# THE CONTROL AND MANIPULATION OF RUMEN FERMENTATION

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The loss of energy which occurs in the rumen fermentation can be very appreciable (Czerkawski, 1969) and it is therefore not surprising that efforts are being made to find ways of manipulating the fermentation to minimise this loss. The area has been reviewed by Marty, (1972), Demeyer and Van Nevel (1975) and Czerkawski (1969). In addition to the energy consideration a number of conditions exist, where the pattern of the major fermentation products, the short chain fatty acids. are unbalanced in terms of animal needs. In the high producing dairy cow a fine balance obtains between the needs for glucose and consequently glucose precursors for milk production (Blaxter, 1967) and the effects of excess of these on milk fat content (Jorgensen, Schultz & Barr, 1965; McCullough, 1966). Very high rates of growth in ruminants have generally been associated with rumen fermentations giving elevated proportions of propionate. Ishaque, Thomas and Rook (1971) have obtained an augmented flow of microbial protein at the level of the duodenum associated with high propionate proportions in the rumen. The rumen fermentation through the production of microbial protein constitutes a major source of essential amino acids to the animal. Possible objectives in altering the fermentation may be:

1) to improve overall energetic efficiency,

2) to improve the balance of glycogenic to non-glycogenic precursors,

3) to increase microbial protein synthesis.

In this review the aim is to examine the energetics of the major types of rumen fermentation in terms of energy to the animal, and energy for microbial growth, to outline the factors normally controlling these fermentations, to indicate susceptible control points, to examine the mode of action of some known agents, which have been used to alter rumen patterns and to give the conditions under which such agents can be helpful.

## Energy available to the animal from the major types of rumen fermentation

Probably the best way to begin examination of the possible value of manipulating rumen fermentation is to look at each of the major fermentation pathways:

anhydrohexose to acetate, methane and $CO_2$;

anhydrohexose to equimolar proportions of acetate and propionate, methane and $CO_2$;

anhydrohexose to acetate and propionate in the proportions 1:2 and $CO_2$;

anhydrohexose to butyrate, methane and $CO_2$;

with respect to their relative theoretical potential efficients in terms of:

1) energy conservation,

2) the provision of glucogenic precursors, and

3) microbial protein synthesis.

The results of the relevant calculations using the relationship of ATP production to microbial growth (Bauchop & Elsden, 1960) and the analytical data for rumen bacteria given by Demeyer and Van Nevel (1975) and Henderickx (1976) are given in table 1. In table 2 some more detail is given of the gluconeogenic precursors in bacteria calculated from Henderickx's (1976) analyses.

A number of points emerge quite clearly from these calculations. In energetic terms conversion to acetate only of the organic acids, results in the highest energy loss, but produces the most favourable protein/energy ratio. The propionate fermentations conserve energy much better but at the price of a decrease in protein/energy ratio of some 15%. The butyrate fermentation is better than the acetate as an energy conserver but is poorest from the protein energy viewpoint.

The diminished protein/energy ratio from propionate production is probably compensated to some extent by a sparing action of propionate, which can compete with amino acids in both catabolism and as a gluconeogenic precursor. Ferriero and I (unpublished observations) have found that with cattle on basal sugar cane:urea diets, which are protein limited, addition of propionate decreases animal performance but when sub-optimal protein supplement is present in the diet propionate increases performance. Although both acetate and butyrate, by providing alternative substrate, could theoretically save amino acid catabolism, their major sites of metabolism are not important in amino acid breakdown so sparing by these is probably negligible.

It is perhaps worth noting that a change to greater energetic efficiency is also a change to higher effective calorie density; a fact which might be used advantageously just below the borderline of 2.6 Cal/g (Baumgardt, 1970).

It is perhaps as well to indicate what are the practical or "real life" boundaries in terms of the fermentations. It is very unlikely that one could obtain a greater shift than that observed in going from a poor quality roughage pattern of Acetate:Propionate:Butyrate 75:15:10 to a high grain pattern of 45:40:15. In table 3 the comparisons are made for protein yield, energy efficiency and protein energy ratio.

It can be seen from these calculations that there are advantages to be had in terms of feed conversion efficiency from directing fermentation towards propionate production at high feeding rates. At or near the maintenance level if diversion is bought at the price of

decreasing overall fermentation rates on a poor quality ration, there may be diminished protein availability, intake and performance. Due to processes which destroy a proportion of the bacteria first synthesised, the yields of bacteria and the overall energetic efficiencies in the animal are lower than the theoretically calculated values of tables 1, 2 and 4. Mathison and Milligan (1971) and Nolan, Norton and Leng (1973) using N15 techniques have demonstrated appreciable return of nitrogen from microbial to the rumen free amino acid and ammonia pools. At the maintenance level of feeding some 30% of biomass synthesized could be lost in this way. Bacteriolysis by phape (Adams *et al.* 1966), destructive bacterial interactions (Hungate, 1966) and protozoa1 engulfment and digestion (Coleman, 1967; 1972) are the main factors in bacterial loss. Lysis by lysogenic phage appears to be an inverse function of bacterial growth, induction being closely associated with processes which slow down DNA replications in the host organism (Watson, 1975). The importance of this process as a source of loss map be expected to diminish rapidly as feeding level is increased above maintenance, provided there are no sharp diurnal variations in food intake.

