The Biochemistry and Microbiology of the Rumen and the Hind Gut (Lectures 4 and 5)

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A GENERAL INTRODUCTION

The stomach of the ruminant animal is part of its gastrointestinal tract and consists of four parts, the rumen, the reticulum, the omasum and the abomasum. The rumen and the reticulum are joined by a fold of tissue and the digesta can flow relatively freely between these two compartments. Therefore these two parts of the ruminant stomach are known collectively as the reticulo-rumen. The ruminant stomach is a large organ, which together with the contents can account for about 15% animal weight, with the reticulo-rumen accounting for 80-90%.

The abomasum corresponds to the simple stomach of monogastric animals in as much as its contents are acid and it appears to have a similar function. The actual function of the omasum has not been clearly defined, but it appears to be involved in the water balance within the ruminant gut. The reticulorumen functions as a large fermentation vat, where the largely lignocellulosic feed of poor quality can be converted to products that can be subsequently utilized by the host animal.

The reticulorumen is a complex multilobe outgrowth of the gut, with strong muscles and efficient blood supply. It is subject to rumen movement brought about by regular muscular contraction, and the movement controls the passage of digesta within and out of the reticulo-rumen. It

also controls the process of rumination, where part of the solid digesta is regurgitated, chewed and swallowed again and the process of eructation (a mechanism for the removal of fermentation gases).

The reticulo-rumen is inhabited by a dense and complex microbial population consisting of bacteria, protozoa and anaerobic fungi. The microorganisms break down feed, convert it to end-products of fermentation and thus obtain energy and nutrients for their own growth. The host animal uses the end-products of microbial metabolism for fattening and it uses the microbial matter as an important source of its own dietary protein and other essential nutrients.

In spite of the diversity of microbial species in the rumen, the important biochemical reactions in their metabolism are common to most species. Therefore, in the study of biochemistry of the microbial system of the rumen, it is possible to use mixed microbial samples and, in fact to treat the rumen as if it was a homogeneous bag of enzymes. This approach will be adopted here to outline the main features of the biochemistry of the rumen.

The fibrous components of the ruminant diet are difficult to digest and although they are broken down extensively in the rumen, this is brought about by the concerted action of different groups of microorganisms and an indirect contribution of the host animal. The efficiency of this process depends on the provision and maintenance of a favourable environment, a proper spatial distribution of microbial populations in a heterogeneous system and suitable differential flows of digesta both within and out of the rumen. Consequently, from a microbiological point of view, the rumen cannot be considered to be a simple bag of enzymes or microbial packets of enzymes.

B BIOCHEMISTRY

1. Conditions in the rumen

The ruminant animal provides an excellent environment for microbial activity and growth in the rumen. The contents are kept at 39^{o} and the pH value is maintained within narrow limits by removal of acidic end-products (absorption and dilution) and by an input of buffering salts in saliva. The contents are mixed and the throughput is maintained by a sophisticated mechanism designed to deal with demands imposed by the heterogeneous and refractory nature of the substrates and the requirement for prolonged residence time within the reaction system.

Although the rumen wall is well supplied with blood and therefore oxygen, deep in the rumen the conditions are anaerobic and most of the biochemical reactions do not require oxygen.

2. Substrates

Essentially all the food of ruminant animals comes from plants. It contains carbohydrates, proteins and other nitrogenous compounds, lipids, phenolic and other acids and inorganic compounds. Quantitatively, the most important constituents are the carbohydrates. They consist of structural and nonstructural carbohydrates, with the former predominanting in forages and the latter in concentrate feeds. These, together with their approximate proportions are listed in Table 1. In general the structural carbohydrates are the most difficult to break down, and the extent will depend on the age and type of the plant. Much of the protein in plant material is derived from plant cell contents, but a proportion of

crude protein (N x 6.25) comes from other nitrogenous compounds and some may not be available to microorganisms. Plant lipids are complex and those that are derived from cuticular material are not readily metabolized in the rumen. Little is known about the contribution of other compounds (e.g. tannins, resins) to ruminant nutrition, except that some could be detrimental because of their physiological effects or because they may reduce the availability of the usual substrates for microbial attack.

