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## SYMPOSIUM ON 'MODEL SYSTEMS IN NUTRITIONAL RESEARCH'

### Microbial fermentation in the rumen

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The cow is of the bovine ilk,
One end is moo—the other milk!
(Ogden Nash)

God showed a little more acumen,
By joining moo and milk with rumen.
(C. L. Czerkawski)

Ogden Nash defined the cow in a simple way and this definition was extended considerably by my daughter, but it is not enough to say that the rumen is simply something with an input and an output. It is a very complex organ, which enables a complex, predominantly anaerobic microbial population to survive inside an animal that depends on a plentiful supply of oxygen to all its tissues, including the rumen. The microbial population of the rumen can break down some of the toughest substrates (cellulose, hemicellulose) and convert them to end-products in order to obtain energy for its own growth. Its activity supplies the host animal with a great deal of energy (as absorbed end-products) and food as microbial protein to be digested lower down in the gut.

The rumen is a complex organ, containing a very dense microbial population, consisting of some twenty species of protozoa and possible 200 species of bacteria, whose main function is to break down very tough solid substrate (Hungate, 1966). Not only are the contents of the rumen heterogeneous, but different parts of the food are degraded at different rates, and those that are not degraded leave the rumen at different rates with the mean residence time ranging from a few hours to several days. Clearly it would be very difficult to study the microbial population and its function *in situ* in such a complex system and therefore, from the beginning, attempts were made to isolate parts of the system. Numerous and

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varied artificial rumens were developed, ranging from very simple techniques, in which small samples of rumen contents are incubated in test-tubes, to very complex types where attempts are made to simulate rumen movement, absorption through the wall and differential flow of heterogeneous digesta. In general, the number of times that any given artificial rumen had been used after its inception varied inversely with its complexity (Czerkawski, 1976).

The simple techniques made it possible to obtain a great deal of basic microbiological and biochemical information and to lay the foundation for the considerable store of knowledge of the pathways of degradation of dietary constituents in the rumen, microbial utilization of nutrients and generation of energy for growth and maintenance. These simple procedures were found to be inadequate for the studies of microbial populations in a flowing system and as a first approximation, the rumen was treated as if it were a continuous culture. It was found that many of the established results from the classical continuous-culture work could be applied to the rumen and this led to further advances in our understanding of the system. However, the classical continuous culture deals with a uniform suspension of micro-organisms and utilization of soluble substrates, while in general the substrate of micro-organisms in the rumen is solid and major proportions of this substrate consist of very tough fibrous material (cellulose and hemicellulose). The bacteriological studies of surface growth had only a limited application since the surface attack could not account for the remarkably efficient degradation of fibrous food in the rumen; a postulation of some kind of 'attack from within' was required. How can one study a complex, heterogeneous and flowing system? An analytical approach would destroy the integrity of the system and the answer was to develop a model system that would incorporate the basic features of the real rumen and yet be sufficiently simple for the research worker to assess and to control all the important parts of the system without disturbing its normal function.

## *The model system*

*Criteria for a practical model system.* Before one tries to develop a practical model system one should consider a number of points. Is it going to be used for routine assays or for speculative work, or is the objective to simulate the real system? The sphere of interest is very important. If one is not interested in gas production or in degradation of solid substrates, a considerable simplification of the apparatus may be possible. The duration of the contemplated experiments should also be considered very carefully; in general long incubations will require special provisions for feeding, removal of end-products of fermentation and maintenance of steady conditions within the reaction vessels.

A common mistake in simulation of a biological system such as the rumen is to oversimulate it. For instance, the end-products of fermentation in the rumen are removed simultaneously in two different ways, by simple dilution down the gut and by absorption through the rumen wall, but this does not mean that such a facility has to be built into the model system. On the contrary, it is important that

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the end-products are removed, irrespective of the means, and this can be done by the continuous dilution with artificial saliva, with great simplification of the design of the apparatus.

*The rumen simulation technique* (*Rusitec*). This technique was developed to give the operator maximum control, with relatively simple and cheap apparatus (Czerkawski & Breckenridge, 1977). It is possible, using this technique, to determine precisely all the inputs and outputs, including gases and, significantly, to investigate what goes on inside the reaction vessels. In routine use, normal solid food is provided and thus the apparatus simulates the heterogenous rumen system. In some sophisticated simulation systems, the relative amounts of undigested