A generalized thesis is developed later in this review that conditions such as energy substrate pressure, availability of other nutrients and pH favour particular kinds of fermentation and that this becomes a major selective influence on the rumen microbiota. The protozoa whichhave little or no capacity for the propionate pathways tend therefore not to occur under conditions favouring high propionate production but are very prominant in high butyrate fermentations and exist to a lesser extent in acetate rich conditions. Manipulation towards propionate formation in removing the protozoa, a potential source of bacterial loss, should on the surface increase energetic and protein efficiency. The role of the protozoa is at present a hotly debated one. Evidence from the literature seems contradictory with respect to the advantages (Abou Akkada & el Shazly, 1964; Christiansen *et al.* 1965; Borhami *et al.* 1967) and disadvantages (Leng, 1973; 1976) of the presence of protozoa. Several authors have found no effect of defaunation on animal performance (Pounden & Hibbs, 1950; Eadie & Gill, 1971; Williams & Dinusson, 1973). In the author's opinion the key point is the source of protozoa1 amino acids. Protozoa that are directly synthesised from the food materials and passed from the rumen are a better product than bacteria because of their more ready digestion and absorption. When however a large proportion of the protozoa1 protein comes from predation of bacteria the effect is deleterious because of the doubled energy cost of synthesising the protozoa1 biomass. Leng (1973; 1976) has stressed the importance of protozoa1 death in the rumen as a cause of rumen inefficiency arising out of the inherent instability of the protozoa1 populations. A possible mechanism for such instability in butyrate rich fermentations linked to high protozoa1 numbers is discussed below (see section below on 'Internal Factors in Primary Fermenters'). When a large part of the N intake is in the form of NPN there can be little doubt that the presence of large numbers of protozoa is disadvantageous and that attempted manipulation towards a propionate rich fermentation is to be recommended. It is doubtful if anything is to be gained in trying to remove the protozoa present in the rumen when good quality forages are fed.

Losses of synthesized microbial biomass are thus likely to alter appreciably the amount of microbial protein available to the animal. With acetate:methane fermentations, which are likely to predominate

Table 1. The Products of Fermentation by the Major Pathways in the Rumen.

Results per 100 g Anhydrohexose fermented.

| | Path.1 | Path.2 | Path.3 | Path.4 |
|---|---|---|---|---|
| Acetate moles | 0.896 | 0.434 | 0.286 | |
| Propionate (moles) | | 0.434 | 0.573 | |
| Butyrate (moles) | | | | 0.454 |
| Bacteria (Grammes) | 24.90 | 26.98 | 27.67 | 24.03 |
| Digestible Bacterial Calories | 87.15 | 94.43 | 96.85 | 84.10 |
| VFA Calories | 187.3 | 250.0 | 270.1 | 237.9 |
| Total Useable Calories | 274.45 | 344.43 | 366.95 | 322.00 |
| Digested protein g | 8.52 | 9.24 | 9.47 | 8.23 |
| Potential Glucose g | 12.01 | 52.07 | 64.92 | 11.59 |
| Digested Protein per Megacal absorbed | 31.04 | 26.83 | 25.81 | 25.56 |

1) 'Acetate: Methane' Pathway:-

$$2HCO_3^- + (C_6H_{10}O_5) = 2CH_3.COO^- + CH_4 + 3CO_2 + 2H_2O$$

2) 'Propionate:Acetate:Methane' Pathway:-

$$2HCO_3^- + (C_6H_{10}O_5) = CH_3.COO^- + CH_3.CH_2.COO^- + 0.25CH_4 + 2.75CO_2 + 1.5H_2O$$

3) 'Propionate:Acetate' Pathway:-

$$2HCO_3^- + (C_6H_{10}O_5) = 2/3CH_3.COO^- + 4/3CH_3.CH_2.COO^- + 8/3CO_2 + 4/3H_2O.$$

4) 'Butyrate:Methane' Pathway:-

$$HCO_3^- + (C_6H_{10}O_5) = CH_3.CH_2.CH_2.COO^- + 0.5CH_4 + 2.5CO_2 + H_2O$$

Calculations on $Y_{ATP}$ = 10.6g/mole (Bauchop & Elsden, 1960) and bacteria as $C_6H_{9.85}O_{2.99}N_{1.20}$ (Demeyer & Van Nevel, 1975).

Table 2. Glucose Precursors in Rumen Bacteria calculated from Henderickx (1976).

| Component | % age of D.M. | Glucose Equivalent g. |
|---|---|---|
| Protein | 47.8 | 27.72 |
| DNA | 1.9 | .41 |
| RNA | 7.6 | 3.26 |
| Glycerol in lipids | 0.15 | 0.15 |
| Galactose in lipids | 0.06 | 0.06 |
| Polysaccharides | 33.4 | 37.11 |
| Total | | 68.71 |

Assumes 100g protein can form 58 g glucose.
Mean m.w. of anhydromononucleotide 350.