3. Reactions in the rumen

In general the biochemical reactions in the rumen are mediated by biological catalysts - the enzymes, with particular steps requiring specific enzymes. Some of these reactions are simple, others result in production of intermediates and may require specific co-factors, which could undergo a change during the reaction and which have to be regenerated (Fig. 1).

(a) Hydrolysis

A large proportion of carbohydrates and proteins in ruminant feeds is in the form of polymers which have to be hydrolysed to smaller units. This is mediated by a variety of hydrolytic enzymes (glycosidases, proteases) and there is evidence that many of these reactions stop before the polymer is broken down into single units. However, the oligosaccharides or polypeptides are attacked further, sometimes by different microbial species equipped with specific enzyme systems. Common intermediates in the degradation of cellulose and hemicellulose are the disaccharides cellobiose and xylobiose.

There are many other hydrolytic reactions in the rumen and some, like the conversion of ATP to ADP, are involved in energetic exchanges.

glucose is converted to 2 moles pyruvic acid with a net production of 2 moles ATP. The energy released during hydrolysis of ATP can be used in synthesis and in other processes. It should be noted that some of the intermediates in this pathway can be used in the synthetic processes and the final product, pyruvic acid (or its phospho-derivative phospho-enolpyruvic acid PEP) plays a central role in many other reactions (Fig. 5), being involved in the synthesis of most amino acids, particularly through the tricarboxylic acid cycle. This cycle (Fig. 6) has a central role in many reactions resulting in the synthesis of not only proteins, but other compounds that constitute the functioning microbial cell.

The C_3 compounds resulting from the cleavage of glucose are converted to the acid end products of fermentation (acetic, propionic and butyric acids, Fig. 7). Propionate is produced by the randomizing pathway through succinate or directly through acrylate. The production of acetate results in a cleavage of PEP to CO_2 or formate and the production of butyrate is in effect a condensation of two C_2 units. The production of acetate and butyrate results in a net output of reducing power, either as reduced cofactors (e.g. NADH) or as hydrogen gas, while the production of propionic acid results in a net uptake of reducing power. Since the production of acetate + butyrate always exceeds that of propionate in the rumen, there is always an excess of reducing power and part of this is used by methanogenic bacteria to reduce CO_2 to CH_4 (on the right of Fig. 7).

(c) Synthetic reactions

These have already been mentioned (Figs 5 and 6); they illustrate de novo synthesis of building blocks that can be used by the microorganisms to grow and reproduce. These building blocks are then used

to synthesise polymers, such as microbial enzymes (protein), storage or structural polysaccharides, lipids, nucleic acids, etc. The synthetic reactions have one thing in common, they all require energy. This is particularly pertinent to the synthesis of microbial protein from carbon fragments and NH_3 produced by degradation of feed proteins. Since the energy released during catabolic reactions is always less than the energy expended in the corresponding synthetic reactions, the degradation and resynthesis of protein (dietary to microbial or microbial to microbial e.g. due to lysis of microorganisms) will lower the efficiency of the system. However, these seemingly inefficient processes may have certain beneficial effects. For example, the microbial protein has a better nutritional value to the host animal than some of the feed proteins and the microbial turnover may be of benefit in the degradation of fibrous materials by the adherent microbial population.

(d) Transfer reactions

These are very important in the biochemistry of the rumen, particularly in relation to the generation of energy and will be discussed in the next section.

4. Reduction and oxidation

Since the microbial system of the rumen functions without oxygen, when one substance is oxidised another must be reduced at the same time. For instance, when ethanol is incubated with rumen contents it is converted to acetic acid, as can be easily shown by incubations with ^{14}C -ethanol. This is accompanied by an increase in production of CH_4 which is not labelled. The reactions can be summarized as follows:

$$CH_3CH_2OH + H_2O \longrightarrow CH_3COOH + 2H_2 \quad \ldots\ldots (1)$$

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \quad \ldots\ldots\ldots (2)$$

In reaction (1) ethanol is oxidized to acetic acid and at the same time water is reduced to hydrogen. In reaction (2) carbon dioxide is reduced to methane and at the same time hydrogen is oxidized to water. Many other examples can be found in the rumen. For instance in Fig. 7 when fumarate is reduced to succinate, the reduced cofactor $FADH_2$ is oxidized to FAD.