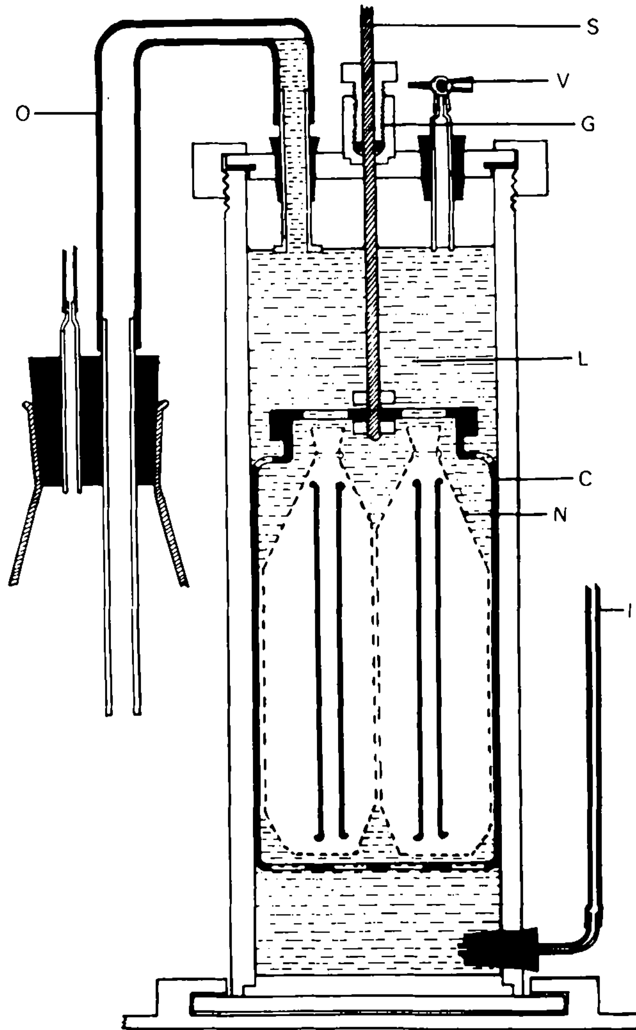


Fig. 1. Outline of a typical unit in the rumen simulation technique (Rusitec) taken from Czerkawski & Breckenridge (1977). L, liquid reaction mixture; N, nylon-gauze bag with solid digesta; G, gland; S, moving shaft; V, sampling vent; C, moving perforated container; I, saliva input; O, effluent output.

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solids leaving the system are largely determined by the apparatus (e.g. Hoover *et al.* 1976). In Rusitec, the amount of solid material that leaves the vessel is known precisely because it is taken out manually.

A typical unit is shown schematically in Fig. 1. The reaction vessel is made of Perspex and is provided with a flange for easy access. Solid digesta are contained in nylon-gauze bags inside a perforated 'cage', which slides inside the reaction vessel with an amplitude of 50–60 mm at 8 cycles/min. Artificial saliva is infused continuously at the bottom of the vessel and the excess liquid and the fermentation gases are forced through an overflow by a small positive pressure in the gas space. An experiment is usually started by placing approximately 80 g solid digesta in one nylon bag, placing a quantity of food to be used in another bag and filling the reaction vessel up to overflow with dilute rumen fluid. After 24 h, the solid inoculum is removed and a new bag of food is placed in the vessel. Subsequently, the older bag is removed each day and a new bag placed in the vessel; this means that the food spends 2 d in the vessel, but other incubation times have been used. The bag to be removed is allowed to drain and then it is placed inside a larger polyethylene bag together with 40 ml artificial saliva. The solid contents are washed by squeezing gently, and then squeezing out excess liquid and repeating the procedure with more artificial saliva. The combined washings are returned to the reaction vessel and the washed solid is dried, weighed and analysed. The output of end-products of fermentation (methane, volatile fatty acids, etc.) decreases slightly during the first 4–6 d of the experiment, and thereafter the steady fermentation can be maintained indefinitely.

It should be stressed that Rusitec is a rumen without a rumen wall (i.e. without a normal layer of epithelial cells); there is no provision for removal of soluble substances except through overflow. However, the fermentation patterns obtained and the outputs of products per unit mass food digested resemble closely the patterns and outputs in sheep kept on the same diet (Czerkawski, 1978).

*Variation on a theme.* It is possible to deviate from the routine procedure outlined previously. For instance, it is possible to have four bags instead of two in each vessel, e.g. two with concentrate and two with roughage (Czerkawski & Breckenridge, 1979*a*). One bag of each component is taken out daily and these are replaced by new ones; the fate of the two dietary components can then be determined independently. Another useful variant of the routine procedure is to leave the fibrous food inside the vessel or to put in a matrix of indigestible material, such as wood shavings, and to supply all the food in solution (Czerkawski & Breckenridge, 1979*b*). As one would expect, the fermentation patterns differ somewhat from the normal patterns (even those obtained with predominantly concentrate diet) but it is not necessary to use a very elaborate cocktail of soluble feed. Other obvious possibilities include the changes in feeding frequency and sequence of bag removal. For instance, with three bags in a vessel, the removal of one bag/d gives an incubation time of 72 h. One investigator in Edinburgh placed bags within bags and another in Ayr introduced small bags to observe changes occurring over a short period of incubation.

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*Control of fermentation in Rusitec.* Two types of control should be considered: (1) to be in control, i.e. to know precisely what is going on in different parts of the system, and (2) to be able to control the system, i.e. to be able to alter its function deliberately. Because of the advances made in surgical techniques it is possible to have access to various parts of the gastrointestinal system of a ruminant animal and to find out what is going on within each of these parts. However, each of these parts, and particularly a large organ like the rumen, is still often treated like a 'black box', with an input, an output and assumed homogeneous contents. Even some of the most recent reports in the literature refer to representative samples of rumen contents taken with a suction tube and yet more than half the space in the rumen is occupied by semi-solid digesta mass (Czerkawski & Clapperton, 1984). In Rusitec, the whole bag of solid digesta is removed, fractionated and subsampled; it would be difficult to obtain a more representative sample. It is also possible to investigate different parts of the microbial system within the vessel and to make quantitative assessments of the relevant contribution of such parts to the whole.

Numerous attempts have been made to alter the relative flow-rates in the rumen by input of water or by adding salt solutions and the results obtained were rather unpredictable. In Rusitec, it is only necessary to adjust the pump setting to choose any required flow-rate. The mean residence time of the solid can also be varied as required. A variety of additives and chemicals can be used in amounts that could not be tolerated by the animal. Having demonstrated definite and reproducible responses, small amounts, e.g. sufficient to give 5–10% change, not normally detected in animal work, could be used in larger trials.

In the majority of experiments with Rusitec, the final control periods gave very similar results to the initial controls and, in a well-constructed apparatus, the results in replicate vessels are identical (Stanier & Davies, 1981).

The system is very stable and can adapt to and tolerate a considerable number of constraints. For instance, in some experiments in India, a very poor but stable fermentation was maintained on a 950 g straw/kg diet. The microbial output was very low and yet the output and pattern of end-products of fermentation was entirely consistent with microbial synthesis (U. Mehra, unpublished results).