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Table 3. Comparison of Products from high Acetate and high Propionate Fermentations

| Proportions: Ac:Pr:Bu | 75:15:10 | 45:40:15 |
|---|---|---|
| Useable Calories per 100g anhydohexose | 320.3 | 335.8 |
| Useable protein per 100g anhydrohexose | 8.67 | 8.95 |
| Glucogenic material g glucose/100g anhydrohexose | 23.14 | 40.12 |
| Energetic Efficiency % | | |
| g useable protein/Megacalorie absorbed | 28.68 | 26.65 |

Assumptions as in table 1.

at relatively low rates of feeding and rumen turnover, losses by bacteriolysis could be serious and there would be small losses by protozoa1 action. Under these conditions one has also to take into consideration the fact that the maintenance reauirements of the bacteria will appropriate a sizable part of ATP production. The calculations in tables 1 and 3 will overestimate the microbial protein available to the host animal, from this type of fermentation. Propionate fermentations associated with high rates of feeding would be expected to show relatively small losses of bacteria, because the fast microbial growth rates and also from the virtual absence of protozoa when conditions *are* appropriate for propionate formation. High butyrate fermentations resulting from ultra rapid dissimilation of carbohydrates are distinguished by elevated numbers of protozoa and favourable to energy storage (an energy consuming process) and are probably therefore prone to some inefficiency in primary synthesis and appreciable losses through protozoa1 action.

The indications are that, in terms of efficiency of net microbial synthesis, conditions favourable to propionate formation are likely to be best. The observations of Ishaque, Thomas and Rook (1971) on increased passage of microbial protein when propionate predominated in the fermentation products have been mentioned earlier.

Factors determining fermentation patterns: Bacterial Interdependence.

Generally it has been considered that the chemical nature of the substrate determines the selection of microorganisms and that these in turn have specific fermentation patterns, diets high in cellulose and hemicellulose leading to predominately acetate production, diets rich in starch giving high propionate fermentations. A number of observations have pointed the need to modify this view. Roughapes ground and pelleted can give 'starch type' fermentations (Meyer, Kromann & Garret, 1965; Thomson, 1972). Thorley, Sharpe and Rryant (1968) have found the microbial distribution between species in the rumen, with ground forages, to resemble that occuring with grain diets. Barley diets fed below *ad Zibitwn* intakes give rise to butyrate rich fatty acid patterns (Eadie, Hyldegarde-Jensen, Mann, Reid & Whitelaw, 1970). The bacterial flora in this case were similar to those of roughage fed animals. Hobson (1965) has shown changes in product pattern with change of dilution rate on continuous culture of *S. rumencantium*. These observations taken together suggest that the rate of presentation of substrate is a major factor in fixing the direction taken by the rumen fermentation. Hungate (1966) and Wolin (1975) have emphasised the importance of interaction between microbial species in the determination of the final pattern. Fermentation to acetate with $H_2$ evolution is dependent on the removal of H2 by the methanobacteriä and other H2 users. Fermentation with succinate as a product is dependent on succinate uptake by other organisms. Fo rmate production may similarly be expected to depend on formate removal. Of the two major processes of acetate:$H_2$ formation and succinate production, the former is probably less immediately adaptable in terms of meeting rapid changes of conditions because of the need to increase methanogen numbers to meet an increase in H2 production. Optimisation of conditions for the overall sequence involves meeting the requirements of all of its members. No pathway can exist far from the conditions, which are needed by the secondary and later fermenters. When the primary fermenter has a choice of pathways, this choice will

be controlled to an appreciable extent by the relative suitability of conditions for the secondary and later stages. Wolin (1975) has studied several two and three organism systems which demonstrate clearly the influence of the secondary fermenters on the nature and speed of the primary reactions. The presence of methanogens diverted fermentations towards acetate production and decreased the occurence of other H2 accepting processes such as succinate, propionate and ethanol formation. Such systems are naturally reciprocal: H2 pressure is the driving force for methanogen growth, succinate pressure will speed the growth of succinate users and so on.

These interactions and relations although extremely useful in understanding stabilisation of rumen fermentation patterns do not enable us to say why a particular pathway is chosen in the first instance.

Internal Factors in Primary Fermenters. Some insight into basic directing factors can be obtained by looking at internal metabolic relationships of organisms adaptable with respect to their fermentation pathways. Since the major rumen substrates are carbohydrates discussion will be mainly round the metabolic routes for these. The Embden-Meyerhof pathway is the almost universal process taking carbohydrate to pyruvate in rumen microorganisms (Baldwin, Wood & Emery, 1963). The direction taken by the pyruvate formed by glycolysis is the key to the ultimate short chain fatty acid pattern. In Fig. 1 the possible routes for pyruvate are indicated.

Some immediate differences can be seen in the requirements for pathway (1) leading to succinate and propionate and pathways (2), (3) and (4) leading to acetate. Pathway (1) initially consumes CO2 and NADH and $H^+$ while pathways (2), (3) and (4) produce CO2 and increase NADH and $H^+$ directly or indirectly. This acts as a stabilising mechanism; increase in pathway (1) leads to conditions favouring the acetate pathways, while an increase of metabolism to acetate creates conditions for the propionate route. This tends to prevent complete switching to the use of one single pathway. Each of the pathways produces ATP but pathway (1) is the more sensitive to ATP changes. In terms of metabolite control of the glycolytic sequence to pyruvate, the acetate and propionate pathways have different characteristics. Through its production of NAD in pathway (1) coupling occurs with the glyceraldehyde-3-phosphate dehydrogenase reaction so that each reaction accelerates the other. On the other hand the tendency to increase NADH by pathways (2), (3) and (4) leads to negative feedback on the glyceraldehyde-3-phosphate reaction. Increasing flow through the glycolytic system caused by increased substrate pressure by increasing the NADH/NAD ratio thus favours the propionate over the acetate pathways. So here we have one important determinant of the final pattern, substrate pressure.

