled every two hours over an eight hour period

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	6.60	6.65	6.60	6.55	6.50	6.58	0.866	6.35	6.45	6.40	6.40	6.35	6.39	0.655
B	6.45	6.40	6.45	6.35	6.45	6.42	0.697	6.25	6.30	6.40	6.30	6.35	6.32	0.902
C	5.95	6.15	6.00	6.10	5.95	6.03	1.506	5.95	6.00	6.00	6.00	6.10	6.01	0.911

* Coefficient of variation of individual measurements

TABLE 4.10

The calculated absorption constants (K_R) for rumen sulphide at rumen pH values and the estimated sulphide absorption half-lives

Sheep No.	pH	% H_2S	% HS^-	K_R	Rumen sulphide half-life (min)
A	6.49	64.5	35.5	-0.0628	11.0
B	6.37	70.3	29.7	-0.0663	10.5
C	6.02	84.1	15.9	-0.0747	9.3
Mean	6.29	73.0	27.0	-0.0679	10.3
± S.E.	0.141	5.82	5.82	0.00353	0.52

TABLE 4.11

Concentration of sulphur in rumen fluid (µg S/mℓ)

	Sheep No. A	B	C	Mean ± S.E.
Total S	188	177	170	178 5.3
Total sulphate S	5.3	5.4	6.0	5.6 0.22
Ester sulphate S	1.4	1.4	1.7	1.5 0.09
Inorganic sulphate S	3.9	4.0	4.3	4.1 0.13
Sulphide S	4.6	4.2	4.1	4.3 0.15
Protein S	179	167	160	169 5.6
Soluble organic S	1.2	1.2	1.3	1.3 0.04
Neutral S	183	171	164	173 5.5
Total reducible S	5.3	5.4	6.0	5.6 0.22

TABLE 4.12

The concentrations of rumen fluid total sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	183	195	193	185	196	191	3.1	181	179	191	189	190	186	2.9
B	167	177	180	169	180	175	3.4	174	171	184	181	183	179	3.2
C	166	175	165	175	176	171	3.2	165	172	174	164	170	169	2.7

* Coefficient of variation of individual measurements

TABLE 4.13

The concentrations of plasma inorganic sulphate in sheep taken at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	53	54	53	52	54	53	2.0	51	49	50	49	51	50	2.3
B	48	50	47	49	46	48	2.8	51	48	51	48	51	50	2.9
C	50	52	50	50	49	50	2.5	49	48	50	48	52	49	3.1

* Coefficient of variation of individual measurements

TABLE 4.14

The concentrations of rumen fluid total sulphate sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	5.1	5.1	5.0	5.3	5.2	5.1	2.02	5.4	5.4	5.3	5.6	5.3	5.4	2.11
B	5.1	5.3	5.4	5.2	5.4	5.3	2.07	5.5	5.6	5.4	5.6	5.6	5.5	1.39
C	5.7	5.7	5.9	5.8	5.9	5.8	1.79	6.0	6.2	6.1	6.2	6.4	6.2	2.32

* Coefficient of variation of individual measurements

TABLE 4.15

The concentrations of rumen fluid ester sulphate sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	1.4	1.3	1.3	1.4	1.4	1.4	3.85	1.4	1.5	1.4	1.5	1.4	1.4	4.45
B	1.3	1.4	1.5	1.3	1.4	1.4	5.37	1.4	1.4	1.4	1.5	1.6	1.5	5.35
C	1.5	1.5	1.7	1.6	1.7	1.6	4.86	1.7	1.8	1.7	1.8	1.9	1.8	5.08

* Coefficient of variation of individual measurements

TABLE 4.16

The concentrations of rumen fluid inorganic sulphate sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	3.7	3.8	3.7	3.9	3.8	3.8	1.59	3.9	4.0	3.9	4.0	3.9	3.9	1.31
B	3.8	3.9	3.9	3.9	3.9	3.9	1.21	4.1	4.1	4.0	4.1	4.1	4.1	0.74
C	4.2	4.1	4.2	4.2	4.2	4.2	0.68	4.3	4.4	4.4	4.4	4.5	4.4	1.33

* Coefficient of variation of individual measurements

TABLE 4.17

The concentrations of rumen fluid sulphide sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	4.5	4.4	4.5	4.6	4.5	4.5	1.40	4.5	4.7	4.6	4.6	4.5	4.6	1.33
B	4.2	4.2	4.3	4.2	4.3	4.2	1.49	4.2	4.2	4.1	4.3	4.1	4.2	1.94
C	4.0	4.0	4.1	4.0	4.1	4.0	1.52	4.0	4.1	4.0	4.2	4.0	4.1	1.77

* Coefficient of variation of individual measurements

TABLE 4.18

The concentrations of rumen fluid protein sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	175	179	177	177	1.0	181	180	183	181	1.0
B	169	169	167	168	0.8	166	165	166	166	0.6
C	157	159	160	159	1.0	161	161	163	162	0.6

* Coefficient of variation of individual measurements

TABLE 4.19

The nitrogen to sulphur ratio (N/S) in ruminal microbial protein fraction of sheep sampled at different times of the day

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	9.01	9.12	9.05	9.06	0.615	9.03	9.07	9.14	9.08	0.613
B	9.57	9.59	9.61	9.59	0.209	9.44	9.57	9.52	9.51	0.690
C	9.49	9.45	9.57	9.50	0.643	9.53	9.48	9.61	9.54	0.687

* Coefficient of variation of individual measurements

TABLE 4.20

The concentrations of microbial protein sulphur in the rumen of sheep sampled at different times of the day (mg S/100 g wet precipitate)

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	76	76	74	75	1.4	76	77	79	77	2.1
B	76	76	79	77	2.3	75	79	76	77	2.7
C	81	78	83	81	2.9	82	80	84	82	2.5

* Coefficient of variation of individual measurements

TABLE 4.21

The concentrations of microbial protein nitrogen in the rumen of sheep sampled at different times of the day (% N in wet precipitate)

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	0.681	0.697	0.673	0.684	1.787	0.685	0.702	0.722	0.703	2.634
B	0.728	0.733	0.762	0.741	2.477	0.704	0.753	0.728	0.728	3.364
C	0.764	0.741	0.796	0.767	3.601	0.786	0.761	0.811	0.786	3.181

* Coefficient of variation of individual measurements

TABLE 4.22

The concentrations of fluid soluble organic sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	1.1	1.3	1.1	1.2	6.62	1.2	1.3	1.2	1.2	5.91
B	1.2	1.3	1.2	1.3	4.45	1.3	1.1	1.2	1.2	6.34
C	1.3	1.4	1.4	1.4	5.26	1.3	1.2	1.3	1.3	4.38

* Coefficient of variation of individual measurements

TABLE 4.23

Rumen fluid sulphur pools (mg S)

	Sheep No. A	B	C	Mean	± S.E.
Total S	606	548	512	555	27.4
Neutral S	589	532	494	538	27.7
Protein S	576	519	481	525	27.8
Total reducible S	17	17	18	17	0.4
Total sulphate S	17	17	18	17	0.4
Ester sulphate S	5	4	5	5	0.2
Inorganic sulphate S	12	13	13	13	0.2
Sulphide S	15	13	12	13	0.7
Soluble organic S	3.9	3.8	4.0	3.9	0.05

TABLE 4.24

Daily flow of sulphur from the rumen (mg S/day)

	Sheep No. A	B	C	Mean	± S.E.
Total S	1531	1415	1398	1448	41.6
Neutral S	1488	1372	1349	1403	43.0
Protein S	1457	1338	1314	1370	44.0
Total reducible S	43	43	49	45	2.0
Total sulphate S	43	43	49	45	2.0
Ester sulphate S	11	11	14	12	0.8
Inorganic sulphate S	32	32	35	33	1.2
Sulphide S	37	34	33	35	1.2
Soluble organic S	9.8	9.8	10.8	10.1	0.36

TABLE 4.25

E.C.F. (% of body weight), plasma inorganic sulphate concentration (µg S/mℓ), body sulphate pools (mg S) and daily excretion flow rates of inorganic sulphate (mg S/day)

	Sheep No. A	B	C	Mean ±	S.E.
E.C.F.	22.7	22.7	25.2	23.6	0.85
Plasma inorganic sulphate concentration	52	49	50	50	0.8
Body sulphate pools	411	376	462	416	24.9
Daily excretion flow rates of inorganic sulphate	768	883	900	850	41.6

with tungstic acid was approximately 9.4/l.

Data pertaining to the digestion of organic matter (OM) are presented in Table 4.7. The apparent digestibility of OM in the whole tract was approximately 71.1% and the apparent OM digested in the reticulo-rumen was equivalent to approximately 47.5% of intake. Of the total dietary OM digested it was estimated that approximately 92.4% was digested in the rumen.

The microbial protein synthesised per 100 g OM "truly digested" in the rumen was approximately 18.1 g, a value similar to that generally accepted.

The protein sulphur that passed to the omasum was equivalent to approximately 51.6% of the total sulphur entering the rumen.

The ester sulphate and soluble organic sulphur that left the rumen in digesta were equivalent to approximately 0.6% of the total sulphate intake and 0.4% of the total sulphur entering the rumen, respectively.

Three models (named A, B and C) were constructed for these experiments, each based on the same assumptions as those described in the low sulphur intake experiment (Part IV). The block diagrams of the models A, B and C are shown in Figures 4.2, 4.3 and 4.4, respectively. The differential equations describing the linear system of each model were the same as those described in the high sulphur intake experiment (Part V) and are presented in Table 3.27.

As the sum of the flow rates F_{06}, F_{26} and F_{36} was calculated (by solving a system of n equations and n + 1 unknowns) to be equivalent to approximately 0.4% of the total flow rate from the rumen, each of the above flow rates were taken as zero for the convenience of calculation.

In these models, the excretion rate which would maintain a constant body sulphate compartment was approximately 591 µg S/min, which is well within the range of excretion rates of 540 - 650 µg S/min from results of Kennedy and Milligan (1978). Taking a mean value of plasma inorganic sulphate concentration of approximately 50 µg S/mℓ, the excretion rate, calculated from results of Bishara and Bray (1978b), is 400 µg S/min, a value less than that reported by Kennedy and Milligan (1978). This is discussed more

FIGURE 4.2 Model A. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

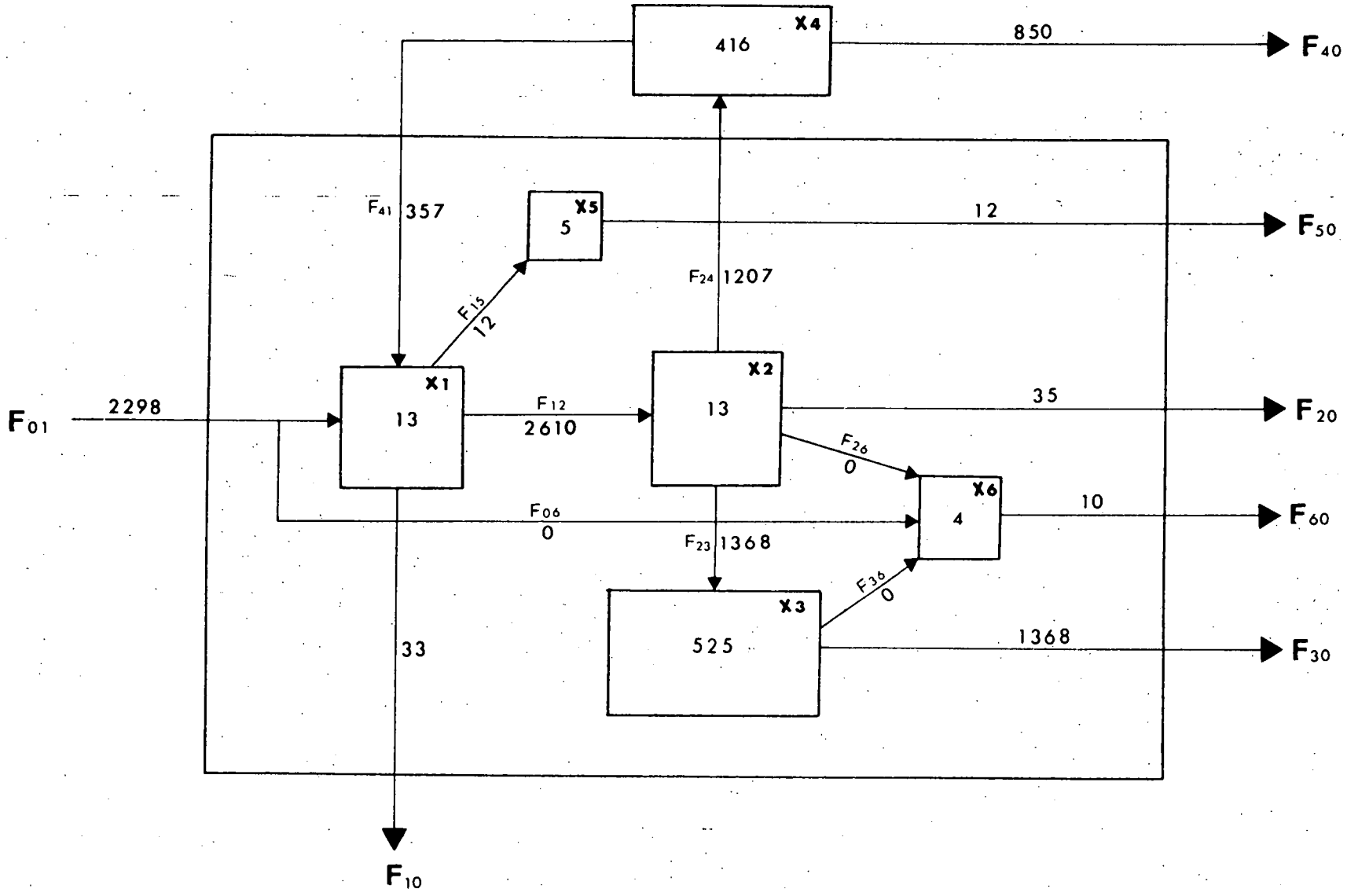
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