Nitrogen is one of the easiest components to determine accurately, and the determination of the N balance in Rusitec gives nearly 100% recovery. It is possible, by supplementing a poor diet of hay with glucose only (N-limiting conditions) to show N recoveries of greater than 100%, i.e. N fixation (Czerkawski & Breckenridge, 1979*a*). Having got such a reliable and controllable system, it is possible to investigate the metabolism of proteins in a very systematic manner. It was shown recently (J. W. Czerkawski, unpublished results) that of three protein supplements tested (soya-bean meal, fish meal and casein), soya-bean meal made the greatest contribution to the output of end-products of fermentation and casein had the smallest effect (see Table 1). It is interesting that although most of the casein was broken down inasmuch as it could not be precipitated with trichloroacetic acid, a large proportion of the products was in the form of polypeptides. With all three supplements the micro-organisms destroyed large

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Table 1. *Fermentation of protein supplements in Rusitec*

| | Change in output (/g supplement) | | |
|---|---|---|---|
| Supplement . . . | Casein | Fish meal | Soya-bean meal |
| Gases (mmol): | | | |
| Methane | 0·2 | 1·3 | 1·8 |
| Carbon dioxide | −0·2 | 1·9 | 4·8 |
| Volatile fatty acids (mmol): | | | |
| Acetic | −0·7 | 2·3 | 3·1 |
| Propionic | 0·7 | 0·6 | 1·3 |
| Butyric | 0·2 | 0·5 | 0·7 |
| $C_5$-acids | 2·1 | 0·9 | 0·6 |
| Microbial matter (mg) | 90 | 110 | 260 |

proportions of protein (measured as total α-amino group) and the extent of this loss was increased by further increasing the supplementation (Table 2), whereas with the hay diet about 90% of the added α-$NH_2$ group was recovered in the undigested food and in the micro-organisms; with casein, fish meal and soya-bean meal supplements, 80, 90 and 150 g/kg diet respectively, the recoveries were 45–57%. When the supplementation was increased further the recoveries of α-$NH_2$ group were even lower (32–46%). It was possible to determine the degradability of the feed protein in the basal diet and in the diet supplemented with casein, fish meal or soya-bean meal and to calculate the protein degradability in the supplement. This was usually very high, even with fish meal (95–100%) and was much higher than the reported results of measurements in vivo and *in situ* (e.g. Ørskov & McDonald, 1979; McAllan & Smith, 1983).

Using Rusitec, it was possible to measure the flows of protein and non-protein-N (NPN) in a systematic manner (see Fig. 2), to define various quantities and to show how some of these quantities are interrelated. The true protein content of the diet (T) is defined as the amount of true protein-N per unit total N in the diet (Tables 3 and 4). This is affected by the amount of NPN added. The degradability of this protein (D) is the net proportion disappearing during the passage through the system. As can be seen in the tables, this quantity was high and did not change much in the model system. The protein conversion ratio (R) is the amount of microbial protein synthesized per unit feed protein disappearing. This interesting factor changed considerably with the type of feed and supplement and in general was high with NPN supplements and low with high protein supplements. Another potentially useful quantity in this system was the efficiency of synthesis of microbial protein (E). This is defined as the amount of microbial

Table 2. *Mean recoveries of total nitrogen and* α*-amino group in Rusitec* (%)

| Protein supplement . . . | Control (hay) | + Casein | + Soya-bean meal | + Fish meal |
|---|---|---|---|---|
| N | 103 | 97 | 96 | 92 |
| α-$NH_2$ group | 93 | 41 | 49 | 37 |

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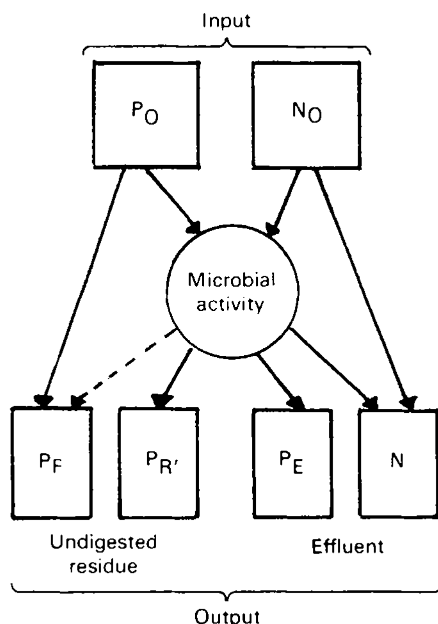


Fig. 2. A diagram to show the flow, distribution and changes in the protein (P) and non-protein-nitrogen (N) in Rusitec. Subscripts: O, original input; F, unchanged food protein; R′ microbial protein in the undigested residue; E microbial protein in the effluent.

protein-N produced per unit total N supplied to the system. With a variety of supplements, this quantity was not very high (0·18–0·33) and it was very simply related to the other three quantities (E = TDR). All this applied to the controlled model system only, but the concepts developed may make it easier to define more precisely the terms used in the experiments in vivo and to understand the rumen better.

Table 3. *True protein content* (*T*), *degradability of protein* (*D*), *protein conversion ratio* (*R*) *and efficiency of microbial protein synthesis* (*E*) *in Rusitec*

(All values are given as ratios of the same units)

| Protein supplement ... | Control (hay) | + Casein | + Soya-bean meal | + Fish meal |
|---|---|---|---|---|
| $T = P_O/(P_O + N_O)$ | 0·49 | 0·78 | 0·76 | 0·74 |
| $D = (P_O - P_F)/P_O$ | 0·81 | 0·96 | 0·98 | 0·91 |
| $R = P_M/(P_O - P_F)$ | 0·84 | 0·25 | 0·41 | 0·27 |
| $E = P_M/(P_O + N_O)$ | 0·33 | 0·19 | 0·31 | 0·18 |

$P_O$, protein in feed; $P_F$, undegraded protein (unchanged food protein); $P_M$, microbial protein; $N_O$, non-protein-nitrogen in feed.