Associated with NADH usage in pathway (1) there is $H^+$ consumption. The effects of pH drops are thus similar to the effects of increased substrate flow and in practice the two will be found together since increased fermentation rate causes pH to fall.

All the pathways from pyruvate are ATP generators but pathway (1) is the most sensitive to changes in ATP concentration or more strictly in phosphorylation potential $\ln \frac{ATP}{ADP.P_i}$. Increase in phosphorylation

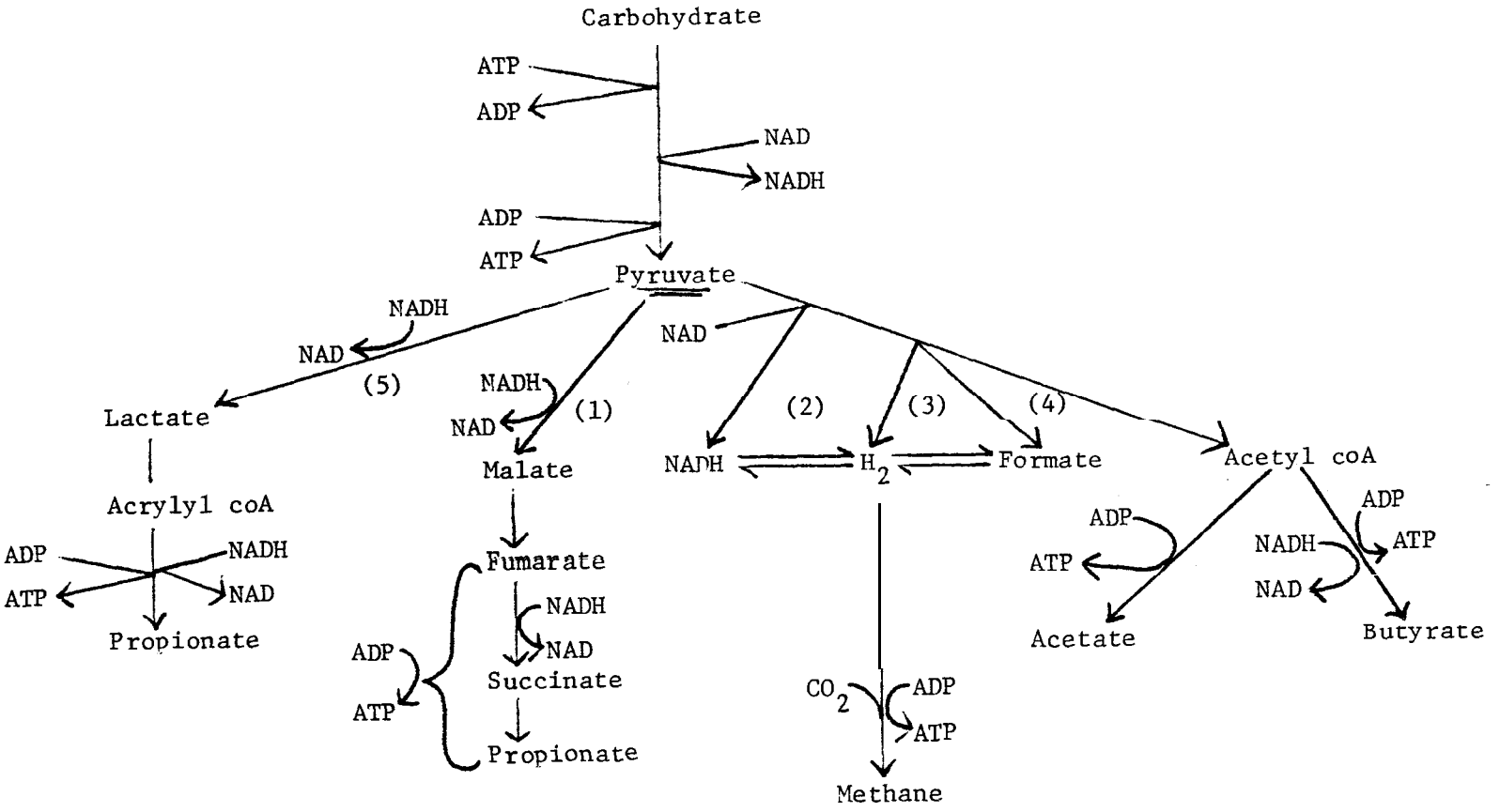


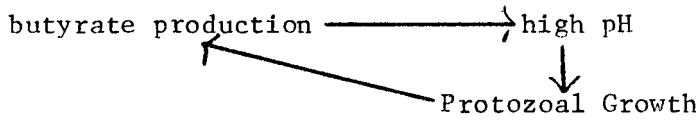

Figure 1. *Pathways of Carbohydrate Fermentation in the Rumen.*

potential acting through the phosphoglycerokinase and phospho-glyceraldehyde-3-phosphate dehydrogenase reactions increases the ratio NAD/NADH and so favours the acetate forming reactions. The main usage of ATP is in the biosynthetic reactions of growth. Optimising the concentrations of the substrates for biosynthetic reactions and growth will maintain the phosphorylation potential low and favour propionate production; nutritional deficiency of any kind will increase the phosphorylation potential and favour acetate formation. Ladzunski and Belaich (1972) have shown the ATP pool to increase in *Zymomonas mobilis* in pantothenate deficiency. It may at first be thought that, as the majority of rumen microorganisms are capable of synthesising nearly all their needs, this effect may be relatively unimportant, but one must realise that most microorganisms maintain allosteric and repressor control of their biosynthetic processes. A small degree of deficiency is necessary to signal the "switching on" of pathways producing amino acids and the monomers necessary to cell synthesis. When preformed nutrients are available in the medium cell levels can be more closely optimised for growth and ATP utilisation. The effects of added amino acids and peptides in the stimulation of growth in organisms which have the biosynthetic potential for the amino acids demonstrates the validity of the thesis. Recent results from Baldwin's laboratory (Maeng, Van Nevel, Raldwin & Morris, 1976) have shown the importance of amino acids as ruminal N sources in increasing microbial growth rates and yields, confirming and extending the earlier observations of Hume (1970). When 25% of N available was as an amino acidmixture,microbial growth yield per unit of carbohydrate fermented was double that obtained with NPN alone.

Thus good nutritional conditions for the primary fermenters will lead to high propionate patterns, poorer conditions will favour acetate and butyrate. The acetate pathway is itself dependent, as we have seen, on the methanogens,which are also auxotrophic. When the acetate pathway is forced by the conditions in the primary fermentation and partially blocked by insufficiency of the secondary reaction of methane formation, the alternative means of hydrogen disposal into butyrate, which also decreases the acetate concentration,tends to become important. In extreme cases formation of higher acids is possible. Because the butyrate fermentation produces only half as much acid as the other main fermentations it tends to lead to rather alkaline conditions in the rumen. The Cuban molasses feeding regime (Preston, Willis, Elias and Sutherland, 1967) with its use of large quantities of urea illustrates the