X4
416
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F40
F41 357
X5
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F24 1207
X1
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X2
13
35
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0
X6
4
10
F60
F06
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F23 1368
F36
0
X3
525
1368
F30
33
F10

Hindgut

FIGURE 4.3 Model B. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

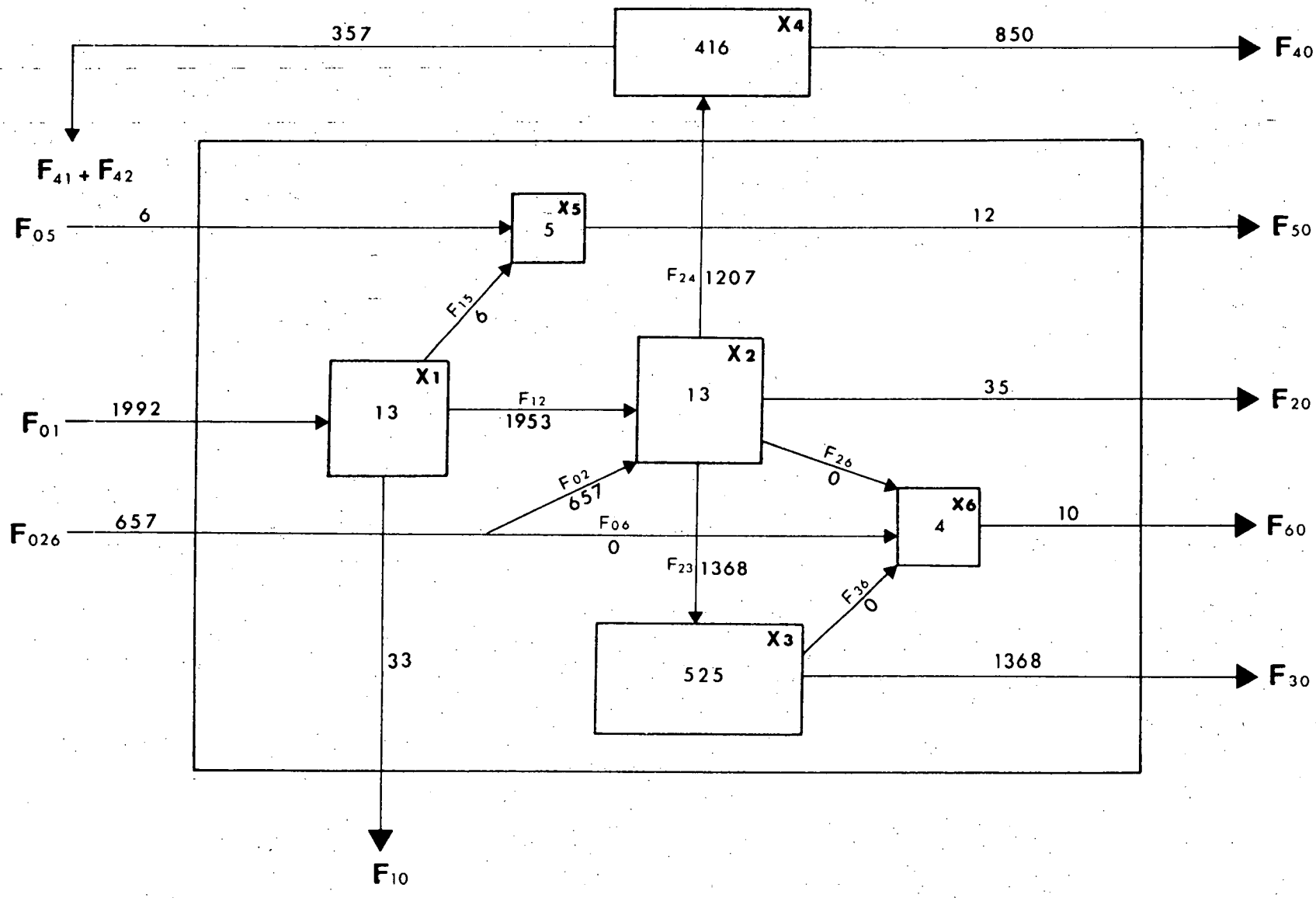
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


357
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F12
1953
X2
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F02
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F60
F026
657
F06
0
F23 1368
F36
0
X3
525
1368
F30
33
F10

Hindgut

FIGURE 4.4 Model C. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

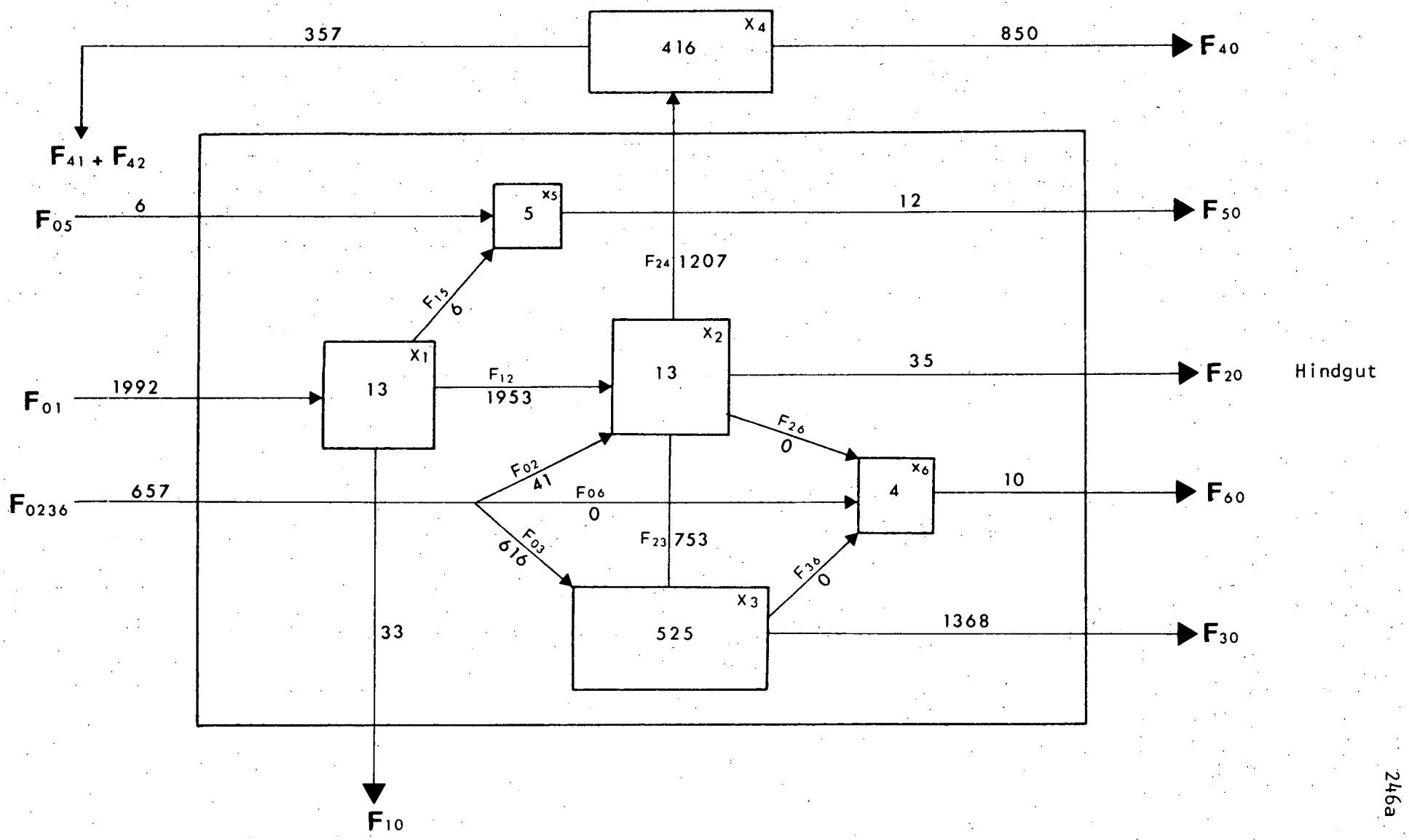
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


357
416
X_4
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$F_{41} + F_{42}$
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X_5
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F_{50}
F_{24} 1207
F_{15}
6
X_2
F_{01}
1992
13
X_1
F_{12}
1953
13
35
F_{20}
Hindgut
F_{26}
0
F_{02}
41
F_{0236}
657
F_{06}
0
4
X_6
10
F_{60}
F_{03}
616
F_{23} 753
F_{36}
0
33
525
X_3
1368
F_{30}
F_{10}

fully in Part VIII. In the present models, the urinary excretion of total sulphur was approximately 887 µg S/min, and since the excretion of inorganic sulphate was taken as 591 µg S/min, the difference of 296 µg S/min has to be accounted for. No attempt was made to differentiate between inorganic sulphate-S, ester sulphate-S and neutral S in urine and faeces. Taking the mean values for neutral-S and ester sulphate-S in urine from various experiments (see high sulphur intake experiment, Part V), the above difference of 296 µg S/min can be accounted for as excreted in urine as ester sulphate-S and neutral S.

The differences in rumen volumes and flow rates between the high S intake and 15% starch intake experiments were not significant. Similarly, the differences in half-times and mean retention times in the rumen of ^{51}Cr-EDTA between the high S intake and 15% starch intake experiments were not significant.

Rumen pH in the high S intake was significantly higher ($P < 0.05$) than in the 15% starch intake. There was a significant increase ($P < 0.02$) in the calculated absorption constant (in absolute value) of rumen sulphide in comparing the 15% starch intake with the high S intake experiment.

There was no significant difference between the high S and 15% starch intake experiments for the estimated rumen sulphide half-life, the N/S ratio in microbial protein, the sulphur retention, the sulphide lost "calculation" and the urinary and faecal excretion of total sulphur. The sulphide lost "prediction" was greater ($P < 0.10$) in the 15% starch intake than in the high S intake experiment.

Both the apparent dry matter (DM) digestibility and the apparent organic matter (OM) digestibility were greater ($P < 0.10$) in the 15% starch intake than in the high S intake experiments. Similarly, the apparent sulphur digestibility, the apparent OM digested in the rumen, the dietary OM "truly digested" in the rumen, the flow of microbial protein from the rumen and the protein synthesised per 100 g OM "truly digested" in the rumen were greater ($P < 0.10$) in the 15% starch intake compared with the high S intake experiment.

The concentration of plasma inorganic sulphate was significantly increased ($P < 0.02$) in the 15% starch diet compared with the high S diet.

There was no significant difference between the high S and 15% starch intake experiments for the concentrations of total S, ester sulphate-S,

soluble organic S and neutral S in rumen liquor. The concentration of sulphide-S in rumen liquor was significantly decreased ($P < 0.01$) and the concentration of protein S in rumen liquor was significantly increased ($P < 0.01$) in the 15% starch intake compared with the high S intake experiment. The concentrations of total sulphate-S, the inorganic sulphate-S and the total reducible S in rumen liquor were greater ($P < 0.10$) in the high S intake than in the 15% starch intake experiment.

There was no significant difference between the high S and 15% starch intake experiments for the daily flow of total S, neutral S, ester sulphate-S and soluble organic S from the rumen. The daily flow of sulphide-S from the rumen was significantly decreased ($P < 0.01$) in the 15% starch diet compared with the high S diet. Similarly, the daily flows of total reducible S, total sulphate-S and inorganic sulphate-S from the rumen were significantly decreased ($P < 0.05$) in the 15% starch diet compared with the high S diet. The daily flow of protein S from the rumen was greater ($P < 0.10$) in the 15% starch intake compared with the high S intake experiment.

The daily flow of recycled sulphur was greater ($P < 0.10$) in the 15% starch intake than in the high S intake experiment.

PART VII

THE EFFECTS OF A HIGH SOLUBLE CARBOHYDRATE CONTENT IN A RATION ON SULPHUR DYNAMICS IN THE RUMEN

A 30% STARCH INTAKE

(a) Introduction

The effects of various carbohydrate sources in promoting microbial protein synthesis have been extensively investigated. Carbohydrates differ widely in the ways in which they fulfil their main function of making energy and carbon skeletons available for the activity of rumen microflora. Helmer and Bartley (1971) have pointed out that the most effective carbohydrate is starch and the least effective cellulose when urea was used as a source of dietary nitrogen. The ability of ruminal micro-organisms to utilise non-protein nitrogen and the influence of different energy sources on urea utilisation have been demonstrated by many authors. Chappell and Fontenot (1968) and Preston *et al.* (1961) in *in vivo* metabolism studies have shown that readily available carbohydrates increased urea utilisation.

Arias *et al.* (1951) found that increasing the energy content of the fermentation medium resulted in increased conversion of urea nitrogen into bacterial protein with six sources (dextrose, cane molasses, sucrose, starch, cellulose and ground corn cobs) of energy tested. They also observed that small amounts of a readily available carbohydrate aided cellulose digestion, which in turn increased urea utilisation for protein synthesis, whereas large amounts of such materials inhibited cellulose digestion.

In these experiments the influence of increasing dietary starch level on the synthesis of protein by rumen microflora and the absorption of sulphur from the rumen was examined.

(b) Materials and Methods

The animals, the feeding regime and the experimental procedures used in the present experiments were the same as those described in the high sulphur intake experiment (Part V). The diet used was also the same, except that 30% of the oat hulls in the high sulphur intake diet were replaced by starch. The ration provided daily 12.3 g total nitrogen and 2.21 g total sulphur with a N/S ratio of 5.6/1. The composition of the diet is shown

in Appendix 5.

(c) Results and Discussion

The results of the present experiments are presented in Tables 5.1 to 5.25. Similar recoveries of ^{51}Cr were obtained as in previous experiments (see Table 5.2).

The mean inorganic sulphate concentration in strained rumen fluid was 3.5 to 3.7 µg S/mℓ (see Table 5.16) and c. 1.5% of the inorganic sulphate intake passed unchanged to the omasum.