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Table 4. *True protein content (T), degradability of protein (D), protein conversion ratio (R) and efficiency of microbial protein synthesis (E) in Rusitec*

(All values are given as ratios of the same unit)

| Diet ... | Hay | Hay + urea | Hay + barley | Hay + barley + urea |
|---|---|---|---|---|
| $T = P_O/(P_O + N_O)$ | 0·38 | 0·25 | 0·63 | 0·44 |
| $D = (P_O - P_F)/P_O$ | 0·71 | 0·66 | 0·95 | 0·94 |
| $R = P_M/(P_O - P_F)$ | 0·72 | 1·37 | 0·48 | 0·63 |
| $E = P_M/(P_O + N_O)$ | 0·22 | 0·23 | 0·29 | 0·26 |

$P_O$, protein in feed; $P_F$, undegraded protein (unchanged food protein); $P_M$, microbial protein; $N_O$, non-protein-nitrogen in feed.

### *Development of a conceptual model (compartmentation)*

*Definition of compartments.* At least three microbial compartments have been identified in Rusitec and the definition stems directly from practical application. The free suspension of micro-organisms (commonly known as the strained rumen contents) forms the population of compartment 1. When a strained sample of solid digesta is washed repeatedly with artificial saliva, a large proportion of the micro-organisms can be removed. These loosely-attached or temporarily-trapped micro-organisms constitute the population of compartment 2. Whereas the consistency of compartment 1 is uniform, the distribution of micro-organisms in compartment 2 is not. The determination of the total amount of microbial matter in compartment 2 (the washings) is straight-forward, but the estimation of the volume of liquid in this compartment (and hence the mean microbial concentration) is more difficult. However, the latter can be readily calculated (Czerkawski & Breckenridge, 1979*b*). Generally, the washing procedure used dilutes the concentrations of the micro-organisms about three times and since the microbial concentrations in the washings are invariably three to four times higher than in compartment 1, it follows that the mean microbial concentration in compartment 2 must be greater than in compartment 1 by an order of magnitude (Wallace *et al.* 1981). Moreover, since the concentration of micro-organisms in compartment 2 increases considerably with 'depth' (see p. 109), parts of this compartment must have a very dense microbial population. The washing procedure cannot remove all the microbial matter from the solid digesta. It is found that when the nylon-gauze bag of digesta is placed inside a polyethylene bag, artificial saliva is added and the contents are washed repeatedly by squeezing, most of the loosely-bound micro-organisms are removed in the first two to three washes. The microbial population that cannot be removed is defined as the population of compartment 3. The microbial matter in compartment 3 can be partly removed by destroying the solid matrix by sonication or, more effectively, by sonication with alkali. This microbial matter is not removed as intact cells, but it is possible to estimate its amount in the solid matrix using bacterial and protozoal markers and it can be shown that microbial dry matter can account for 10–15% of the dry matter of the washed

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Table 5. *Some of the compartmental properties in Rusitec*

| Compartment ... | 1 | 2 | 3 |
|---|---|---|---|
| Microbial concentration (mg/ml) | 0·7–1·0 | 20–30 | 40–60 |
| Protozoa (% total microbial matter) | 10–20 | 30–40 | 40–50 |
| Dilution rate (/d) (nominal dilution rate 0·9/d) | 1·7 | 0·8 | 0·5 |
| Estimated yield of ATP (g microbial matter/ mol ATP) | 6·2 | 12·0 | 12·9 |

undigested matrix. The volume of compartment 3 can be calculated from the exclusion volume of polyethelene glycol (PEG) (Czerkawski & Breckenridge, 1969) or it can be determined more directly by suspending the washed solid in a nylon-gauze bag in a vessel containing hexane, agitating gently to remove extraneous water, allowing the hexane to evaporate and measuring the moisture content of the solid. The two methods give similar volumes of compartment 3 of 2·5–2·7 ml/g dry matter. In general, the mean concentration of micro-organisms in compartment 3 appears to be greater by a higher order of magnitude than in compartment 2. In fact, under certain conditions, the microbial volume accounts for most of the liquid space in compartment 3 (see Table 5).

The 'structure' of compartment 2 can be studied by a very simple procedure (Czerkawski & Breckenridge, 1982). Instead of washing the bags of digesta with artificial saliva, one corner of the polyethylene bag is cut with scissors, the solid is squeezed slowly and the fractions of the associated liquid are collected (e.g. eight to ten fractions of 3–4 g). It is found that whereas the concentrations of microbial matter and some enzymes (e.g. alkaline phosphatase (*EC* 3.1.3.1)) increase with the depth of the compartment, the concentrations of other substances (e.g. urease (*EC* 3.5.1.5) and ammonia) remain roughly constant (see Fig. 3). This shows that compartment 2 may be homogeneous in some respects and heterogeneous in others. Moreover, there is no sharp division between compartments.

No doubt further studies will reveal even greater complexity of the system but at this stage a lot can be learned by assuming that there are three well-defined and distinct compartments.