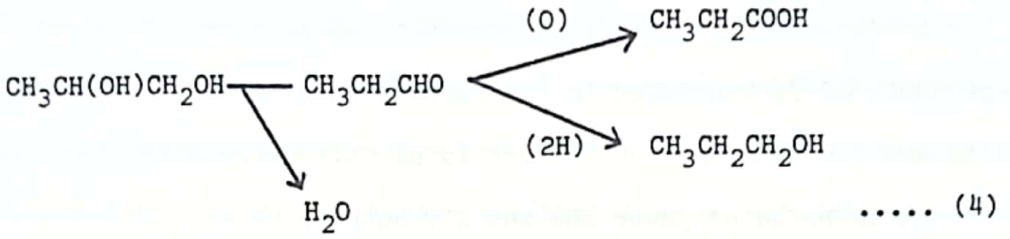
Except for abnormal conditions, hydrogen gas does not accumulate in the rumen. The equations (1) and (2) above and the reactions summarized in Fig. 7 show why this is so. The reactions in many rumen bacteria and protozoa are such that there is a net production of hydrogen but fortunately there are other microorganisms that can use this hydrogen and benefit from it. The reaction summarized by equation (2) is incomplete; it is associated with release of energy which the methanogen can use for growth.

Metabolic hydrogen and its fate can be used as a unifying principle when one considers the complex interactions in the rumen. The identification and measurement of products and the use of known stoichiometric relations can often confirm our hypotheses and even lead to the discovery of hitherto unsuspected pathways. For instance, when 1,2-propanediol was incubated with rumen contents the main product was propionic acid.

$$CH_3CH(OH)CH_2OH \longrightarrow CH_3CH_2COOH + "H_2" \quad \ldots\ldots (3)$$

However, there was no accumulation of hydrogen and there was no increase in methane production (hydrogen sink). In fact there was a small inhibition of methane production (compare reactions (1) and (2). A search

for another end-product revealed an accumulation of n-propanol and the reaction was shown to be a dehydration followed by a dismutation:



..... (4)

where some of the intermediate (propionaldehyde) is oxidized to propionic acid and some is reduced to propanol.

5. Generation of energy

When the so-called high energy compounds are hydrolysed, for example when ATP is converted to ADP + P, there is a release of free energy, which in the absence of a coupled energy-requiring reaction would be converted to heat. We have seen in the foregoing discussion (e.g. Figs 4 and 7) that the degradation of certain substrates is associated with production of ATP from ADP and phosphate and the energy released could be used in coupled synthetic reactions. ATP obtained in this way is produced by substrate level phosphorylation (SLP).

The high-energy phosphate bonds can be also produced in association with some reduction-oxidation reactions, such as the conversion of fumarate to succinate in the production of propionic acid. The view that this type of reaction may result in ATP formation is supported by the large change in free energy for this reaction, by the new ideas on the electro-chemical potential across bacterial membranes and the electron transport. The ATP or equivalent produced in this way is said to be due to electron transport phosphorylation (ETP).

It is possible, from stoichiometric relations and from known or likely metabolic pathways to calculate the changes in the reducing power and the production of ATP during conversion of glucose to the main end-products of fermentation in the rumen. This has been done and the results are given in Table 2. There is no obvious relation between the net change in reducing power and the ATP output.

Since we can measure the production of end-products of fermentation in the rumen, we can in fact calculate the ATP yield using the values in Table 2.

The microbial yield Y_{ATP} is defined as the weight of microbial dry matter produced (g) per mole of ATP used. It is often assumed to have a value of about 10, but other values have been reported. Thus, if the microbial output in the rumen is measured together with the output of end-products of fermentation it is possible to calculate the microbial yield Y_{ATP} or if the value of Y_{ATP} is assumed it is possible to predict the microbial output from the fermentation data.

C MICROBIOLOGY

1. The microbial system in the rumen

Although rumen contents are in a state of flux they are very heterogeneous, consisting of a free suspension of microorganisms (rumen liquor) and a large fibrous mass of digesta that has a very concentrated microbial population closely associated with it. The raft of digesta occupies the central constricted part of the rumen (the boundary between the dorsal and ventral rumen) and it is washed by rumen liquor and by reciprocating flows brought about by ruminal contractions. Portions of

the fibrous mass are regurgitated, chewed, squeezed and swallowed. The sharp contraction of the reticulum propels the food and the bolus to the rear of the rumen, and therefore the raft of digesta acts like a continuously renewable reversible filter. The result of this process is that the fibrous feed particles are broken down due to alternating fermentative and mechanical activity and that they do not leave the rumen unless they are reduced to a small size (1-2 mm).