The calculated absorption constants for rumen sulphide at the measured rumen pH values and the estimated sulphide absorption half-lives are presented in Table 5.10. In these experiments only small fluctuations were noted in rumen pH (see Table 5.9) and sulphide-sulphur concentrations (see Table 5.17). The mean rumen pH was 5.50 to 5.81 and the sulphide-sulphur concentration 3.6 to 3.9 µg S/mℓ strained rumen fluid. Little sulphide passed from the rumen in the digesta which was approximately 1.1% of the total sulphur entering the rumen, which is similar to the values of 0.4% - 2.8% found by Bird and Hume (1971).

The absorption of sulphide from the rumen was of the order of 1210 to 1332 mg S/day, thus c. 42.0% to 46.0% of the total sulphur entering the rumen respectively was absorbed as sulphide. In the present experiments it was estimated by difference that 621 - 702 mg S/day (total sulphur) was recycled to the rumen.

The nitrogen to sulphur ratio (N/S) found in microbial protein precipitated with tungstic acid was approximately 9.5/1.

Data pertaining to the digestion of organic matter (OM) are presented in Table 5.7. The apparent digestibility of OM in the whole tract was approximately 77.4% and the apparent OM digested in the reticulo-rumen was equivalent to approximately 47% of intake. Of the total dietary OM digested it was estimated that approximately 87.7% was digested in the rumen.

The microbial protein synthesised per 100 g OM "truly digested" in the rumen was approximately 20.1 g, a value similar to that generally accepted.

The protein sulphur that passed to the omasum was equivalent to approximately

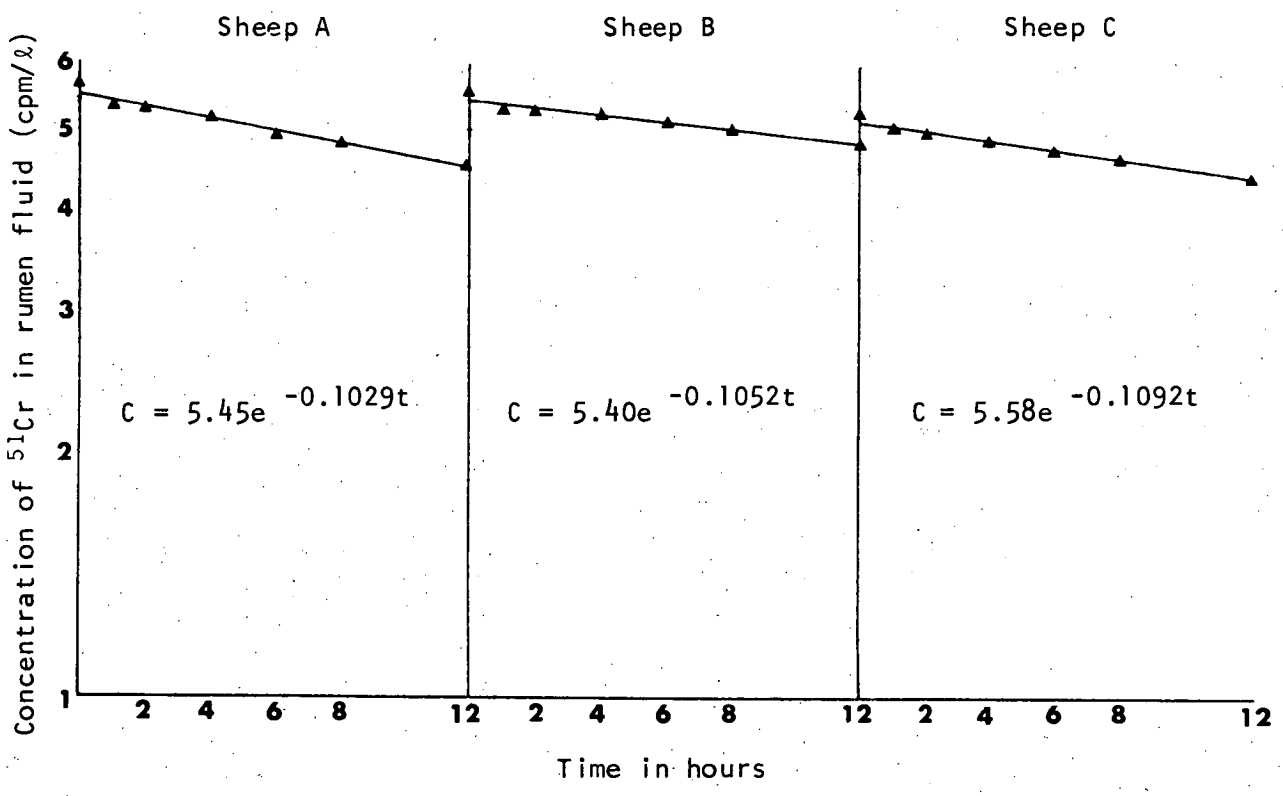


FIGURE 5.1 Changes in the rumen concentration of ^{51}Cr-EDTA following administration on successive days. Each curve represents the mean result of three successive days of the ^{51}Cr-EDTA infusion into the rumen.

TABLE 5.1

Rumen fluid volumes and flow rates. The volumes are calculated at 0, 24, 48 hours. The flow rates are given in litres per day

Sheep No.	A		B		C	
Day	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d	Volume ℓ	Flow Rate ℓ/d
1	3.302	8.122	3.230	8.170	3.114	8.199
2	3.349	8.272	3.205	8.121	3.171	8.262
3	3.315	8.221	3.195	8.021	3.189	8.367
Mean	3.322	8.205	3.210	8.104	3.158	8.276
CV*	0.7310	0.9301	0.5622	0.9373	1.2401	1.0262

* Coefficient of variation of individual measurements

TABLE 5.2

The cumulative recovery of ^{51}Cr in excreta following intraruminal administration of ^{51}Cr-EDTA to sheep fed 800 g pellets daily. The collection period was 9 days.

Sheep No.	Recovery (% of dose)		
	Faeces	Urine	Total
A	99.2	2.1	101.3
B	98.1	2.9	101.0
C	98.1	2.4	100.5
Mean	98.5	2.4	100.9
± S.E. of the mean	0.37	0.26	0.23

TABLE 5.3

^{51}Cr-EDTA half-time in the rumen (h), ^{51}Cr-EDTA mean retention time in the rumen (h), average daily water intake during the markers experimental period and digestibility trial (mℓ) and apparent sulphur digestibility (%)

	Sheep No. A	B	C	Mean ± S.E.
^{51}Cr-EDTA half-time in the rumen	6.7	6.6	6.3	6.6 0.11
^{51}Cr-EDTA mean retention time in the rumen	9.7	9.5	9.2	9.5 0.16
Average daily water intake during the markers experimental period	2716	2484	2282	2494 125.4
Average daily water intake during the digestibility trial	2503	2391	2216	2370 83.5
Apparent sulphur digestibility	80.1	78.6	82.3	80.3 1.07

TABLE 5.4

Intakes and Digestibility of Dry matter (DM) and Organic matter (OM)

	Sheep No. A	B	C	Mean ± S.E.
DM				
Intake (g/day):	733	733	733	733 0
Faecal output (g/day):	198	150	177	175 14.0
Apparently digested (g/day):	535	583	556	558 14.0
Apparent digestibility (%):	73.0	79.6	75.9	76.1 1.92
OM				
Intake (g/day):	675	675	675	675 0
Faecal output (g/day):	173	129	155	153 12.6
Apparently digested (g/day):	502	546	520	522 12.6
Apparent digestibility (%):	74.4	80.8	77.0	77.4 1.87
Digestible OM intake (g/day/kg $BW^{0.75}$)	34.8	38.8	34.7	36.1 1.33
BW = sheep wt (kg):	35.1	34.0	36.9	35.3 0.84

TABLE 5.5

Sulphur intake and excretion data

	Sheep No. A	B	C	Mean ± S.E.
Sulphur intake (mg/day):	2212	2212	2212	2212 0
Faecal sulphur (mg/day):	440	474	391	435 24.2
Urine sulphur (mg/day):	1128	1157	1232	1173 31.1
Sulphur retention:				
mg/day:	644	581	589	604 20.1
mg/100 mg S intake:	29.1	26.2	26.6	27.3 0.91

TABLE 5.6

Sulphur lost from the rumen (mg S/day)

	Sheep No. A	B	C	Mean ± S.E.
Sulphur intake (1)	2212	2212	2212	2212 0
Daily flow of total S from rumen (2)	1582	1623	1609	1605 11.9
Sulphur lost from the rumen other than flow down the tract (S^{2-}lost "calculation") (1)-(2) = (3)	630	589	603	607 11.9
Faecal S	440	474	391	435 24.2
Urinary S	1128	1157	1232	1173 31.1
Intake S - Faecal S	1772	1738	1821	1777 24.2
Sulphide lost "prediction" (4)	1332	1210	1291	1278 35.7
Estimated recycled S (4) - (3)	702	621	688	670 25.0

TABLE 5.7

Digestion of organic matter (OM) in the rumen of sheep

	Sheep No. A	B	C	Mean ± S.E.
OM intake (g/day)	675	675	675	675 0
Faecal OM output (g/day)	173	129	155	153 12.6
OM apparently digested: Amount (g/day)	502	546	520	522 12.6
as % of intake	74.4	80.8	77.0	77.4 1.87
OM in digesta leaving rumen (%)	4.37	4.44	4.29	4.37 0.043
OM leaving rumen (g/day)	358	360	355	358 1.4
Apparent OM digested in rumen: Amount (g/day)	317	315	320	317 1.4
as % of intake	46.9	46.7	47.4	47.0 0.21
Microbial nitrogen leaving rumen (g/day)	14.29	15.10	14.82	14.74 0.238
Microbial OM leaving rumen (g/day)	136	144	141	140 2.3
Dietary OM leaving rumen (g/day)	222	216	214	217 2.6
Dietary OM "truly digested" in rumen: Amount (g/day)	453	459	461	458 2.6
as % of OM intake	67.1	68.0	68.3	67.8 0.38
as % of total OM digested	90.1	84.1	88.8	87.7 1.81

TABLE 5.8

Mean flow rate and protein production data

	Sheep No. A	B	C	Mean ± S.E.
Rumen volume (ℓ)	3.322	3.210	3.158	3.230 0.0484
Flow of digesta from rumen (ℓ/day)	8.205	8.104	8.276	8.195 0.0499
Flow of TA-nitrogen (g/day)	14.29	15.10	14.82	14.74 0.238
Flow of microbial protein (TA-nitrogenx6.25) (g/day)	89.3	94.4	92.6	92.1 1.49
Protein synthesised per 100 g OM "truly digested" in rumen (g)	19.7	20.6	20.1	20.1 0.24

TABLE 5.9

Rumen fluid pH sampled every two hours over an eight hour period

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	5.80	5.70	5.80	5.75	5.80	5.77	0.775	5.90	5.85	5.90	5.80	5.80	5.85	0.855
B	5.75	5.70	5.80	5.70	5.75	5.74	0.729	5.70	5.60	5.65	5.75	5.70	5.68	1.004
C	5.60	5.50	5.60	5.50	5.60	5.56	0.985	5.55	5.40	5.40	5.35	5.50	5.44	1.510

* Coefficient of variation of individual measurements

TABLE 5.10

The calculated absorption constants (K_R) for rumen sulphide at rumen pH values and the estimated sulphide absorption half-lives

Sheep No.	pH	% H_2S	% HS^-	K_R	Rumen sulphide half-life (min)
A	5.81	89.6	10.4	-0.0780	8.9
B	5.71	91.5	8.5	-0.0792	8.8
C	5.50	94.6	5.4	-0.0810	8.6
Mean	5.67	91.9	8.1	-0.0794	8.7
± S.E.	0.091	1.46	1.46	0.00089	0.10

TABLE 5.11

Concentration of sulphur in rumen fluid (µg S/mℓ)

	Sheep No. A	B	C	Mean ±	S.E.
Total S	193	200	194	196	2.3
Total sulphate S	4.1	3.9	4.0	4.0	0.04
Ester sulphate S	0.5	0.3	0.4	0.4	0.06
Inorganic sulphate S	3.5	3.6	3.6	3.6	0.01
Sulphide S	3.9	3.6	3.8	3.8	0.09
Protein S	187	195	190	190	2.3
Soluble organic S	1.1	1.0	1.0	1.0	0.02
Neutral S	189	196	190	192	2.3
Total reducible S	4.1	3.9	4.0	4.0	0.04

TABLE 5.12

The concentrations of rumen fluid total sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	184	194	196	187	196	191	2.9	190	192	194	198	196	194	1.6
B	195	197	200	195	204	198	2.0	198	202	199	204	208	202	1.9
C	190	189	192	199	197	193	2.2	192	190	201	194	200	196	2.3

* Coefficient of variation of individual measurements

TABLE 5.13

The concentrations of plasma inorganic sulphate in sheep taken at different times of the day (μg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	59	58	56	56	58	57	2.0	57	55	57	56	54	56	2.0
B	54	55	52	53	52	54	2.4	53	54	57	55	53	55	2.6
C	57	55	55	53	53	55	2.5	57	57	54	57	56	56	2.1

* Coefficient of variation of individual measurements

TABLE 5.14

The concentrations of rumen fluid total sulphate sulphur in sheep sampled at different times of the day (μg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	3.9	3.8	4.1	4.0	4.2	4.0	4.10	4.1	4.2	4.1	4.4	4.3	4.2	2.50
B	4.0	4.1	3.9	3.9	4.1	4.0	2.22	3.8	3.9	3.9	4.0	3.9	3.9	2.31
C	3.9	3.9	3.8	4.0	4.0	3.9	2.33	4.1	4.1	4.1	3.9	4.2	4.1	2.61

* Coefficient of variation of individual measurements

TABLE 5.15

The concentrations of rumen fluid ester sulphate sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	0.4	0.4	0.5	0.6	0.4	0.5	16.48	0.5	0.5	0.6	0.6	0.6	0.6	12.66
B	0.3	0.3	0.4	0.3	0.3	0.3	14.53	0.3	0.4	0.4	0.3	0.3	0.3	12.30
C	0.4	0.4	0.5	0.3	0.4	0.4	15.61	0.4	0.3	0.3	0.4	0.4	0.4	18.29

* Coefficient of variation of individual measurements

TABLE 5.16

The concentrations of rumen fluid inorganic sulphate sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day							2nd Day						
	0900h	1100h	1300h	1500h	1700h	Mean	CV*	0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	3.5	3.3	3.6	3.4	3.8	3.5	4.91	3.7	3.7	3.6	3.7	3.7	3.7	1.72
B	3.8	3.9	3.6	3.6	3.8	3.7	3.47	3.4	3.6	3.5	3.7	3.6	3.6	2.87
C	3.5	3.5	3.3	3.7	3.5	3.5	4.09	3.7	3.8	3.9	3.5	3.8	3.7	3.39

* Coefficient of variation of individual measurements.