situation of nutritional limitation diverting fermentation from propionate and butyrate becoming a major hydrogen sink. When butyrate is a major product, the resulting high pH further disfavours propionate formation but does creata conditions suitable for protozoa. The original observations of Purser and Moir (1959) of the limitation of protozoa to middle and higher ranges of pH in the rumen have been confirmed many times. These organisms seem to be restricted in their biochemical potential with respect to propionate formation. Of the organisms listed by Hungate (1966) only Entodinia and Ophyroscolex produced propionate and in both of these it constituted only a small proportion of total acids. All of the protozoa produced acetate, butyrate, $CO_2$ and $H_2$. With the protozoa therefore pathways are mainly confined to acetate and butyrate formation with the latter being favoured by substrate pressure.

As discussed above for acetate and propionate formation there is

some degree of co-operative interaction in that the products of one favours the other. Butyrate and propionate formation are diametrically opposed. With butyrate production we have the accelerating cycle shown below:




Self amplifying systems of this kind are extremely sensitive to minor changes in imputs and conditions, and may be expected to show appreciable instability.

Adaptation. When a new set of conditions are imposed in the rumen by a change of dietary regime, there will generally be an immediate shift in fermentation pattern towards that appropriate to these new conditions of substrate pressure, microbial nutrient availability and pH followed by a gradual selection of organisms most efficient in carrying out the preferred metabolic sequence. An exceptional case is concerned with a fifth pathway not discussed above the formation of lactic acid. This pathway has feed forward characteristics in that the glyceraldehyde-3-phosphate dehydrogenase reaction can be coupled to the reaction of pyruvate to lactate. Because of its rapidity and the virtual absence of product inhibition effects and consequent independence of secondary fermenters this pathway becomes important in the changeover from herbage to diets containing high concentrations of freely fermentable carbohydrates. The pathway is succeeded by lactate utilisation by organisms such as Megasphaera elsdenii and Veillonella spp. and propionibacter spp. With high starch diets lactate can remain important as an intermediate (Wallnofer *et al. 1966)* although its production tends to be diminished somewhat by competition with primary propionate and succinate formation. After the initial phase of adautation rumen concentrations lactate concentrations remain low. Marty and Sutherland (1970) found that with molasses based diets that diminution of lactate concentration with time of adaptation was not due to a decrease in the rate of production (which actually increased) but to an even greater increase in the rate of disappearance.

Lactate may be metabolised in two principal ways:-

1) by reoxidation to pyruvate and then further processing by the routes 1), 2) and 3) as outlined above (see Fig. 1). The same general rules for acetate:propionate butyrate partition apply.

2) by metabolism through the acrylate pathway (pathway 5. of Fig. 1) with propionate the unique product.