*A three-compartment model.* It is reasonable, on the basis of the above discussion, to assume that there must be movement between compartments 1 and 2 and between compartments 2 and 3, but not between compartments 1 and 3 directly. Therefore, it is possible to suggest a simple model as shown diagramatically in Fig. 4, which is largely self-explanatory. Clearly, the properties of the compartments change during routine incubation when new food is added every day. However, it is possible to simulate the three-compartment nature of the system more simply, by introducing an inert solid matrix and supplying all the food in solution. The solid matrix could be in the form of clean wood shavings or it could be hay which had been allowed to remain in the reaction vessels for a few days until there is no further loss of fibre. It was found that this leaves about 20% of the original material, which is not degraded any more (Czerkawski & Breckenridge, 1979*b*). An acceptable steady fermentation was maintained in the

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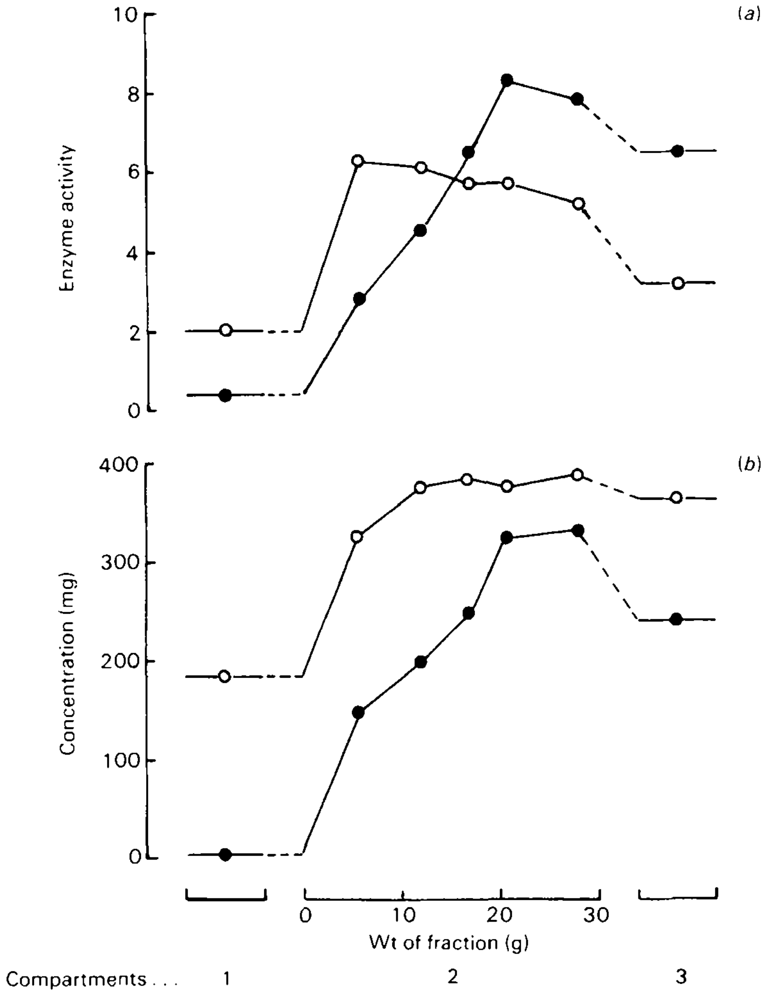


Fig. 3. (*a*) Activity of (○), urease (*EC* 3.5.1.5; μg ammonia-nitrogen/ml per min); (●), alkaline phosphatase (*EC* 3.1.3.1; μmol/ml per h); (*b*) concentration of (○), ammonia-N (mg/l) and (●) protein (mg/10 ml) in compartments 1, 2 and 3 in Rusitec.

reaction vessels, using a relatively simple, but balanced soluble food 'cocktail' and it was possible to calculate the rates of synthesis of microbial matter in each compartment and the rates of flow of microbial matter and water between compartments.

The analyses showed that the flows of liquid between compartments 1 and 2 (assumed equal, since the system was in a steady-state) were smaller than the outflow from the reaction vessel; the flows between compartments 2 and 3 were even smaller. The dilution rates were different in each compartment and decreased with the 'depth' of the compartment, being generally higher in compartment 1 than the nominal dilution rates for the whole system (volume of daily outflow from the vessel/volume of vessel up to the overflow).

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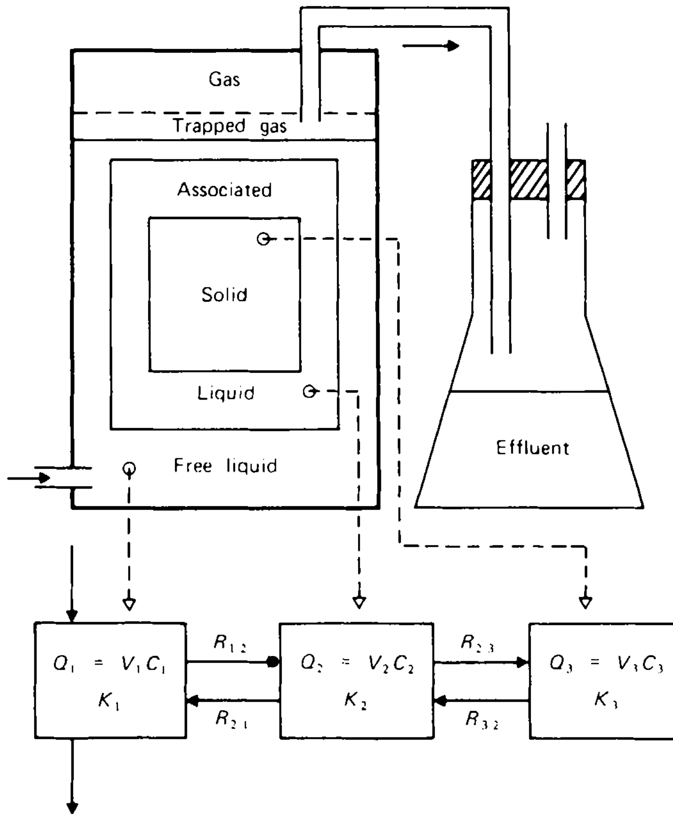


Fig. 4. Schematic representation of Rusitec and a flow diagram to illustrate the three-compartment model of the system. *Q*, *C*, *K* are quantities, concentrations and specific rates of synthesis of microbial matter respectively. *V* is the volume and *R* is the specific rate of flow of water. Thus, the flow of water from compartment 1 to 2 is equal to $R_{1\ 2}.V_1$. (Czerkawski & Breckenridge, 1979*b*).