TABLE 5.17

The concentrations of rumen fluid sulphide sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*	2nd Day 0900h	1100h	1300h	1500h	1700h	Mean	CV*
A	3.8	3.8	3.9	3.9	3.9	3.8	1.64	3.8	3.9	3.8	3.9	4.0	3.9	1.40
B	3.6	3.5	3.6	3.6	3.6	3.6	1.11	3.6	3.6	3.5	3.5	3.6	3.6	1.30
C	3.8	3.8	3.8	3.9	3.9	3.8	1.37	3.7	3.8	3.8	3.8	3.8	3.8	1.42

* Coefficient of variation of individual measurements

TABLE 5.18

The concentrations of rumen fluid protein sulphur in sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	184	188	184	185	1.4	188	186	190	188	1.0
B	194	198	199	197	1.3	193	189	195	192	1.4
C	185	188	191	188	1.5	191	188	194	191	1.4

* Coefficient of variation of individual measurements

TABLE 5.19

The nitrogen to sulphur ratio (N/S) in ruminal microbial protein fraction of sheep sampled at different times of the day

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	9.24	9.38	9.28	9.30	0.775	9.30	9.40	9.32	9.34	0.567
B	9.58	9.61	9.52	9.57	0.479	9.52	9.56	9.63	9.57	0.582
C	9.36	9.44	9.49	9.43	0.695	9.46	9.38	9.51	9.45	0.694

* Coefficient of variation of individual measurements

TABLE 5.20

The concentrations of microbial protein sulphur in the rumen of sheep sampled at different times of the day (mg S/100 g wet precipitate)

Sheep No.	1st Day					2nd Day				
	0900h	1300h	1700h	Mean	CV*	0900h	1300h	1700h	Mean	CV*
A	77	82	79	79	2.9	80	83	81	81	2.4
B	79	84	78	81	4.0	79	79	85	81	3.9
C	80	83	85	83	3.4	84	82	86	84	2.7

* Coefficient of variation of individual measurements

TABLE 5.21

The concentrations of microbial protein nitrogen in the rumen of sheep sampled at different times of the day (% N in wet precipitate)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	0.714	0.768	0.732	0.738	3.726	0.741	0.784	0.752	0.759	2.943
B	0.759	0.811	0.747	0.772	4.405	0.751	0.756	0.814	0.774	4.526
C	0.745	0.787	0.809	0.780	4.167	0.799	0.768	0.822	0.796	3.403

* Coefficient of variation of individual measurements

TABLE 5.22

The concentrations of fluid soluble organic sulphur in the rumen of sheep sampled at different times of the day (µg S/mℓ)

Sheep No.	1st Day 0900h	1300h	1700h	Mean	CV*	2nd Day 0900h	1300h	1700h	Mean	CV*
A	1.0	1.0	1.1	1.0	3.50	1.0	1.1	1.1	1.1	2.47
B	1.0	1.0	1.1	1.0	3.50	1.0	1.0	0.9	1.0	3.64
C	1.0	1.1	1.0	1.0	3.50	0.9	1.0	1.0	1.0	3.64

* Coefficient of variation of individual measurements

TABLE 5.23

Rumen fluid sulphur pools (mg S)

	Sheep No.				
	A	B	C	Mean ±	S.E.
Total S	641	643	614	633	9.3
Neutral S	627	630	601	620	9.1
Protein S	620	625	599	615	8.1
Total reducible S	14	13	13	13	0.3
Total sulphate S	14	13	13	13	0.3
Ester sulphate S	1.7	1.0	1.2	1.3	0.19
Inorganic sulphate S	12	12	11	12	0.1
Sulphide S	13	11	12	12	0.4
Soluble organic S	3.5	3.2	3.2	3.3	0.09

TABLE 5.24

Daily flow of sulphur from the rumen (mg S/day)

	Sheep No.				
	A	B	C	Mean ±	S.E.
Total S	1582	1623	1609	1605	11.9
Neutral S	1549	1591	1576	1572	12.4
Protein S	1533	1578	1569	1560	13.8
Total reducible S	34	32	33	33	0.5
Total sulphate S	34	32	33	33	0.5
Ester sulphate S	4	3	3	3	0.4
Inorganic sulphate S	29	29	30	30	0.2
Sulphide S	32	29	31	31	0.9
Soluble organic S	8.6	8.1	8.4	8.4	0.15

TABLE 5.25

E.C.F. (% of body weight), plasma inorganic sulphate concentration (μg S/mℓ), body sulphate pools (mg S) and daily excretion flow rates of inorganic sulphate (mg S/day).

	Sheep No. A	B	C	Mean ± S.E.
E.C.F.	22.7	22.7	25.2	23.6 0.85
Plasma inorganic sulphate concentration	57	54	55	55 0.7
Body sulphate pools	452	417	515	462 28.6
Daily excretion flow rates of inorganic sulphate	630	589	603	607 11.9

54.1% of the total sulphur entering the rumen.

The ester sulphate and soluble organic sulphur that left the rumen in digesta were equivalent to approximately 0.2% of the total sulphate intake and 0.3% of the total sulphur entering the rumen, respectively.

Three models (named A, B and C) were constructed for the present experiments, each based on the same assumptions as those described in the low sulphur intake experiment (Part IV). The block diagrams of the models A, B and C are shown in Figures 5.2, 5.3 and 5.4, respectively. The differential equations describing the linear system of each model were the same as those described in the high sulphur intake experiment (Part V) and are presented in Table 3.27.

As the sum of the flow rates F_{06}, F_{26} and F_{36} was calculated (by solving a system of n equations and n + 1 unknowns) to be equivalent to approximately 0.3% of the total flow rate from the rumen, each of the above flow rates were taken as zero for the convenience of calculation.

In these models, the value of the excretion rate which would maintain a constant body sulphate compartment was approximately 422 µg S/min, which is somewhat lower than the excretion rates of 540 - 650 µg S/min from results of Kennedy and Milligan (1978). Taking a mean value of plasma inorganic sulphate concentration of approximately 55 µg S/mℓ, the excretion rate, calculated from results of Bishara and Bray (1978b) is 420 µg S/min, which fits well with these models. In these models, the urinary excretion of total sulphur was approximately 814 µg S/min, and since the excretion of inorganic sulphate was taken as 422 µg S/min, the difference of 392 µg S/min has to be accounted for. No attempt was made to differentiate between inorganic sulphate-S, ester sulphate-S and neutral S in urine and faeces. Using the mean values for neutral-S and ester sulphate-S in urine from various experiments (see high sulphur intake experiment, Part V), the estimated urinary excretion of ester sulphate-S is 192 µg S/min and the urinary excretion of neutral S is 89 µg S/min, which is a total of 281 µg S/min. Thus 111 µg S/min or 13.6% of the total urinary sulphur could not be accounted for. However, taking into consideration the standard error of the means used above the value of 13.6% is reduced to 7.6%. Thus 7.6% of the total urinary sulphur could not be accounted

FIGURE 5.2 Model A. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

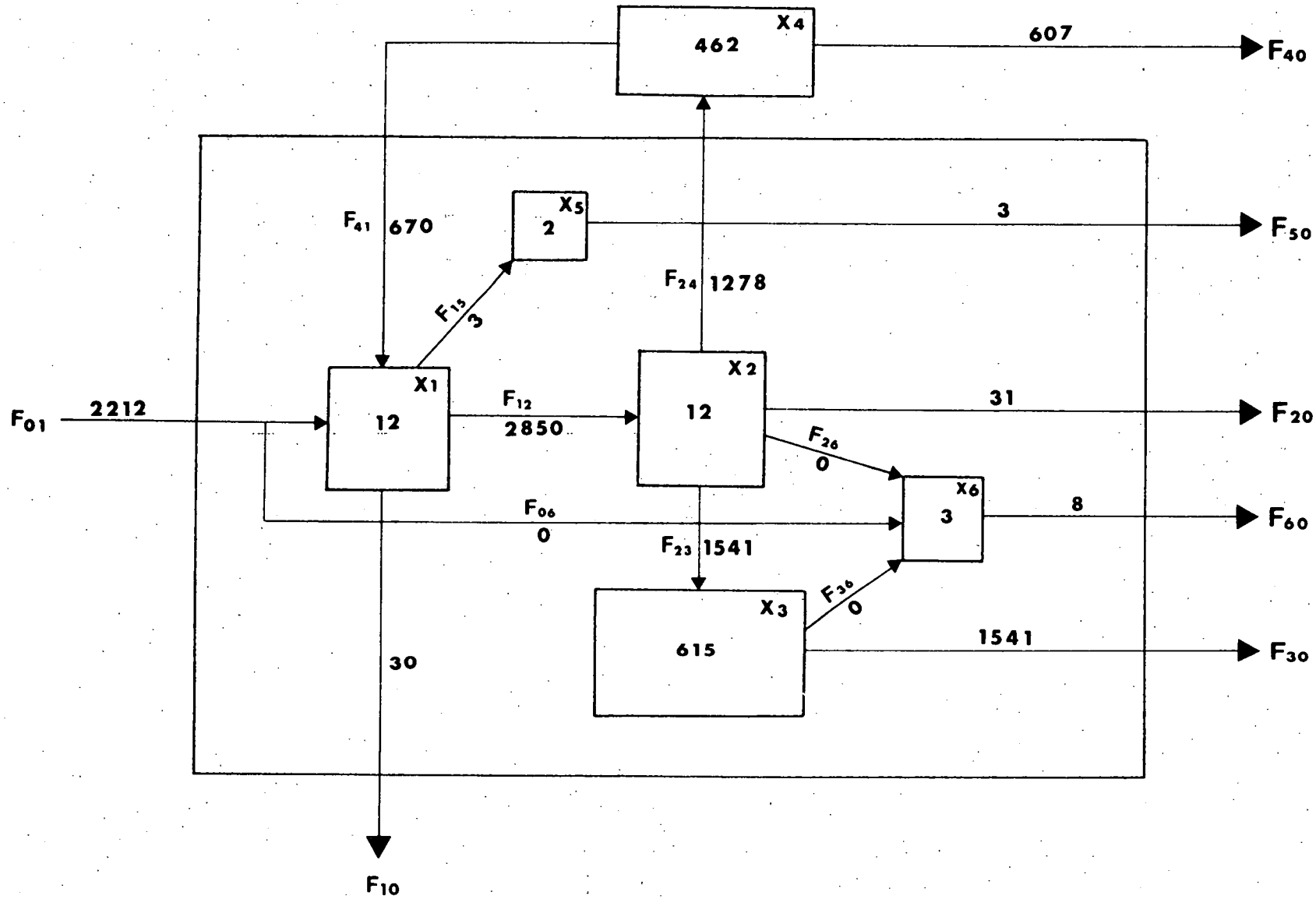
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


X4
462
607
F40
F41 670
X5
2
3
F50
F24 1278
F13
3
X1
12
F01
2212
F12
2850
X2
12
31
F20
F26
0
X6
3
8
F60
F06
0
F23 1541
F36
0
X3
615
1541
F30
30
F10

Hindgut

FIGURE 5.3 Model B. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

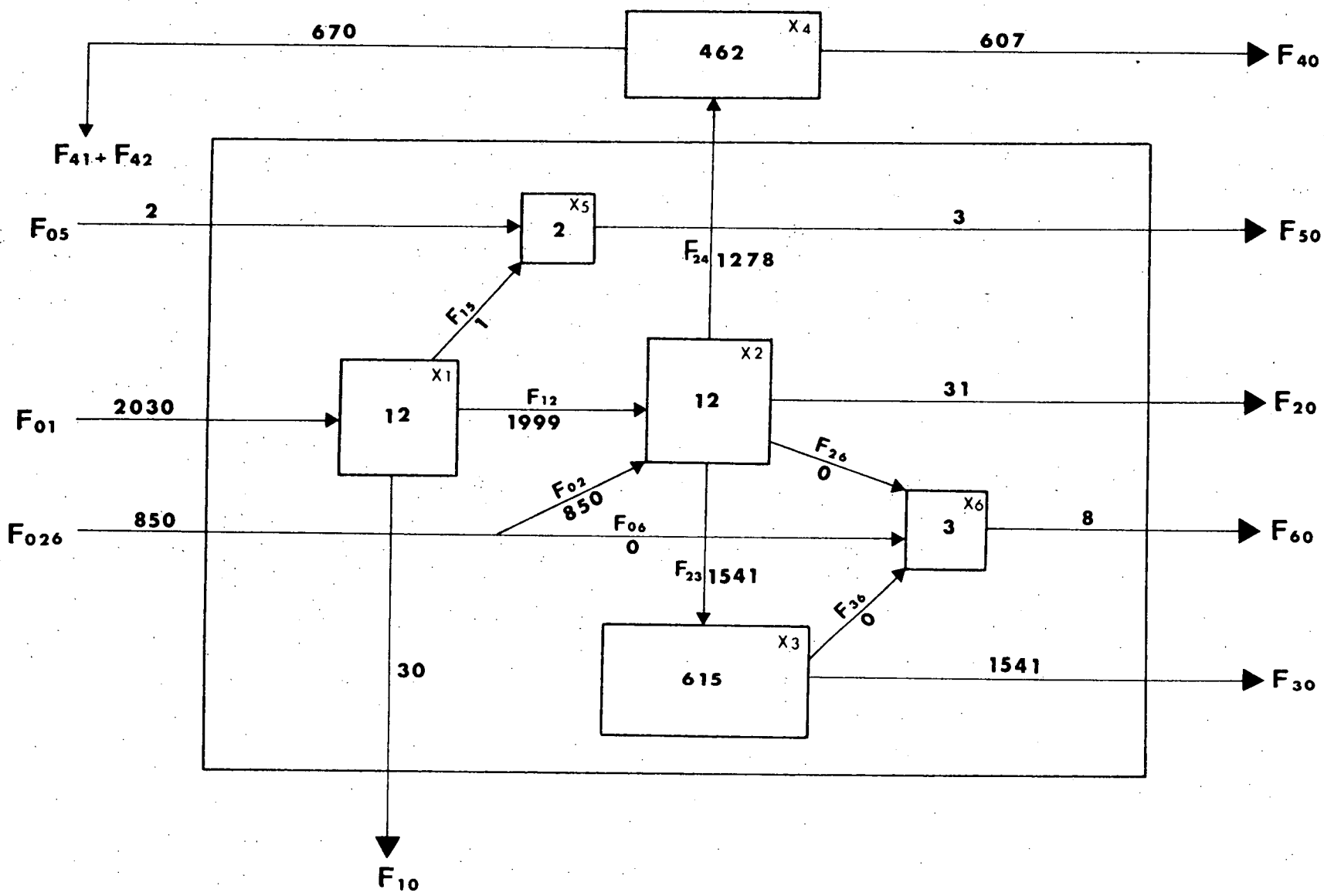
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