Switching of propionate formation between the acrylate and the succinate pathways is probably a pH function with the former predominating at low pH, because of the requirement for $CO_2$ as bicarbonate in malate formation (Schwartz & Gilchrist, 1975). It should be stressed that lactate does not inevitably end up as propionate. When the acrylate pathway is of limited occurence, the distribution of lactate carbon in fatty acids will not be very different from the distribution of carbon from hexoses.

The Free Energies of Interconversion. As shown in the preceding section

the key to the final fatty acid pattern lies in the metabolic routes taken from pyruvate. In an adaptable organism the selection of route will depend on the Free Energy decrease by that route. This is equivalent to saying that the selection of propionate rather than acetate will occur if there is a decrease in Free Energy attendant on the conversion of acetate to propionate under the cellular conditions.

If we define "P" as $\frac{(ATP)}{(ADP).(P_i)}$

and "I" as $\frac{(NADH).(H^+)}{(NAD)}$

the Free Energy $G_{ap}$ of the conversion acetate to propionate is

$$G_{ap} = G'_{apo} + RT \ln. \frac{(prop)}{(acet)} . \frac{P}{I^3} \quad (CO_2)$$

Increase of "P" makes this more positive that is favours acetate formation. Increase of "I" makes this more negative and so favours propionate production.

For the reaction butyrate to propionate the Free Energy $G_{bp}$ is

$$G_{bp} = G'_{bpo} + RT \ln. \frac{(prop)^2}{(buty)} . \frac{P}{I^4} \quad (CO_2)^2$$

Increase of "P" favours butyrate formation.
Increase of "I" strongly favours propionate production.

For the reaction acetate to butyrate the Free Energy is given by

$$G_{ab} = G'_{abo} + RT \ln \frac{(buty)}{(acet)^2} . \frac{1}{I^2}$$

The relationship here is governed solely by I, increase of which drives butyrate formation.

The general conclusions are as in the preceding section. Substrate pressure, increasing NADH/NAD, $H^+$ and $CO_2$ leads to propionate formation but increase of the ratio ATP/ADP.$P_i$ forces fermentation towards the acetate:butyrate axis the pronortions of these being dependent on the NADH/NAD ratio. Acetate is favoured by efficient hydrogen removal, high ATP/ADP ratios and slow fermentation. An additional point worth noting is that factors which lead to fatty acid accumulation such as decreased motility, hyperkeratinisation of the rumen wall, increase of pH will all tend to increase the importance of butyrate as a product.

Effective Substrate Concentration. The discussion above stresses the importance of substrate pressure as a determinant of fermentation patterns, but unfortunately it is difficult to put an numerical value for the effective concentration of insoluble substrates such as starch and cellulose in the rumen fermentation. If the effective concentrat-

ion of the substrate is defined as that concentration of glucose which gives the same rate of fermentation as the substrate we can proceed as follows.

$$\text{Rate} = (\text{Substrate concentration}) \times K_{\text{substrate}}$$

$$= (\text{Effective concentration}) \times K_{\text{glucose}}$$

$$\text{or (Effective concentration)} = (\text{Substrate concentration}) \times \frac{t_{\frac{1}{2}}\ \text{glucose}}{t_{\frac{1}{2}}\ \text{substrate}}$$

$t_{\frac{1}{2}}$ glucose = 1 minute

A sheep at maintenance will eat 1000g/diem of feed of 60% digestibility. Of the 600 g digested, 540 g will be fermented in the rumen assuming an apparent digestibility coefficient for the rumen of 70% and that 20 g of microbial biomass are produced from each 100 g of digestible organic matter. If fermentation is at a steady rate, for a rumen of 51 volume, the rate of fermentation is 4.5g/1/h. A $t_{\frac{1}{2}}$ glucose of 1 min corresponds to a $K_{\text{glucose}}$ (h-l) of 41.58.

$$\text{Effective substrate concentration} = \frac{4.5}{162} \times \frac{1000}{41.58}\ \text{mM}.$$

$$= 0.668\ \text{mM}.$$

We can now look at the effect of placing 100 g of substrate of various kinds into the rumen.

For soluble carbohydrates of grass $t_{\frac{1}{2}}$ = 4 min.

$$\text{Immediate Effective concentration} = \frac{100}{5 \times 162} \cdot \frac{1}{4} \times 1000\ \text{mM} = 30.86\text{mM}$$

For starch $t_{\frac{1}{2}}$ = 60 min.

$$\text{Immediate effective concentration} = \frac{100}{5 \times 162} \cdot \frac{1}{60} \cdot \frac{1000}{\text{mM}} = 2.06\text{mM}$$

For cellulose $t_{\frac{1}{2}}$ = 360 min

$$\text{Immediate Effective concentration} = \frac{100}{5 \times 162} \cdot \frac{1}{360} \cdot \frac{1000}{\text{mM}} = 0.34\text{mM}$$

Nearly 99% of the soluble carbohydrate and some 83% of the starch would have to be fermented before the substrate pressure fell to that caused by the cellulose addition. The bulk of the three substrates are thus metabolised at quite different effective substrate concentrations.

Improving the substrate access to the cellulose by a factor of six would bring its effective substrate concentration up to that of the starch. Ingestion of the grass soluble carbohydrates in grazing with slower steady intakes could bring the effective substrate concentration down into similar regions to the starch. Feeding molasses where the soluble carbohydrates are in free solution and immediately accessible

would lead to very high substrate pressures of short duration. Feeding chopped sugar cane the rate of release of the contained supar would tend to restrict to some degree the attainment of ultra high substrate pressures.