Since food was supplied in solution by infusion into compartment 1, it was concluded that there was a decreasing concentration of substrate from compartment 1 to 3 and that the flow of bacteria was towards increasing concentrations of substrate. This was consistent with the observation that the flows of diaminopimelic acid (DAP; bacterial matter) were always greater in the direction compartment 3 to 1 than in the opposite direction. In the rumen, or in Rusitec given solid food, the flow of micro-organisms would be from compartment 1 to 3 soon after feeding since the flow of soluble food components is in the opposite direction (a possible aid to colonization). In general in Rusitec, the mean residence time of DAP increased with the depth of the compartment, particularly at high nominal dilution rates. This illustrates the important phenomenon of sequestration of microbial matter in the solid matrix. One of the advantages of Rusitec is that, unlike an ordinary continuous culture, it is capable of maintaining indefinitely a normal protozoal population when balanced solid food is used. Under these conditions the protozoa accounted for about 20% of the microbial mass in

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the effluent and compartment 1. The percentage of protozoa in the undigested, washed residue (compartment 3) was always greater (40–50%) than in the effluent, again demonstrating sequestration.

When the simplified system was used, with all the food supplied in solution, protozoa disappeared from the effluent. Occasionally, a few protozoa could be found in compartment 1, but there were many protozoa in compartment 2 and even greater concentrations (measured in terms of amino ethyl phosphonic acid) in compartment 3. This is an example of sequestration par excellence since no protozoa leave the system and yet a healthy population is being maintained. Since the net synthesis of protozoa under these conditions is zero, the rate of total synthesis must be equal to the rate of degradation as defined by Van Nevel & Demeyer (1977).

*Other compartments and their interrelations.* When solid food is used in Rusitec, there is a single compartment 1, but there are two compartments 2 and two compartments 3 (Fig. 5). This is because at any given time there is the new bag and the bag that had already been incubated for 24 h. In general the properties of compartments 2a and 3a will be different from those of compartments 2b and 3b and they will change during incubation. Thus, strictly speaking, Rusitec given solid food should be described by a five-compartment model, but since most of the changes take place during the first 24 h of incubation, the conditions in the 'new' bag can be estimated from analysis of the bag after 48 h of incubation.

The situation is much more complicated in the rumen, where another microbial compartment can be identified. This is the microbial population that is close to the rumen wall. It has been shown that the microbial population close to the rumen epithelium is different from the rest of the population in the rumen (Wallace *et al.* 1979). There is apparently a lot of ureolytic activity associated with this microbial population and a large proportion of these micro-organisms are facultatively aerobic. The micro-organisms close to the rumen wall would be expected to use substances that are known to pass through the wall (e.g. urea, $O_2$), and they may also degrade dead epithelial tissue. Thus, this region of the rumen contains a microbial population that is different from that found deep in the rumen (compartments 1, 2 and 3) and it is not unreasonable to assume that it forms another compartment and to call it compartment 4.

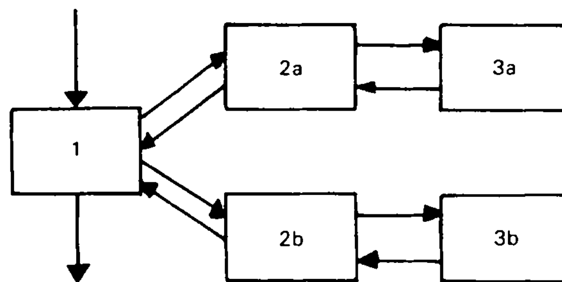


Fig. 5. Flow diagram to illustrate a five-compartment system in Rusitec when normal solid food is used. a, b, Bags that were incubated for 1–2 d respectively (routine use of Rusitec, new solid food every day). Clearly, there is only one compartment 1 (the strained rumen contents).

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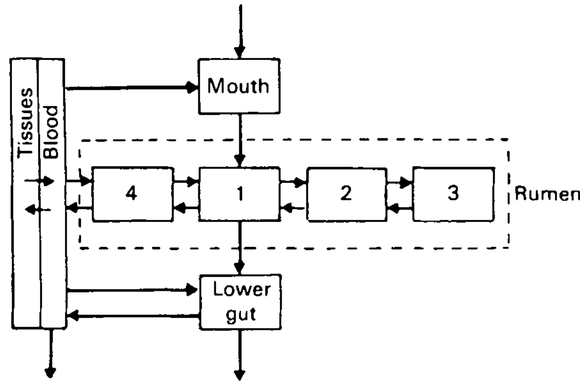


Fig. 6. A conceptual model of the rumen as a basic four-compartment system. Particular conditions, not applicable during rumination. Compartments 1, 2, 3 and 4 are defined on p. 108.