670
X4
462
607
F40
F41 + F42
F05
2
X5
2
3
F50
F24 1278
F13
1
X2
12
31
F20
X1
12
F01
2030
F12
1999
F26
0
F02
850
F026
850
F06
0
X6
3
8
F60
F23 1541
F36
0
X3
615
1541
F30
30
F10

Hindgut

FIGURE 5.4 Model C. The state variables (compartments) are:

x_1 = inorganic sulphate

x_2 = sulphide

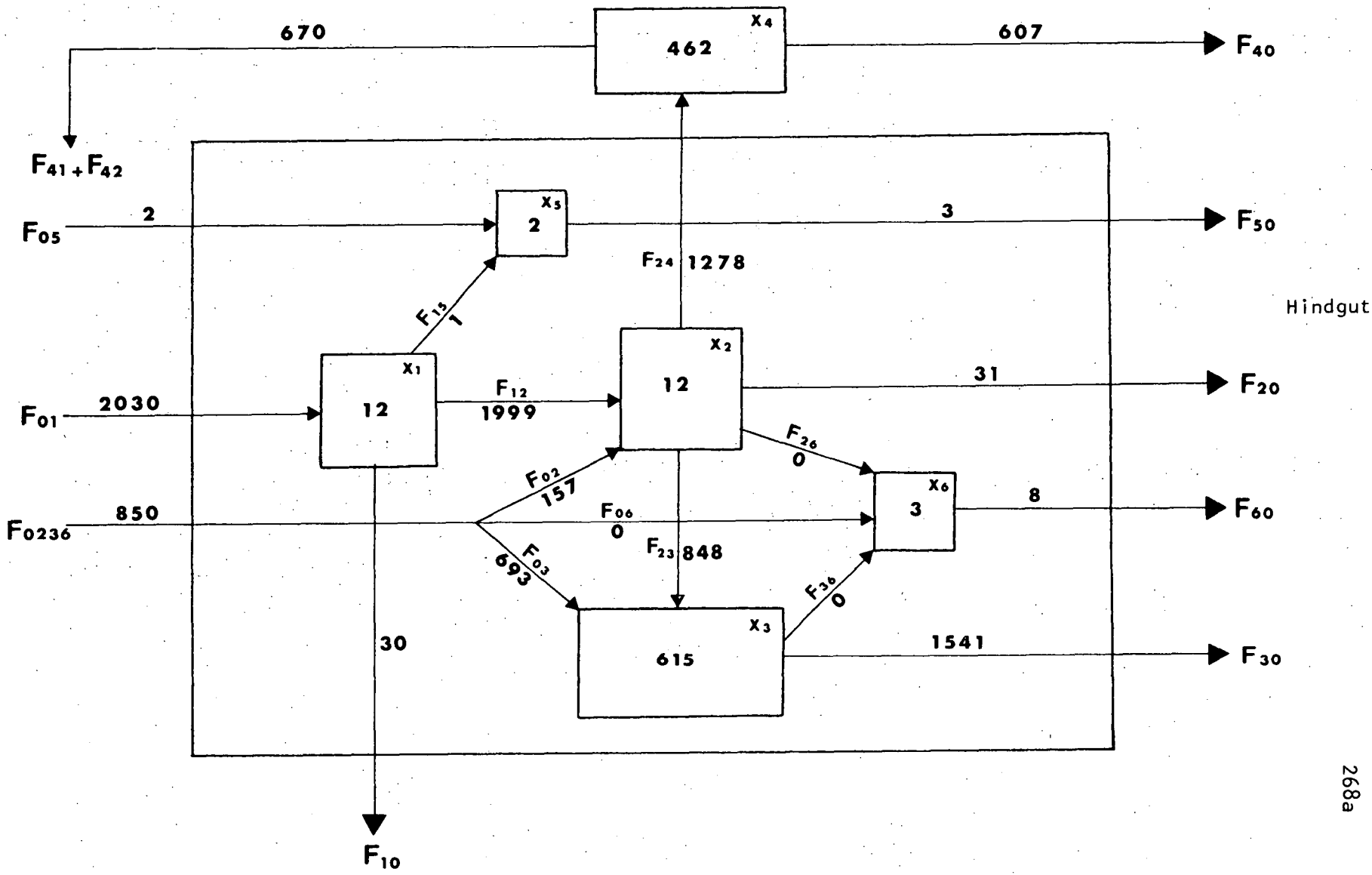
x_3 = protein sulphur

x_4 = body sulphate

x_5 = ester sulphate

x_6 = soluble organic sulphur

Compartment values and flow rate values of sulphur are expressed in mg and mg/day respectively.


670
462
x_4
607
F_{40}
$F_{41}+F_{42}$
F_{05}
2
2
x_5
3
F_{50}
F_{24} 1278
F_{15} 1
Hindgut
F_{01}
2030
12
x_1
F_{12} 1999
12
x_2
31
F_{20}
F_{26} 0
F_{02} 157
F_{0236}
850
F_{06} 0
3
x_6
8
F_{60}
F_{23} 848
F_{03} 693
F_{36} 0
30
615
x_3
1541
F_{30}
F_{10}

for.

The rumen volumes and flow rates from the rumen were both significantly increased ($P < 0.05$) in the 30% starch intake compared with the 15% starch intake experiments. Similarly, there was a significant increase ($P < 0.05$) in the daily water intake during the digestibility trial. Both the half-times and mean retention times in the rumen of ^{51}Cr-EDTA were greater ($P < 0.10$) in the 30% starch intake than in the 15% starch intake experiment.

Rumen pH in the 15% starch intake was significantly higher ($P < 0.02$) than in the 30% starch intake. The calculated absorption constant (in absolute value) of rumen sulphide was greater ($P < 0.10$) in the 30% starch intake than in the 15% starch intake experiment. The estimated rumen sulphide half-life was greater ($P < 0.10$) in the 15% starch intake compared with the 30% starch intake experiment.

There was no significant difference between the 15% starch and 30% starch intake experiments for the N/S ratio in microbial protein, the sulphur retention, the apparent sulphur digestibility and the urinary and faecal excretion of total sulphur.

The sulphide lost "prediction", the apparent dry matter digestibility and the apparent organic matter (OM) digestibility were all greater ($P < 0.10$) in the 30% starch intake than in the 15% starch intake experiments. The sulphide lost "calculation" was greater ($P < 0.10$) with the 15% starch intake experiment.

The dietary OM "truly digested" in the rumen was significantly increased ($P < 0.02$) in the 30% starch diet compared with the 15% starch diet. Similarly, both the flow of microbial protein from the rumen and the protein synthesised per 100 g OM "truly digested" in the rumen were significantly increased ($P < 0.01$) in the 30% starch intake compared with the 15% starch intake experiment.

The concentration of plasma inorganic sulphate was significantly increased ($P < 0.05$) in the 30% starch diet compared with the 15% starch diet.

There was no significant difference between the 15% starch and 30% starch

intake experiments for the concentrations of total S, soluble organic S and neutral S in rumen liquor. The concentration of ester sulphate-S in rumen liquor was significantly increased ($P < 0.02$) in the 15% starch intake compared with the 30% starch intake experiment. Similarly, both the concentration of total sulphate-S and the concentration of total reducible S in rumen liquor was significantly increased in the 15% starch intake compared with the 30% starch intake experiment. The concentrations of inorganic sulphate-S and sulphide-S in rumen liquor were greater ($P < 0.10$) in the 15% starch intake than in the 30% starch intake experiment. The concentration of protein S in rumen liquor was greater ($P < 0.10$) with the 30% starch intake experiment.

There was no significant difference between the 15% starch and 30% starch intake experiments for the daily flow of total S and neutral S from the rumen. The daily flow of ester sulphate-S from the rumen was significantly decreased ($P < 0.05$) in the 30% starch diet compared with the 15% starch diet. The daily flows of total reducible S, total sulphate-S, inorganic sulphate-S, sulphide-S and soluble organic S from the rumen were all greater ($P < 0.10$) in the 15% starch intake compared with the 30% starch intake experiment. The daily flow of protein S from the rumen was greater ($P < 0.10$) with the 30% starch intake experiment.

The daily flow of recycled sulphur was significantly increased ($P < 0.05$) in the 30% starch intake compared with the 15% starch intake experiment.

PART VIII

GENERAL DISCUSSION AND CONCLUSIONS

The main objectives of this research were: (1) to develop a simple-steady state-mathematical model of the sulphur metabolism in the rumen; (2) in the course of its development, to identify weaknesses and limitations of concepts and data regarding the ruminant system; (3) to indicate particular areas where further, more detailed, experimental data are required; and (4) to bring this model to the stage where it was useful as a research tool.

Sulphur models to be considered in this work are mainly intended as predictive tools; each model aims to be a simple one as compared with previous models, i.e. it predicts experimental evidence on sulphur metabolism in the rumen under a variety of sulphur and energy inputs with a mathematical structure of low complexity. Modelling has the added advantages that it helps to focus attention on the fundamental properties of the system and enables predictions to be made under a limitless range of conditions (Prosser and Trinci, 1979). It may be advisable to start with a simple model and to develop it further as more information becomes available, or as the problem demands-always on a sound conceptual framework; a small model with a few good assumptions is likely to be more useful than a large one with many flows.

The results reported here depend only upon the linearity of the transport of sulphur between pools 1-6 and the net losses from them. In this study, non-linear systems are not considered because their mathematical models can become unstable with increasing complexity (see Mazanov and Nolan, 1976). The rationale of this donor-controlled formulation of feeding fluxes ($F_{ji} = \phi_{ji}\, x_i$, see Part I, Section 8) seems grossly oversimplified: the fluxes depend solely on sizes of the donor compartments. Consequently, it is believed from the outset that each of these models would represent the system inadequately, and furthermore would be in the simplest form. The primary motivation for exploring it was to test the adequacy of the data and to gain experience in various techniques of simulation. However, each of these models although highly oversimplified worked well with the assumptions proposed. With the system in steady state and change equal to

zero, system equations serve no immediate purpose, however, when a model is used to simulate change in a biological system the system equations provide a means for computing these changes.

Mazanov and Nolan (1976) suggest that whole-animal models should be considered and that animals should be studied as complete entities. A maximum number of estimates of independently measured processes may then be compared simultaneously with predicted results. In this way the strengths and weaknesses both of the models and of the level of understanding of metabolic processes are highlighted and research programmes expedited.

In the present work, it was found that the maximum microbial protein synthesis occurred at relatively low rumen sulphide-S concentration. Similarly, Mercer and Annison (1976) found that the maximum microbial protein synthesis occurred at relatively low rumen ammonia-N concentration, even when energy was not limiting. The amount of microbial protein synthesised in the rumen was found to be highly correlated with the amount of organic matter (OM) and dry matter (DM) apparently digested and with the amount of dietary OM "truly digested" in the rumen. The relationship between protein synthesised per 100 g OM "truly digested" in the rumen (y,g) with (1) OM apparently digested (x, g/day), (2) dietary OM "truly digested" in the rumen (x, g/day) and (3) DM apparently digested (x, g/day) is shown in Figures I, II and III respectively and is described by the equations 1, 2 and 3 respectively:

(1) $y = -8.1 + 0.05\ x \quad (r = 0.99,\ P < 0.001)$

(2) $y = -12.4 + 0.07\ x \quad (r = 0.99,\ P < 0.001)$

(3) $y = -6.1 + 0.05\ x \quad (r = 0.99,\ P < 0.001)$

Maximum microbial protein production occurred when the amounts of OM apparently digested and dietary OM "truly digested" in the rumen were increased. Also DM apparently digested had the same effect on microbial protein production.