There are three main groups of microorganisms in the rumen bacteria, protozoa and fungi. There are some 200 species of bacteria with varied sizes (0.5-5μm) and morphology (cocci, rods, vibrios etc). There are some 15 species of protozoa, again varying greatly in size (5-100μm) and in morphology, including two major groups the holotrichs and entodinia. Some of the important bacteria and protozoa are listed in Table 3. There are several species of anaerobic fungi (e.g. neocalimastrix frontalis, spheromonas communis) but these are not listed in the Table. In general the fungal zoospores are motile and the vegetative stage of the fungi is closely associated with fibrous mass. These microorganisms should be classed as cellulolytic. An examination of Table 3 leads to a number of general conclusions.

(a) It is not important what the microorganisms look like, but it is important what they do. However, a more detailed study of the adherent microbial population may reveal that the microbial shapes could be important too. The cellulolytic microorganisms are likely to attack plant cell wall, while the saccharolytic ones will use simpler carbohydrates as their substrate. Again, there are exceptions to this rule (see secondary substrates for Bacteroides succinogenes and Butyrivibrios fibrisolvens).

(b) There is considerable similarity in the type of end-products of fermentation of various microbial species. For instance of the 16 species listed in the Table, 14 produce acetic acid, 9 produce propionic acid directly or through succinate, 5 produce butyric acid and 1 produces valeric acid. This relative incidence of microbial types is consistent with the proportions of acetic, propionic, butyric and C_5 acids that are normally found in the rumen (65:20:10:5). The microorganisms that produce acetic acid also produce $CO_2 + H_2$ or formic acid.

(c) Many microorganisms require specific nutrients (e.g. branched fatty acids) and a source of N, which, particularly in the fibre digesters must be in the form of NH_3.

(d) The Table shows several examples of substrate chains. The most obvious one is the use of CO_2 and H_2 by methanogens. Some cellulolytic microorganisms will not degrade the polymer further than the disaccharides which then become the substrate for other organisms. Lactate is another substance that could be a link in a substrate chain.

2. Microbial interactions in the rumen (Table 4).

(a) Cooperative interactions

Rumen microorganisms exhibit a great deal of substrate specificity and it is unlikely that the same microbial species will attack such diverse substrates as cellulose, hemicellulose or pectin. Since these substrates are part of a solid plant wall matrix, the accessibility of a particular component may depend on the removal of another component

possibly by a different microorganism. Some microorganisms will degrade a polymer (e.g. cellulose) to a disaccharide (e.g. cellobiose) but will not use the products. On the other hand, the closely associated saccharolytic microorganisms will degrade these intermediates to the usual end products including branched chain fatty acids which in turn may be required by the primary cellulose digester.

There are many examples of cooperative interaction during the transfer of reducing power. An example is given by the apparent degradation of acetic acid to CH_4 and CO_2. This was shown to be due to activity of two closely associated microorganisms; one converting acetate to CO_2 and H_2 and another using part of the CO_2 and the H_2 to form CH_4.

$$CH_3COOH + 2H_2O \longrightarrow 2CO_2 + 4H_2 \quad \ldots\ldots (5)$$

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \quad \ldots\ldots\ldots (6)$$

Since reaction (5) is inhibited by accumulation of H_2 gas, the presence of a methanogen is of great benefit to this couplet. Many other couplets that involve methane bacteria have been identified and they explain the close association of cellulolysis and methanogenesis. However, there are associations where methanogens are not involved. For instance Vibrio succinogenes depends on production of H_2 from glucose by Ruminococcus albus to reduce fumarate to succinate and thus obtain energy for growth. Sometimes one of the partners in a couplet will grow in the absence of the other but there is usually a shift in the fermentation products and a reduced energetic efficiency.

(b) Other types of interactions

In some microbial interactions that have been demonstrated in the rumen, the interaction may benefit one partner only. For instance a large increase in the concentrations of Streptococcus bovis with high

starch diet, may result in a large accumulation of lactic acid (end product), a marked drop in the pH value of the contents and a depressing effect on other microbial species. Another example is the well known predation of protozoa on small bacteria or even on small protozoa, where the 'partnership' appears to be onesided!