It may be noted here that high acetate fermentations are associated with long fibre diets and the consequent low effective substrate pressures. Intermediate substrate pressures as with starch diets fed *ad libitum* ground and Pelleted roughages; good quality spring grass leads toincreases in propionate. Feeding sugar cane gives fermentation patterns with around 20-25% propionate (Leng & Preston, 1976) and appreciable butyrate, while molasses feeding leads to auite high proportions of butyrate in the end products (Marty & Preston, 1970). Because of the extremely rapid dissimilation of soluble carbohydrates and simple sugars, the rate of ATP production can exceed the rate at which it can be used for growth so that the resulting high phosphorylation potentials cause preferential blocking of the succinate pathway of propionate formation and consequent butyrate accumulation. This situation will be intensified if there are nutritional limitations to growth imposed by the use of NPN as urea rather than protein as a dietary source of nitrogen.

It can be argued that with insoluble substrates, substrate pressure in the immediate environment of digesting organisms must be appreciably higher than the value calculated above as effective substrate concentration. This is undoubtedly true but the most important parameters are the rate of potential hydrogen generation and its interaction with the hydrogen removing process of methanogenesis. For these the effective substrate concentration as calculated is appropriate.

Options in Rumen Manipulation

Manipulation of rumen fermentation is more readily performed under intensive rather than extensive conditions and the ensueing discussion is predicatedon this basis. Possible methods may be conveniently divided into:

1) component selection and processing, and

2) the use of additives such as inhibitors, antibiotics and uncouplers.

Selection of components and processing. The primary control over rumen fermentation is in the selection of the major dietary components, which for stall feeding are likely to be selected from dried forages, silage, grains, molasses and in tropical regions nossibly sugar cane, with or without NPN and protein supplements of animal or vegetable origin and possibly some small auantity of fat. For growth or fattening the objective is to obtain as elevated a proportion of propionate as is possible. The nature of the material ingested and its rate of administration determine the effective substrate concentration and processing is designed to put this in the "grain region" where propionate formation is favoured.

For fibrous components processing required is such as to substantially increase accessability of the substrate to the rumen microorganisms. Grinding and pelleting have been used to achieve this

by physical means, Alkali treatment, though penerally used to increase digestibility, should also increase rate of fermentation. Acid treatment aimed directly at decreasing the chain length and increasing the solubility of B-linked polysaccharides seems largely unexplored in feed stuff preparation, although in the case of hagasse (cane fibre) the sugar mill has the energy excess to do this. Production and testing of hexitols from wood pulp residues has recently been reported.

At the opposite extreme,in terms of rate of attack on the substrate component,is molasses and here the processing required should be such as to diminish the rate of availability. A possibility here is the occlusion of the molasses in suitable particulate material such as bagacillo (cane fine fibre fraction) followed by pelleting. Optimisation of conditions for propionate formation requires the presence of peptides, amino acids and ammonia to prevent limitation of microbial growth and elevation of ATP concentrations. This could be done by incorporation of urea, protein or protein hydrolysate (such as fish silage) with the molasses. Use of a protein with tendencies to gelatinize could also control rate of release. The presentation of molasses in pelleted form should decrease appreciably salivary secretion and so help to lower rumen pH into a region more favourable to propionate formation. Kay (1963) found pelleting of more conventional diets to lead to low ruminal pH. Perkins and his co-workers (1975) have shown successful replacement of part of the concentrate component of dairy rations with pelleted molasses sugar beet pulp.

Processing of grains has been extensively reviewed recently (Armstrong, 1972). The only comment I wish to make here is on the need to examine closely the type of starch in relation to the degree of processing. Starches which by their nature are readily attacked may become over available on processing and give butyrate type fermentations when fed at levels insufficient to maintain a low rumen pH.

Manipulation by Inhibitors and Uncouplers. Modification of the rumen ecosystem by chemical addition is subject to the obvious limitation that the added material must be non-toxic to the host. Attempts to change the fermentation pattern by inhibition at reaction stages, which are closely similar to stens in metabolic pathways in the animal itself are likely to lead to problems. Logically therefore reagents should be aimed at those critical stages which are not paralleled in subsequent host metabolism. Though diversion of fermentation towards propionate might be rather better achieved by blocking pyruvate decarboxylation and so preventing both acetate and butyrate formation it is much safer to concentrate attention on blocking methanogenesis, a unique process.

Although a large number of compounds have been shown to decrease methanogenesis (see the review by Demeyer and Van Nevel, 1975) probably only long chain fatty acids, halogenated analopues of methane and uncoupling agents promise at present any practical application. It should be realised at the outset that, except possibly in the case of uncoupling agents, that changes of rumen VFA distribution will be bought at the price of diminished overall fermentation rate and that therefore such manipulation must be limited to diets the nrincinal components of which are fermented rapidly compared with rumen turnover time or where the decreased intraruminal digestibility can be compensated by postruminal digestion and absorption or where physical processing of the diet has been carried out to offset the effects on ferment-

ation rate.