In recent years, ruminal protein synthesis has been the subject of many investigations. Hogan and Weston (1970) have suggested that, because of the anaerobic nature of the rumen, protein synthesis is usually limited by available energy; further, Kropp *et al.* (1977a) showed that protein

FIGURE 1. Relationship between protein synthesised per 100 g OM "truly digested" in rumen (g;y) and OM apparently digested (g/day;x)
$y = -8.1 + 0.05x$, $r = 0.99$

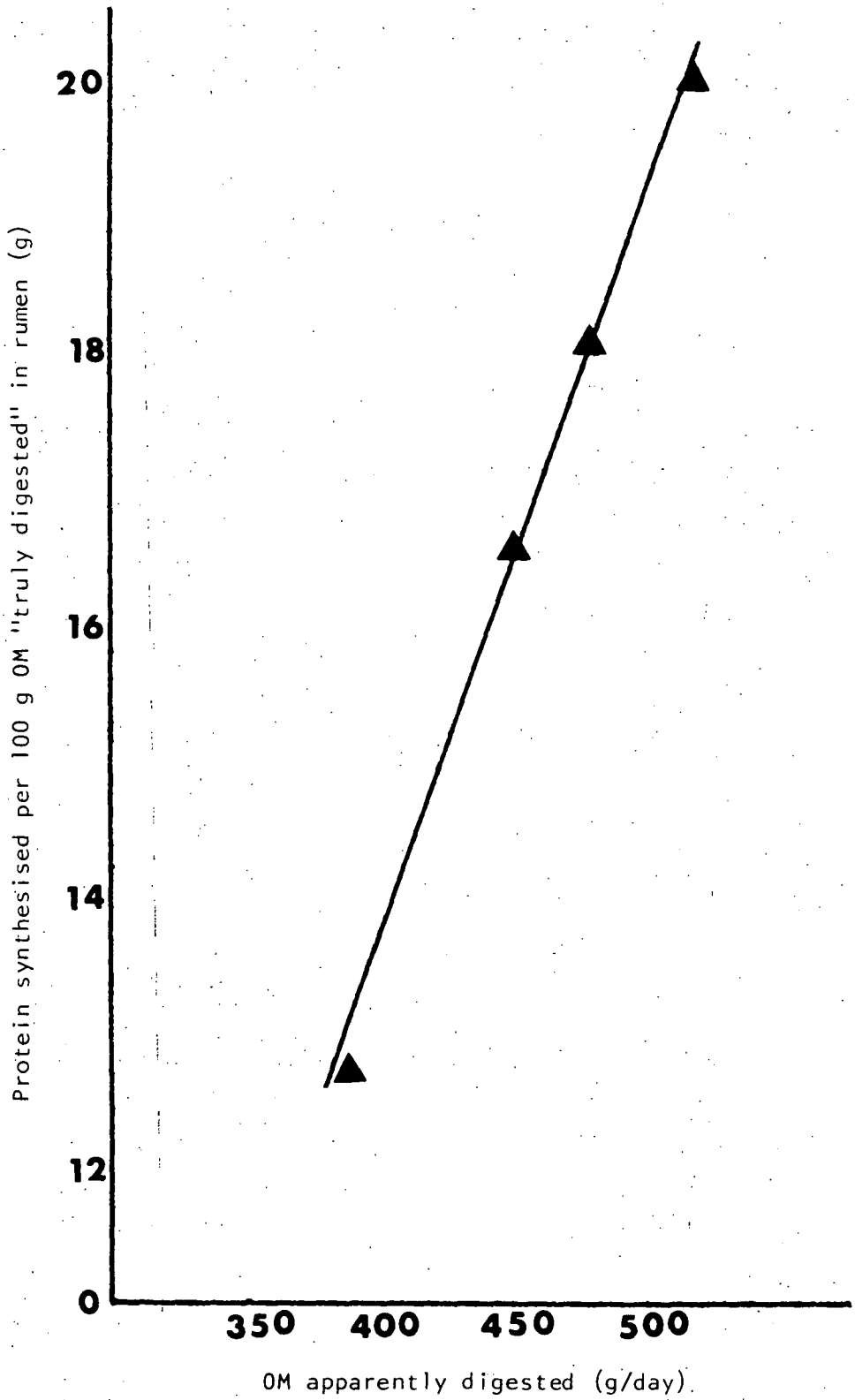

20
18
16
14
12
0
Protein synthesised per 100 g OM "truly digested" in rumen (g)
350
400
450
500
OM apparently digested (g/day)

FIGURE 11. Relationship between protein synthesised per 100 g OM "truly digested" in rumen (g;y) and dietary OM "truly digested" in rumen (g/day;x)

$y = -12.4 + 0.07x$, $r = 0.99$

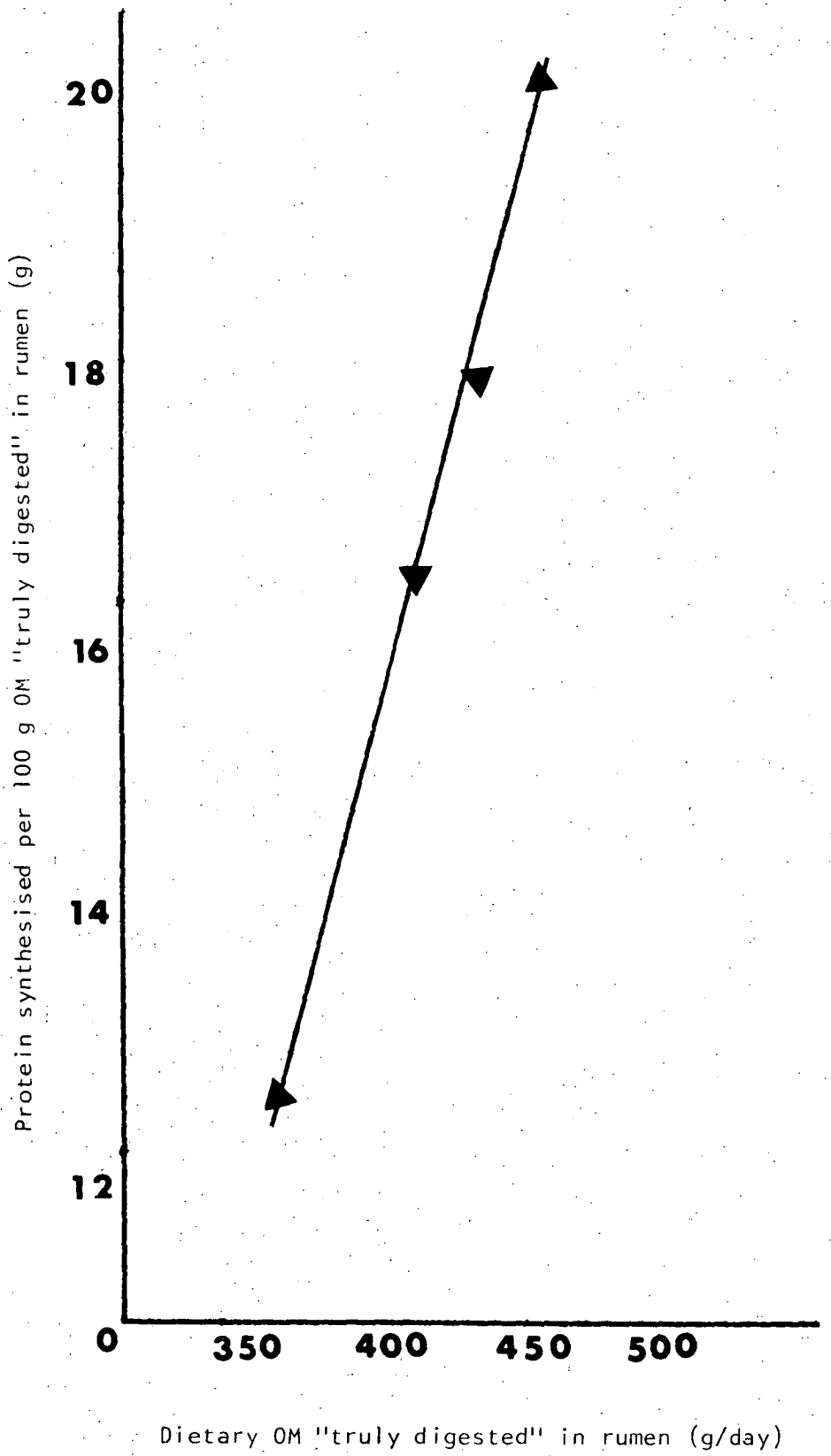
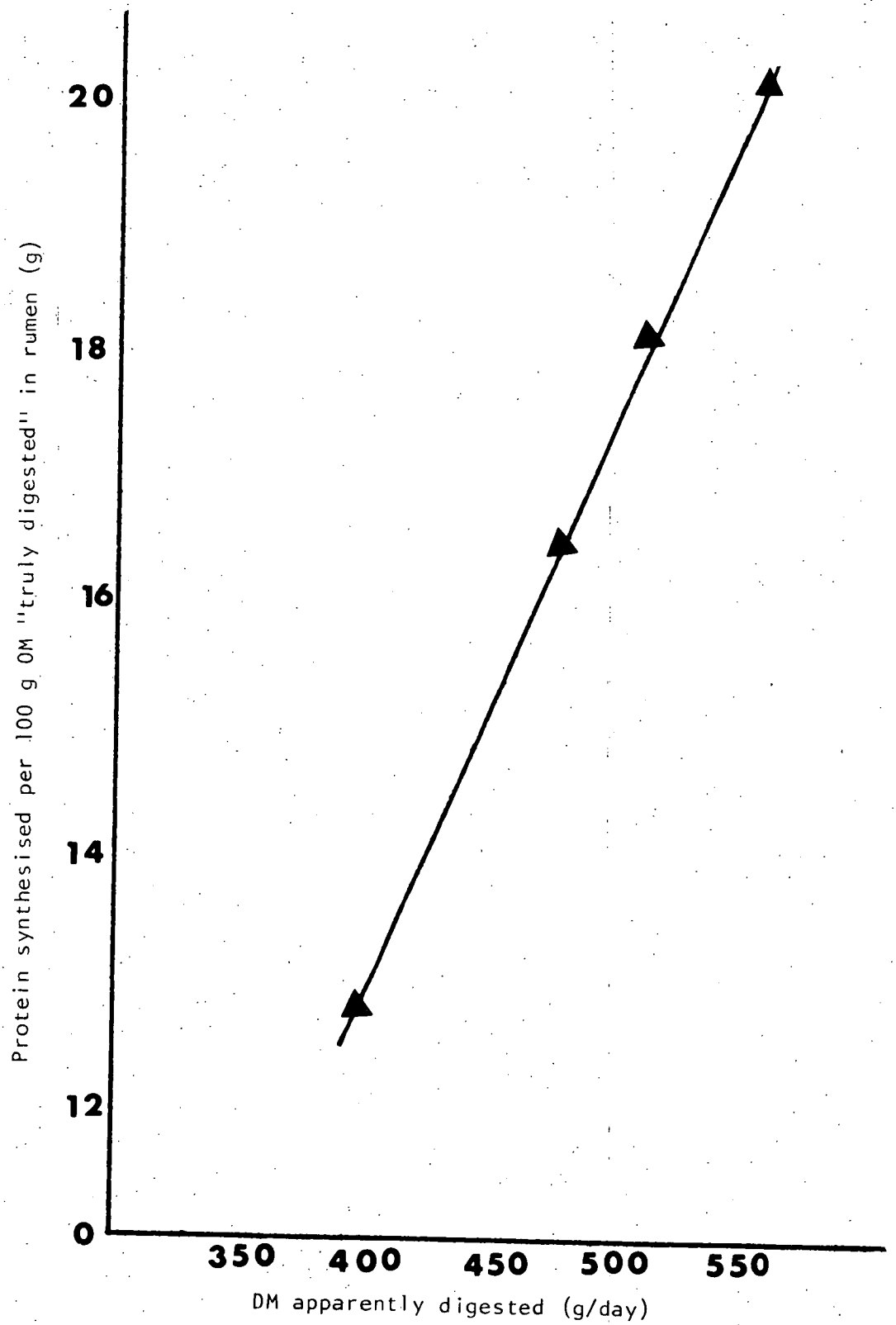

20
18
16
14
12
0
350
400
450
500
Protein synthesised per 100 g OM "truly digested" in rumen (g)
Dietary OM "truly digested" in rumen (g/day)

FIGURE III. Relationship between protein synthesised per 100 g OM "truly digested" in rumen (g;y) and DM apparently digested (g/day;x)
$y = -6.1 + 0.05x$, $r = 0.99$.


20
18
16
14
12
0
Protein synthesised per 100 g OM "truly digested" in rumen (g)
350
400
450
500
550
DM apparently digested (g/day)

synthesis appeared to be limited by available energy and rumen turnover time rather than by nitrogen availability. Protein synthesis in the rumen is closely associated with microbial growth, which is naturally dependent upon the supply of energy-yielding substances, e.g. ATP. The amount of fermentable energy available to ruminal bacteria influences their growth rate and, consequently, the quantity of ammonia converted to microbial protein. Baldwin *et al.* (1977) found that total microbial growth increased with intake level and decreased as feeding frequency decreased. These responses are due, in part, to differences in the proportions of organic matter digested in the rumen and in part to differences in apparent microbial efficiences of ATP utilisation. Recently, Smith (1979) suggests that the best way of achieving greater efficiency of microbial protein production in the rumen was to provide in the diet an adequate supply of starch, preferably cooked, together with a readily available non-protein nitrogen source such as urea. Although molasses has often been used to provide an energy source to encourage use of urea, it appears that it is not very effective for this purpose (Oldham *et al.*, 1977). The reason for this is not clear; it is probably related to the rapid metabolism of soluble sugars in the rumen (although the effect of this may be offset to some extent by their partial conversion to microbial storage polysaccharide, McAllan and Smith, 1977), but how this affects ATP yield or utilisation for protein synthesis is a matter for speculation (Demeyer and Van Nevel, 1975).