The relationship between compartment 4 and the other three compartments in the rumen is shown in Fig. 6. As with the simple three-compartment system in Rusitec, it is not unreasonable to assume that it is impossible for substances to pass from compartment 4 to 2 or 3 directly. The general properties of the four compartments are summarized in Table 6. Clearly, each compartment may have a different function in the rumen, but each has a vital role in the process of efficient breakdown of ruminant food. However, the divisions are not quite clear. For instance, although the main function of compartment 1 is to allow transport in and out of the system, it is a microbial suspension with a great deal of metabolic activity, particularly with soluble food. Moreover, the transport will include microbial matter and although there are gross differences between the populations of compartments 1 and 2, there are also many microbial species that are common to both compartments. This is fortunate and it is this property that made it possible to find out so much about the rumen by studying the population of compartment 1 only (strained rumen contents). The description of the role of compartment 2 as a shuttle between 1 and 3 is probably insufficient. There is no doubt that there is microbial flow in both directions, but at any given time this

Table 6. *Properties of compartments in the four-compartment model of the rumen*

| Compartment . . . | 4 | 1 | 2 | 3 |
|---|---|---|---|---|
| General purpose | Control of inputs and outputs in the system | | Control of sequestration of particular components | |
| Particular role | Strategic control | Transport medium | Shuttle service | Fibre degradation |
| Predominant population | Facultatively aerobic | Utilizers of soluble substrates | Complex, links in substrate chains | Cellulolytic |
| Host influence | Strong | Fairly strong | Some influence | Very little influence |

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compartment contains a very concentrated microbial population which would soon starve if it were not provided with a flow of nutrients both from compartments 1 and 3. The substrates flowing from compartment 3 would include soluble sugars, amino acids and peptides, while compartment 1 would contribute available N (in the form of urea) and buffering salts.

It is unlikely that micro-organisms can act at a distance. The micro-organisms responsible for initial degradation of fibre must be attached to the fibre or at least be in close proximity to it. Very high concentrations of micro-organisms in confined spaces would soon lead to stagnant conditions unless there were special provisions for removal of products and supply of nutrients that cannot be obtained from the fibrous mass. It is known that many cellulolytic micro-organisms do not break down cellulose beyond cellobiose, which is probably less harmful than the usual acidic end-products of fermentation and that a proportion of N in fibrous plant material is not available to micro-organisms. This underlines the importance of the relationship between compartments 2 and 3 of the model system.

### *Consequences of compartmentation*

*Mechanism of adequate N supply.* N can enter the rumen with food as part of the normal diet or as a supplement and there is an endogenous supply, mainly as urea, through the rumen wall and with saliva. There is no doubt that the endogenous supply of N is very important, but there is a great deal of disagreement on what proportions of endogenous N are supplied by these two paths (Kennedy & Milligan, 1980). Rusitec has no rumen wall, there is only one entry point (with saliva) and this can be controlled. A series of experiments with urea (Czerkawski & Breckenridge, 1982) have shown that the high urease activity of the inoculum declined to low, but not negligible values in Rusitec and that the urease activity could be induced at will by infusion of urea in artificial saliva; the activity was a direct function of the rate of input of urea. The addition of urea as a solid or as a concentrated solution had no effect on urease activity. Furthermore, it was shown that the urease activity per unit volume did not change appreciably through compartment 2 and that the urease activity in that compartment was considerably higher than that in compartment 1 (see Fig. 3). As one would expect, the concentrations of ammonia followed a similar pattern. This seems to be a very sensible scheme. Had the urease activity been high in compartment 1, the urea entering the rumen through the wall or with saliva would be quickly hydrolysed to ammonia which would be absorbed through the wall and possibly result in toxicity. The high urease activity in compartment 2 helps the flow of urea by keeping its concentration low there and ensures a high concentration of ammonia where it is needed most, close to compartment 3.

*Microbial attachment and sequestration.* Microbial attachment to solid food has been amply demonstrated (e.g. Orpin & Letcher, 1978; Amos & Akin, 1978; Clarke, 1979; Bauchop, 1979*a*; for review, see Akin, 1979) but little is known about the mechanism of the attachment. The association is not accidental but an integral and necessary characteristic of the rumen (Czerkawski, 1979). The dilution rate of

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liquid in the rumen is about 1·0–1·5/d. This means that those micro-organisms that have a mean generation time longer than 1 d would be expected to be washed out of the rumen. Yet many slow-growing microbial species have been isolated from the rumen and the only reasonable explanation is that these micro-organisms are not removed at the same rate as those in free suspension; in other words, they are sequestrated. In Rusitec and in the rumen, the relative proportions of protozoa in total microbial mass increase from 10–20% in compartment 1 to about 40–50% in compartment 3. Thus protozoa are sequestrated to a greater extent than bacteria and this is consistent with generally slower growth rates of these micro-organisms. Microbial attachment allows an orderly removal of end-products of digestive activity, better than by simple diffusion, which in confined spaces within the solid matrix would soon result in stagnant conditions, cessation of flow and harmful build-up of solutes.

The microbial attachment concentrates microbial activity where it is needed most and makes the microbial system very resistant to adverse changes in conditions. For instance, in the rumen, compartments 2 and 3 account for about 50% of the liquid space, while in Rusitec these compartments account for 10–15% and yet, for any given diet, the outputs of products per unit food weight digested are very similar in the two systems. Moreover, as already stated, in some experiments in India when a very poor diet of straw was used, the microbial concentrations in the effluent were so low that it was quite transparent and yet the fermentation could be maintained throughout the experiment (30 d). This was possible only because microbial concentrations could be maintained within the solid matrix.

Bacteria and protozoa are the predominant microbial groups in the rumen. However, appreciable numbers of fungal zoospores attached to fibrous plant fragments have been demonstrated (Bauchop, 1979*b*). The life cycle of these micro-organisms is about 24 h and the primary function of zoospores is to attach to a suitable surface. It would appear that the anaerobic fungi are confined to compartments 2 and 3. It is uncertain whether these micro-organisms contribute significantly to the nutrition of the host animal either in terms of microbial mass or through the end-products of their metabolism, but there is no doubt that they are involved in the degradation of fibrous material.