Of the halogenated compounds chloral hydrate is probably the best studied. It has been shown to be converted to chloroform (Prins & Seekles, 1968), which is known to be converted to chloroform, an inhibitor of methanogenesis in *Mb.ruminantium* (Bauchop, 1967). In the rumen, mathanogenesis is diminished, hydrogen accumulates and propionate proportions are increased (Prins & Seekles, 1968). Inhibition of methanogenesis is probably through reaction with reduced cobamide (Wood, Kennedy & Wolfe, 1968). Chloral hydrate also causes a depression in the rate of proteolysis in the rumen (Sinph & Trei, 1971) and consequently of ammonia levels (Van Mevel, Henderickx, Demeyer & Martin, 1969).

Positive responses to chloral hydrate fed as the hemiacetal with starch have been reported for steers and fattening lambs (Trei & Scott, 1971; Trei, Scott & Parish, 1972) but Johnson et *al*. (1972) found no beneficial effects.

At present the case for inclusion of chloral hydrate remains not proven.

Long chain fatty acids have been shown to inhibit methanogenesis (Czerkawski, Flaxter & Wainman, 1968) probably by interference with membrane function and uncoupling of ATP production (Galbraith & Miller, 1973). Propionate proportions are increased (Czerkawski, 1973) and there is a depression of butyrate formation possibly by competition for CoA and related to a decrease in protozoa, which are butyrate formers. Major propionate formers are not affected by the presence of long chain fatty acids (Henderson, 1973). Although cellulolysis is diminished by the long chain fatty acids, the proportionate effect is smaller than in the case of methanogenesis. Hydrolysis of dietary fat to form the free fatty acids, which are the effective inhibitors of methanogenesis may be itself decreased when high levels of easily fermented carbohydrate are present in the diet (Latham, Storry & Sharpe, 1972).

Despite the promise of fatty acids in directing fermentation in *vitro* and *invivo* towards propionate formation, favourable effects on animal growth and performance have not been demonstrated: experiments in this area having given disappointing results (Cotton, Boucque, Van Nevel, Demeyer & Buysse, 1973; Johnson, Wood, Stone & Moran, 1972).

Susceptability of the methanogenic bacteria to uncoupling agents may indicate that electron transport phosphorplation is the uniaue means of ATP generation in these organisms. Propionate formation by the succinate and acrylate pathways, although probably also involving electron transport phosnhorylation is supnorted by substrate level phosphorylation so that decreased ATP yield on partial uncoupling can be compensated by increased metabolite flow. This is also true for butyrate formation. Uncoupling agents in general may thus be expected to decrease methanogenesis and acetate proportions and increase propionate and butyrate formation. Lactate formation may also be expected to rise since this type of fermentation is exclusively by substrate level phosphorylation. In general one would expect preater diversion to propionate than to butyrate with uncoupling apents but this will depend also on factors such as substrate pressure, pH and long chain fatty acid levels.

Rumensin (monensin) is probably the most frequently used uncoupling agent and increases of propionate proportions and beneficial effects on animal performance on grain based diets are well documented (Richardson, Raun, Potter, Cooley & Rathmacher, 1976; Raun, Cooley, Potter, Rathmacher & Richardson, 1976). Practical advantages are generally seen as improvements in feed conversion efficiency rather than increases in rate of gain. Information on other diets is more sparse though increases in propionate and live weight gain have been reported for animals at pasture (Potter, Cooley, Richardson, Raun & Rathmacher, 1976).

Interestingly Lopez (J.M. Lopez pers. comm.) has found rumensin to increase butyrate with negligible effects on propionate when sugar cane based diets are fed.

The alkylated phenols (Marco & Erwin, 1967) and DDT, which is dechlorinated in the rumen (Kutches, Church & Duryee, 1970) probably function in a similar way to rumensin as uncoupling agents.

## Summary and Conclusions

Substrate pressure, pH and the availability of subsidiary (non-energy) microbial nutrients are seen as the primary determinants of rumen fermentation patterns. Energetic efficiency is likely to be highest with fermentations in which propionate is the predominant product. Such fermentations are associated with intermediate substrate pressures, good availability of subsidiary microbial nutrients and relatively low pH. Very high substrate pressures at high pH and low availability of subsidiary microbial nutrients favour butyrate formation. Low well sustained substrate pressures at intermediate pH with moderate to poor subsidiary microbial nutrient levels give the conditions for acetate:methane fermentations.

With roughages improvement towards pronionate increase and greater animal performance can best be achieved by better substrate access through decrease of particle size and possibly chemical pretreatment with acid or alkali. Attempts to divert to nropionate formation by chemical inhibition of methanogenesis, when the preponderance of substrate is in the form of cell wall materials, are unlikely to be very productive because of the effects of diminished rate of attack on materials, which are already slow to be attacked.

Some economies in grain use can be obtained by the use of ground forage materials and by the use of rumensin in grain based feed lot rations.

It is suggested that manipulation of molasses based diets might be obtained by compounding for slow release and diminished salivation on acubedmicoparticulate base with urea and minimal quantities of protein or protein'hydrolysate. Investigation of the combined use of long chain fatty acids and rumensin on molasses and cane based diets might also merit consideration.

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