Bauchop and Elsden (1960) suggested for a series of anaerobic bacteria that on average 10.5 g of cellular dry matter were produced per mole of ATP generated (the so-called Y-ATP value). There are however many exceptions to this norm, for example, Hobson and Summers (1967) produced data from studies with *Selenomonas rumenantium* which suggested a Y-ATP value of 20. Mixed cultures of bacteria yield higher Y-ATP values than pure cultures, indicating that the mixed culture, as is found in the rumen, is more efficient (Hungate *et al.*, 1971). It is also possible to describe the energetics of protein synthesis in the rumen using other conventions, for example g microbial crude protein per 100 g apparently digested organic matter. A wide range of values can be found in the literature (see also Part I, Section 5); the data available suggest that, on forage diets, the yield of microbial protein is likely to be in the range of 15-20 g

protein per 100 g OM fermented. However, on non-forage diets the yield could vary more widely (see Hume, 1976). One feature that must be emphasised in this context is that for an efficient fermentation to occur energy and nitrogen supply must be balanced. Too little nitrogen will induce uncoupled fermentation (i.e., fermentation without useful ATP production) whilst too much nitrogen and not enough available energy will result in an inefficient use of the nitrogen. It is not appropriate to discuss this matter at length but it is interesting to note that using simulation techniques, Baldwin *et al.* (1970) predicted that the microbial yield would fall when rumen ammonia concentrations were low, i.e. when low protein diets were considered. Factors affecting rumen microbial protein synthesis are fully discussed in Part I, Section 5.

The reliability of the estimates of ruminal flow and of the fluid volume and all derived values depends on the suitability of the markers used in the stomach and the achievement of "steady state" conditions, in which it must be assumed that the volume of the water in the reticulo-rumen (rumen volume) is constant and that the rates of inflow and outflow of water are equal and constant. It also assumes instantaneous and complete mixing, even though it is known that in practice mixing is relatively slow and, owing to the continual influx of water in the saliva, never complete (Warner and Stacy, 1968).

Previous studies have shown that ruminal fluid volume estimated using Cr-EDTA is greater than when estimated using polyethylene glycol (PEG) (Goodall and Kay, 1973). On the other hand PEG may be precipitated in ruminant animals when forages rich in tannins are consumed (Kay, 1969a). These factors, together with the more laborious and less specific determination of PEG compared with ^{51}Cr-EDTA, suggest that the latter is a more suitable marker in studies of digestion in the ruminant stomach. The only theoretical disadvantage of ^{51}Cr-EDTA is the slight absorption and subsequent excretion in the urine. In practice, this is not likely to be troublesome, as appropriate corrections can be made. There are, however, the usual difficulties associated with the use of any radioactive substance, but it is noteworthy that ^{51}Cr is considered to be one of the least hazardous isotopes (International Atomic Energy Agency, 1958). It should be noted that solute markers can deviate from ideal behaviour of the rumen. For ex-

ample, Warner (1969a) found that ^{51}Cr-EDTA associated to a small extent with the digesta dry matter. This can lead to overestimates of fluid flow. In addition, incomplete mixing may introduce errors. Warner and Stacy (1968) reported that in their experiment, the high coefficient of variation found in the ruminal samples from sheep was due to the sample from one site being conspicuously lower in marker (^{51}Cr-EDTA) concentration than the others. This indicated that the sample was drawn from a local 'pocket' of relatively unmixed material. In three instances, post-feeding samples showed evidence of similar 'pockets', all in the anterior part of the rumen where saliva enters from the oesophagus.

The possibility that backflow of labelled micro-organisms from the omasum to the rumen could occur and thus invalidate the conclusions drawn from this work, must be considered. Bird (1972e) suggests that it is unlikely that backflow of labelled cells from the omasum could occur, and, in fact, in another experiment, continuous infusion of Cr-EDTA solution into the omasum of each of four sheep, at a rate of 290 mg Cr/day over 2 days, did not elevate the concentration of chromium in the rumen fluid. However, Hauffe and Engelhardt (1975) showed that in sheep, several hours after administration of radio-cerium (^{144}Ce) into the omasal-abomasal orifice, considerable radioactivity could be detected in the reticular contents. They suggested that some of the longer particles which have entered the omasum seem to be able to be carried back into the reticulum. The occasional flow of barium, placed in the omasal canal, back into the reticulum has also been observed in unanesthetised dairy cattle (Stevens *et al.*, 1960) and seemed to occur when the omasal body contracted during closure of the omaso-abomasal orifice.

In the present experiments, it has been assumed that the fluid flow, estimated from the concentration of marker in ruminal samples is the same as the actual flow through the reticulo-omasal orifice. However, this assumption does not hold if saliva does not fully mix with the entire contents of the reticulo-rumen. In addition, the composition of the reticular contents may differ from that sampled in the ventral region of the rumen; Hauffe (1972), Engelhardt and Hauffe (1975b) suggested that, because of the continuous passage of saliva into the forestomachs the concentration of the marker (^{51}Cr-EDTA) in the reticulum, and consequently in the fluid entering

the omasum, is usually lower than in the rumen fluid. This was shown in goats and sheep.

Bird (1971, 1972a) reported that only small amounts of sulphate escaped from the stomach of sheep given sulphate in the diet or as an intraruminal infusion. Bird and Hume (1971) reported that the inorganic sulphate sulphur contribution was c. 2.2% - 2.6% of the total sulphur flowing to the omasum, when 1.4 g sulphate sulphur was added daily to the basal diet which contained 0.6 g S/day. Gawthorne and Nader (1976) reported that, in their experiments although comparatively large amounts of sulphate were infused daily (four sheep were intraruminally infused with 10 g sodium sulphate/day) the sulphate concentration was so low that 16 mls fluid had to be used to give sufficient sulphate-S for accurate analysis. In relation to determination of sulphate in strained ruminal fluid Gawthorne and Nader (1976) suggest that some sulphate may have escaped analysis if it was converted to sparingly-soluble sulphates, for example calcium sulphate, and remained attached to large plant particles. Doyle (1977) has found that inorganic sulphate was evenly distributed in Fractions A and B (see Doyle, 1977) of ruminal digesta, but ester sulphate was contained largely in the particulate material despite several washings. This could explain the very low concentrations of ester sulphate-S in strained rumen fluid found in these experiments.

In these models the unknown values are the inputs into the reticulo-rumen of sulphur in epithelial sheddings, saliva or sulphate diffusion across the rumen wall, and also the absorption of sulphur compounds. Till *et al.* (1973) estimated that the gut wall contains about 70 mg S/kg body weight and the net turnover was estimated to be 2 - 7 mg S/day/kg body weight. From these estimates, the maximum possible input of sulphur into the gastrointestinal tract from the turnover of mucosal cells in a 40 kg sheep would be 280 mg/day. However, the proportion of this sulphur directly entering the digestive tract is unknown. Since the epithelium of the reticulo-rumen is a stratified squamous tissue somewhat keratinised and subject to abrasion, but not secretion, it may represent a substantial proportion of the endogenous sulphur input to this organ (Doyle and Moir, 1979b). Bray (1969c) estimated the sulphur inputs into the rumen by sulphate movement across the rumen wall in sheep fed diets adequate and deficient in sulphur to be only 20 and 5 mg

S/day respectively. A more recent estimate of sulphur transfer from the plasma to the rumen is 29 mg S/day in sheep fed a low-sulphur roughage based ration (Kennedy *et al.*, 1976). These estimates suggest that input of sulphur from sulphate movement across the rumen wall is of minor importance and in the models was taken as zero. Further, the absorption of inorganic sulphate (Bray, 1969a) and sulphur amino acids (Annison, 1956; Liebholz, 1971) from the rumen is small and for the purposes of these models was taken as zero.

The loss of sulphur from the rumen other than by passage down the tract can best be explained by the rapid rate of sulphide absorption providing that the absorption of inorganic sulphate and sulphur amino acids is negligible. Gawthorne and Nader (1976) however, have suggested that substantial amounts of sulphate may be lost by an as yet unidentified pathway. In the experiments reported here the predictive equations for sulphide absorption using the absorption constants of Bray and Till (1975) seemed to be in agreement within the flow rates calculated by difference.

Published estimates of sulphur recycled to the rumen of sheep in saliva have been listed by Bray and Till (1975). From these it was estimated that recycling to the reticulo-rumen would contribute about 2 - 5 mg S/day/kg BW and that 23% - 26% of this would be as sulphate sulphur. The estimated recycled sulphur inputs to the rumen for high sulphur intake (93 mg S/day), 15% starch intake (357 mg S/day) and 30% starch intake (670 mg S/day) illustrate the possibility of wide ranges in sulphur added to the ruminal digesta. Bird and Hume (1971) estimated a net daily addition to ruminal digesta of 446 mg S above that ingested in a sulphur-deficient ration. However, this value is substantially higher than the maximum values estimated by Bray (1969b, 1969c), i.e. 200 mg S/day. Bray's estimates were based upon the sulphur-content of saliva collections and on the passage of ^{35}S-sulphate from the blood into the rumen in which the digesta had been replaced by a buffer solution. As Doyle (1977) suggests, the absence of stimulation to the mouth, oesophagus and rumen by the presence of feed and ruminal digesta may have resulted in considerable alteration in both the flow and composition of the saliva compared with sheep ingesting roughage (see Kay, 1966). The mucous component in particular may be

low under such artificial conditions. A more recent estimate indicates that endogenous sulphate inputs contribute 130-160 mg S/day to the rumen (Kennedy and Milligan, 1978) while endogenous organic sulphur contributes 300-340 mg S/day. Presumably this endogenous organic sulphur input may be from saliva and the turnover of mucosal cells as it is determined by difference between known inputs and outflows. Gawthorne and Nader (1976) estimated that the apparent recycling of sulphate to the ruminal contents by way of the saliva or across the rumen wall was between 640 and 880 mg S/day. Recently, Doyle (1977) using the "first approximations", derived from the sulphur concentration in "true digesta", estimated that sulphur recycling to the reticulo-rumen ranged from indeterminate values above zero up to 1227 mg S/day. This is higher than any of the estimates of total sulphur recycling used in these models. The variation of these estimates of the recycled sulphur stresses the need for more extensive investigation about the amount and composition of sulphur recycled to the reticulo-rumen. The value of the recycled sulphur will depend on its form; only that sulphur which is not in the form of protein can result in the net production of protein in the rumen (see Hume and Bird, 1970).

There appears to be no reason to expect that ruminal fluid volume and flow rate from the rumen would change significantly in the 30% starch intake experiment from those observed in the 15% starch intake experiment, particularly as each sheep regularly received a one-hourly feed and a relatively constant dry matter intake. Hyden (1961) stated that "in spite of differences in the diet and a time interval of about one year between the experiments, there is surprisingly good agreement between the estimated flow rates in each animal, and also between animals". However, the increased rumen volumes and flow rates from the rumen in the 30% starch intake experiment were associated with increased daily water intake ($P < 0.05$) during the period of digestibility trial. Hemsley (1975) observed that shorter rumen retention times were associated with greater water intake of sheep consuming the high salt diets.

Rumen turnover is a function of the nature of the food ingested, the level of feeding and also the pattern of feeding (Sutherland, 1976). The relative outflow appears to be appreciably higher on diets of fresh forage than on concentrate feeds. Roughage, at least at moderate levels, seems

to accelerate throughput. When increasing levels of the same food are ingested, rumen volume may show little change but outflow and therefore turnover are increased (Hogan, 1957). Pattern of feeding may affect both rumen volume and outflow. In some early work (Murray *et al.*, 1962) observed a relatively high rumen liquid volume in sheep fed cubed dried grass twice daily but this was accompanied by a low rumen turnover time. Supplying the same ration from a continuous feeding device gave much smaller rumen liquid volumes but increased the rate of turnover. With the majority of the diets salivary input is very closely tied to ruminal output since water intakes are generally relatively low compared to salivary flow and imbibed water seems to be readily and rapidly absorbed (Warner and Stacey, 1968). Sutherland (1976) suggests that the control of rumen outflow cannot be considered solely at the level of the rumen itself and it is clear that a series of feedback mechanisms exist such that distension of any region of post-ruminal gut has the effect of decreasing rumen outflow. Increase in the volume of any part of the intestinal tract beyond the rumen appears to increase outflow from and to decrease inflow to that part of the gut. The effects of these individual controls terminate in a controlling effect on rumen outflow (Phillipson and Ash, 1965).

Doyle and Moir (1979a) found in their models that comparison of the sulphur concentrations in the particulate and fluid fractions of ruminal digesta samples indicated that the sulphur content in the fluid was only a small proportion (c. 15%) of the total. If this is the case, then the ruminal fluid pools in the present models are gross underestimates of true ruminal sulphur pools. Doyle and Moir (1979a) state that "the non-protein neutral sulphur and the reducible sulphur forms present in ruminal digesta should all be capable of passing through the reticulo-omasal orifice in the fluid fraction". However, sequestration of these compounds may occur within the reticulo-rumen. A substantial part of the a-amino nitrogen of ruminal contents is associated with microbial cells (Annison, 1956), and a proportion of free sulphur amino acids is precipitated by trichloroacetic acid (Bird and Hume, 1971). It seems likely therefore that non-protein organic sulphur flows from the reticulo-rumen will also be inflated by these ruminal retentions.

In the present models it is assumed that microbial matter contains c. 10.5%

of N (Hungate, 1966). However, Czerkawski (1978) reported a lower value (8.0% N) and suggests that much of the variation is from variable proportions of polysaccharide, and that much of the variability in N and other components disappears when polysaccharides are excluded. Smith (1975) also quoted 18 results in which the mean N content was 7.2% of microbial dry matter.

In this work, the flux of microbial nitrogen from the rumen pool to the intestine is given by the product of the microbial protein in the pool and the passage rate or the turnover of this pool. As there are no reliable estimates of the extent of lysis of microbes in the rumen, the rumen pool of microbial protein may be overestimated, which would be reflected in the computed value for the passage of microbial protein. Production of sulphide in the rumen would be underestimated if some rumen sulphide does not pass through the fluid pool. If other 'isolated' compartments exist (e.g. inter-cellular pools or local concentrations of sulphide, perhaps in crypts formed by microbial colonies as they digest plant materials), these would not be 'seen' when only rumen fluid is sampled. Whilst there is no evidence to suggest that such pools do occur (see Nolan and Leng, 1974), the possibility needs to be considered during interpretation of the quantitative rumen models.