*Attack from within.* Normal chopped but not ground roughage is used in Rusitec and the digestibility of dry matter is not very different from that of the same diets in donor animals. There is no chewing action in Rusitec, but the flow of the liquid portion of digesta through the solid matrix has similar linear speed to the one observed in the rumen, due to rumen movement. The washing and squeezing of digesta, and the return of the washings to the reaction vessel in the model system may partly simulate rumination. The washed and dried fibre matrix, after 48 h of incubation, becomes discoloured and rather brittle, but the size and shape of the particles (both stem and leaf) look unchanged. Therefore, in the first experiments with Rusitec, the visual observations indicated disappointing results. When the material was dried and weighed, it became apparent that more than

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50% of the dry matter had been digested. These observations suggest that the three-dimensional matrix structure should be preserved and that this may improve the efficiency of the digestion of fibre by a microbial attack from within the matrix.

There is little doubt that the degradation of solid food in the rumen is due to enzyme action and that the enzymes are produced by micro-organisms, but an important question to be answered is whether all these enzymes are produced endogenously or whether there is a more efficient mechanism. The synthesis of protein in the rumen is an expensive process and micro-organisms are known to regulate strictly the synthesis of enzymes by a series of complex mechanisms (induction, repression, etc.). It is unlikely that micro-organisms would release large amounts of enzymes into a medium that contains a very varied and concentrated microbial population. The probability that these enzymes would attack a suitable solid substrate and provide nutrients for the cells that had produced these enzymes must be negligible. Exogenously-produced enzymes that attack plant-cell-wall components have been isolated, but often they account for only 2–3% of the total enzyme activity that can be released when the cells are fractured. There is a great deal of evidence of microbial turnover, i.e. lysis and resynthesis in the rumen, particularly in the solid matrix. It would be of great interest to investigate the role of the enzymes that are released when cells are lysed and if these enzymes are more important quantitatively than the enzymes produced exogenously.

Work with Rusitec showed that the dilution rate decreases with depth of compartments ($1>2>3$) and there is evidence that both the efficiency of microbial synthesis and the degree of recycling increase with depth of compartments ($3>2>1$) (Czerkawski, 1982). These are consistent with the hypothesis that in compartment 3, where most of the degradation of fibre takes place, microbial lysis is not as harmful as it is thought to be, but is an essential part of the system.

*Compartmentation in the rumen.* Rusitec is a model system and, although it simulates the rumen well, it cannot be identified with the rumen. The output of end-products of fermentation per unit dry matter digested is the same as in the rumen on a similar diet, the microbial output is similar and it is possible to maintain a reasonable protozoal population. The distribution of micro-organisms and many other substances (e.g. urease, ammonia) throughout the compartments is the same as in the rumen, but the extent of compartmentation is different. In Rusitec, the volumes of compartments 2 and 3 account for 15–20% of the total volume of the reacting system, while in the rumen, particularly when the animals are given roughage diets, the volume of these two compartments accounts for 50–60% of the total volume. It can be argued that many of the important principles that have been established in Rusitec must be even more important in the rumen. For instance, if it can be established that in Rusitec, microbial lysis plays a vital role in the cleavage of fibrous feeds from within, then this process must be even more important in the rumen.

### *Where do we go from here?*

*The existing system.* A lot of information can be gained from studies with the

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existing system. We know a great deal about compartment 1 since this (the strained rumen contents) has been the subject of widespread investigations over the last 20–30 years. We have also learned a lot about some of the properties of compartment 2 but we know little about the very important compartment 3, except that the microbial population there is very concentrated, that it is closely associated with the solid and that some of the properties are different from those of the other two compartments. By its very nature, this compartment will be the most difficult to study but such a study would certainly repay the effort.

Much additional work can be done on the compartmental properties and the effect of manipulation of various factors to alter and possibly improve these properties. Although much progress has been made on lipid metabolism in the rumen, most of this relates to strained rumen contents (compartment 1). In general, lipids are surface active and undoubtedly they could have marked effects on the distribution, movement and activity of micro-organisms within the compartments (e.g. Czerkawski & Clapperton, 1984). Another line of research within the existing system would be to explore the possibility of improving digestion by introducing additional functional groups in the fibrous substrate.

*Further developments.* The number of Rusitec users is increasing and already research workers have begun to introduce interesting variations on the basic theme. Since the basic technique is essentially a three compartment system and since the rumen has at least four compartments, attempts have been made to introduce the fourth compartment in Rusitec by providing an artificial rumen wall.

The introduction of a proper semi-permeable wall would result in a complex apparatus with considerable loss of control. On the other hand, it is relatively easy to simulate some of the known properties of the rumen wall. For instance, it is known that the rumen epithilium is well supplied with a capillary network and thus with $O_2$. It is a simple matter to introduce about 2 m of fine silicone tubing into each vessel (Czerkawski & Breckenridge, 1979*b*), with the entry and exit outside the vessel, and to infuse $O_2$. It was shown that when about 100 ml $O_2$/d was infused continuously, none could be detected at the exit end of the tube. This means that all the $O_2$ diffused through the silicone tube and was utilized by the micro-organisms, presumably by those close to the silicone tubing. The $O_2$ had a slight but consistent effect on methane production, but its disappearance could not be accounted for by the known end-products of fermentation. A microbiological study of the surface growth on the silicone tube would certainly repay the effort.

## *Concluding remarks*

Many model systems have been developed and used in rumen studies (Czerkawski, 1976). Some were very simple and yet they gave an enormous amount of basic information, others were very complicated. Rusitec is more complicated than a simple incubation in a test-tube, but the technique is easy to master and use. Its value is not in a solution of numerous independent problems, as was the case with simpler incubation techniques. The use of Rusitec led directly to the development of simple concepts on compartmentation and made it possible to

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approach the complex problem of microbial degradation of tough fibrous substrate in an orderly and logical manner. It gave a definite 'frame' to work on, rather than an empirical description or a purely abstract model. In other words, the practical model system turned out to be a good conceptual model.

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