Figure IV represents the filtration and the maximum reabsorption rate of sulphate by the kidney tubules. The straight line of sulphate reabsorbed (maximum) against plasma inorganic sulphate concentration indicates that the maximum reabsorption rate is proportional to the plasma inorganic sulphate concentration. In Figure V the maximum value (Tm) of reabsorption of sulphate per plasma inorganic sulphate concentration is plotted against the plasma inorganic sulphate concentration. The solid line represents results from the literature (Bishara and Bray, 1978b; Kennedy and Milligan, 1978) and the dotted line represents the reabsorption rates derived from the models. The reason for the discrepancy between the excretion of inorganic sulphate proposed by the model and that calculated using the data from the literature for the low sulphur intake experiment is not clear. This value is not included in Figure V because no blood samples were taken in the low sulphur intake experiment. However, it should be noted that the main objective of this work was to concentrate on the rumen system, but

FIGURE IV. Filtration and maximum reabsorption rate of inorganic sulphate by the kidney tubules.

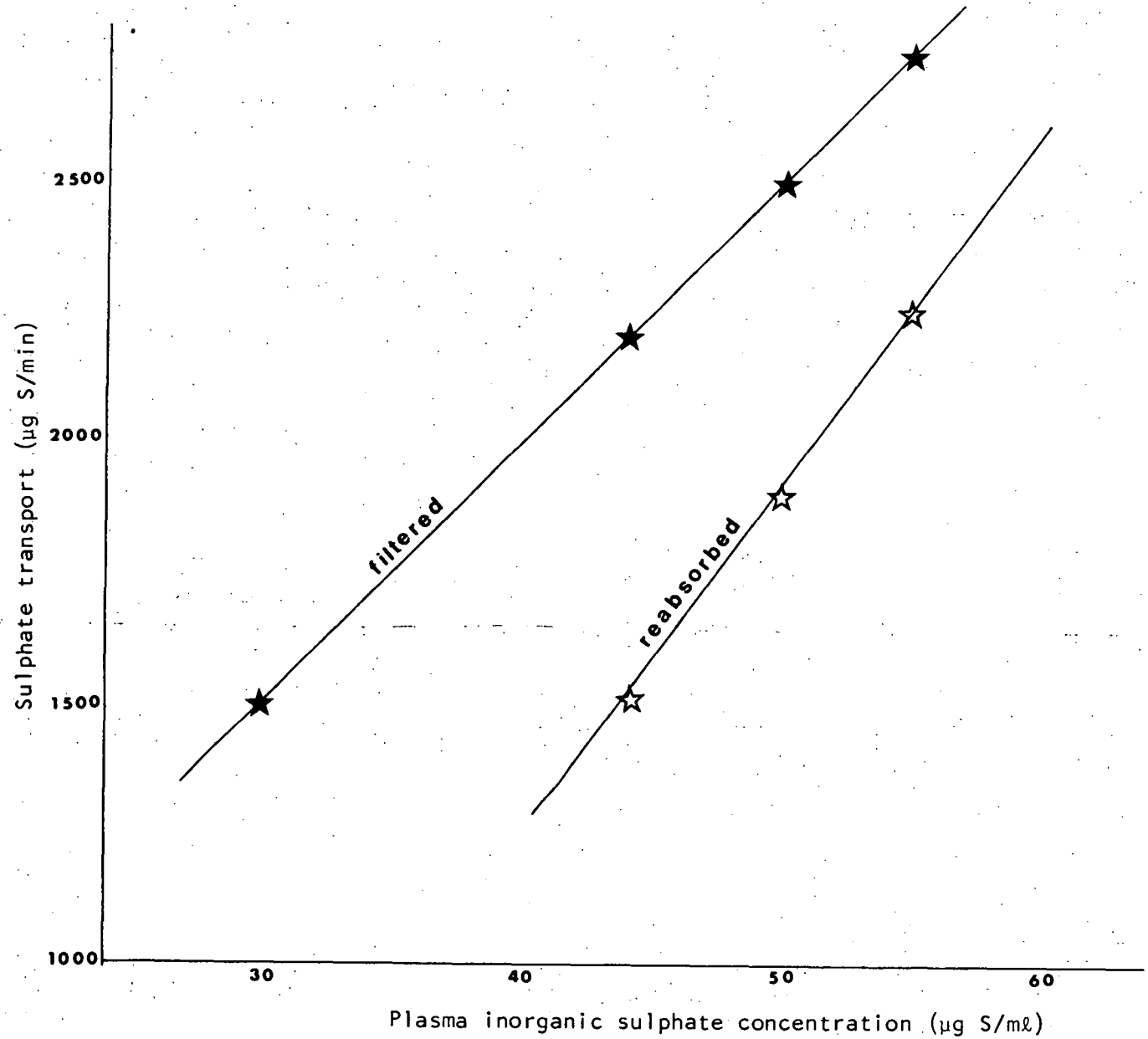

2500
2000
1500
1000
Sulphate transport (µg S/min)
filtered
reabsorbed
30
40
50
60
Plasma inorganic sulphate concentration (µg S/mℓ)

FIGURE V. Maximum value (Tm) of reabsorption of sulphate per plasma inorganic sulphate concentration against plasma inorganic sulphate concentration.

Tm of sulphate/plasma inorganic sulphate concentration (mℓ/min)
44
42
40
38
36
34
32
30
30
40
50
60
Plasma inorganic sulphate concentration (µg S/mℓ)
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during the course of the model development it became obvious that the excretion of inorganic sulphate from the kidney greatly affected the body sulphate pool and consequently the state variables of the rumen system. The above graph of Figure V indicates that the reabsorption maxima (Tm) for sulphate used in the high sulphur, 15% starch intake and 30% starch intake experiments approximate the Tm proposed by the literature. Therefore it was proposed that the excretion rates used for the models are valid. The two lines were tested for coincidence using Student's t (Steel and Torrie, 1960). The test could not detect a significant difference between the two lines, but it must be emphasised that there is only one residual degree of freedom for each line. Thus, within the limits of the two data sets there were no detectable differences between the model and literature lines.

When information on sulphur intake and chemical composition of the diet is given the model predicts the inflow of inorganic and organic sulphur from saliva, the amount of sulphur absorbed from the rumen, the amount of sulphur incorporated into microbial protein, the amount of microbial protein synthesised per 100 g OM "truly digested" in the rumen, the flow of microbial protein and the flows of sulphur from the rumen.

The extent to which potentially degradable dietary constituents are broken down in the rumen and the efficiency with which the microbes use the available energy for growth are particularly important in determining nutrient supply to the sheep. These two factors are interdependent and are part of the outcome of the complex interactions that occur in the rumen. The prime determinants of rumen function are listed by Faichney and Black (1979). They are (1) the intake of feed, its chemical composition and physical form; (2) the inflow of endogenous material; (3) the potential degradability of feed and endogenous constituents; (4) the rates of degradation and outflow of these constituents; and (5) the mass of the microbial population, its requirements for maintenance and growth, and its fractional outflow rate. It is clear that there is insufficient published information to predict quantitatively the outcome of all the interactions occurring in the rumen for the complete range of dietary, physiological and environmental conditions to which sheep may be exposed. In particular, research is needed to provide better definition of diets in terms of

chemical composition, physical form and potential degradability of structural carbohydrate and protein.

Information on degradation rates of the different dietary and endogenous constituents entering the rumen is also lacking. This is particularly important for moieties whose degradation rates and outflow rates from the rumen are of the same order of magnitude (Beever *et al.*, 1980). In addition, the factors which control the fractional outflow rates of the components of rumen digesta (see Faichney and Black, 1979) have not been studied. The effects of physiological state and environmental factors on fractional outflow rates could not be represented in these models because there was no basis for a reasonable hypothesis. A considerable research effort is required in this area.

When considering whether microbial growth is limited by the availability of specific nutrients rather than the availability of energy, information is needed on the recycling of nitrogen and sulphur to the rumen and on the availability of these substances, and of amino acids, for microbial growth. There are few reports from which values can be obtained for microbial utilisation of ammonia (Roffler and Satter, 1975) and inorganic sulphur (Bray and Till, 1975), and the effects of intake and diet composition on the recycling of nitrogen and of sulphur are not well understood. Model predictions have been shown to be very sensitive to these factors for diets in which amino acids, ammonia or inorganic sulphur limit microbial growth (Beever *et al.*,1980). The present models consider only recycling via the saliva and takes no account of transfer of sulphur across the rumen wall. There is only a limited amount of data on the control of saliva flow and composition, and more work is needed for an adequate description of recycling by this route; information on the magnitude of recycling across the rumen wall in relation to intake and diet composition is also required.

Finally, the significant points to be drawn from these studies are that:

(1) The fundamental assumption that the system described was in a steady state, that is the compartment sizes and turnover rates were constant during the experimental period, was largely fulfilled as rumen pH was relatively constant and there were fairly constant concentrations of sulphide in ruminal fluid and inorganic sulphate in plasma under the

hourly-feeding conditions used. Also relative constancy of faecal and urinary outputs during the digestibility trials support the necessary assumption that between-day variations in metabolism were minimal.

(2) The water-soluble marker ^{51}Cr-EDTA used in the high sulphur and 15% starch intake experiments gave similar results for rumen volumes and flow rates as the combination of the two markers ^{51}Cr-EDTA and ^{103}Ru-phen which were used in the low sulphur intake experiment. This apparent contradiction of Faichney's (1975) statement that the use of the two markers ^{51}Cr-EDTA and ^{103}Ru-phen cannot be applied to the reticulo-rumen because many of the particles sampled are much too large to pass into the omasum can probably be explained by the physical nature of the diet. It seems that the particle size of the pelleted diet used in these experiments had small enough dimensions not to impede passage from the rumen.

(3) The level of sulphur and energy intake affected the absorption rate of sulphide from the rumen by altering rumen pH and sulphide concentration. The net microbial protein flow rate and the amount of microbial protein synthesised per 100 g OM "truly digested" in the rumen was affected by the different proportions of OM digested in the rumen and the sulphur and energy content of the diet.

(4) A large amount of blood inorganic sulphate was apparently recycled to the rumen in the 15% starch intake and 30% starch intake experiments.

PART IX

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PART X

APPENDICES

APPENDIX 1

Composition of Experimental Diet and Dietary Components

Diet		Mineral Mix*		Trace Element Mix+	
Component	%	Component	%	Component	%
Oaten hulls	85.7	NaCl	45.6	$C_6H_5O_7Fe.5H_2O$	15
Pollard	7.3	$CaCO_3$	19.4	$CuSO_4.5H_2O$	6
Urea	1.8	$Ca_3(PO_4)_2$	22.8	$ZnSO_4.7H_2O$	3.8
Mineral Mix*	2.8	MgO	2.3	$MnSO_4.H_2O$	0.9
Molasses	0.9	KCl	9.2	KI	0.15
Anhydrous Na_2SO_4	1.5	Trace Element Mix+	0.7	$CoCl_2.6H_2O$	0.60
				H_2SeO_3	0.045
Total nitrogen	1.98			$Na_2MoO_4.2H_2O$	0.95
Total sulphur	0.395				
(% dry matter basis)					
N:S = 5:1					

*, + The mineral mix and the trace element mix were taken from Bray and Hemsley (1969).

Composition of Dietary Components (% of dry matter)

Component	Nitrogen	Sulphur
Oaten hulls	0.60	0.078
Pollard	2.54	0.203
Urea	44.0	0.003
Mineral Mix	0.004	0.106
Molasses	1.05	0.633
Anhydrous Na_2SO_4	-	22.6

APPENDIX 2

Composition of Experimental Diet (Low Sulphur Intake Experiment)

Diet Component	%
Oat hulls	89.9
Pollard	3.8
Urea	2.0
Mineral Mix	3.0
Molasses	1.0
Anhydrous Na_2SO_4	0.36

Total nitrogen = 1.417% of dry matter
Total sulphur = 0.159% of dry matter
N/S = 8.9/1

The composition of the mineral mix and trace element mix was the same as in Appendix 1.

Composition of the sulphur intake (% of total S)

Organic S = 41.2
Inorganic sulphate-S = 58.2
Ester sulphate-S = 0.6

APPENDIX 3

Composition of Experimental Diet (High Sulphur Intake Experiment)

Diet Component	%
Oat hulls	88.9
Pollard	3.8
Urea	3.0
Mineral Mix	3.0
Molasses	1.0
Anhydrous Na_2SO_4	1.08

Total nitrogen = 1.826% of dry matter
Total sulphur = 0.321% of dry matter
N/S = 5.7/1

The composition of the mineral mix and trace element mix was the same as in Appendix 1.

Composition of the sulphur intake (% of total S)

Organic S = 18.2
Inorganic sulphate-S = 81.5
Ester sulphate-S = 0.3

APPENDIX 4

Composition of Experimental Diet (15% Starch Intake Experiment)

Diet Component	%
Oat hulls	75.6
Starch	13.3
Pollard	3.8
Urea	3.0
Mineral Mix	3.0
Molasses	1.0
Anhydrous Na_2SO_4	1.08

Total nitrogen = 1.753% of dry matter
Total sulphur = 0.312% of dry matter
N/S = 5.6/1

The composition of the mineral mix and trace element mix was the same as in Appendix 1.

Composition of the sulphur intake (% of total S)

Organic S = 16.9
Inorganic sulphate-S = 82.8
Ester sulphate-S = 0.3

APPENDIX 5

Composition of Experimental Diet (30% Starch Intake Experiment)

Diet Component	%
Oat hulls	62.3
Starch	26.6
Pollard	3.8
Urea	3.0
Mineral Mix	3.0
Molasses	1.0
Anhydrous Na_2SO_4	1.08

Total nitrogen = 1.681% of dry matter
Total sulphur = 0.302% of dry matter
N/S = 5.6/1

The composition of the mineral mix and trace element mix was the same as in Appendix 1.

Composition of the sulphur intake (% of total S)

Organic S = 15.7
Inorganic sulphate-S = 84.2
Ester sulphate-S = 0.1