3. Compartmentation of microorganisms

Although rumen contents are in a state of flux, they are very heterogeneous and the conditions in the rumen are quite different from those in a homogeneous continuous culture. The foregoing discussion showed that there is a wide distribution of microbial function in the rumen and this is often dictated by the complexity of substrates that are used by the microorganisms. There is no doubt that different locations within the rumen will have functionally different microorganisms and this is particularly true for the microorganisms that are responsible for fibre digestion. The complexity of these problems necessitated an introduction of new concepts and these will be dealt with in lecture No. 8.

D BIOCHEMISTRY AND MICROBIOLOGY OF THE HIND GUT

There is surprisingly little research work on the biochemistry and microbiology of the hind gut. In some animals, e.g. horse or rabbit, the caecum is the main site of microbial activity, while in ruminants the rumen fermentation predominates. However, in ruminant animals, although of secondary importance, the contribution of caecal fermentation should not be ingnored.

First of all, judging from the conditions in the caecum and the end-products of fermentation found there , the metabolic pathways should not differ radicaly from those in the rumen. In general the substrates will be different and because large particles do not leave the rumen, the physical state of the substrates will be different. It has been estimated that of all the microbial activity in the ruminant gastrointestinal tract, between 10 and 20% may be in the caecum. The attempts to manipulate rumen fermentation have met with considerable success but this may be more difficult in the caecum. It is found occasionally that a particular dietary constituent is poorly digested in the rumen and yet it appears to have a good overall digestibility in the animal. The contribution of the microbial activity in the caecum may become of importance under these conditions.

E GENERAL READING

(Lectures 4 and 5)

A. General Biochemistry and microbiology

1. "Rumen and its microbes". (1965) R.E. Hungate. Academic Press, New York.

2. "Rumen microorganisms". (1969) P. Hobson. Process Biochemistry, 4, 53.

3. "Normal flora - rumen bacteria". (1970). M.P. Bryant. An. J. Clin. Nutr., 23, 1440.

4. "Microbial rumen fermentation". (1981). J.B. Russel and R.B. Hespell. J. Dairy Sci., 64, 1153.

5. "Microbial ecology and activities in the rumen". (1982). P.N. Hobson & R.J. Wallace. Part I & II, CRC Critical Reviews in

6. "Rumen metabolism". (1983). R.L. Baldwin & M.J. Allison. J. Anim. Sci., 57, 461.

7. Proceedings of rumen symposia.

"Digestive Physiology and Nutrition of the Ruminant". (1961). Ed. D. Lewis. Butterworths, London.

"Physiology of Digestion in the Ruminant". (1965). Ed. R.W. Dougherty. Butterworths, Washington.

"Physiology of Digestion and Metabolism in the Ruminant". (1970). Ed. A.T. Phillipson. Oriel Press, Newcastle-upon-Tyne.

"Digestion and Metabolism in the Ruminant". (1975). Ed. I.W. McDonald & A.C.I. Warner. The University of New England Publishing Unit, Armidale, N.S.W., Australia.

"Proceedings of the Vth International Symposium on Digestion in the Ruminant". (1980). Clermont-Ferrand, France.

"Digestion and metabolism in ruminants". (1984). Banff, Canada.

B. Microbial interactions

1. "Symbiotic associations: the rumen bacteria". (1963). R.E. Hungate. In "Symbiotic associations". Cambridge University Press.
2. "Interrelationships in the rumen microbiota". (1970). R.E. Hungate. In "Physiology of Digestion and Metabolism in the Ruminant". Ed. A.T. Phillipson. Oriel Press td.
3. "Biologie und Biochemie der Mikrobiellen Verdanung". (1973). Ed. D. Giesecke and M.K. Henderickx. BLV Verlagsgesellschaft, Muchen, Bern, Wien.
4. "Metabolic interactions among intestinal micro-organisms". (1976). M.J. Wolin. Am. J. Clin. Nutr., 27, 1320.
5. "Digestion and metabolism in the ruminant". (1975). Ed. I.W. McDonald and A.C.I. Warner. University of New England Publishing Unit, N.S.W., Armidale, Australia. (a) "Interaction between the bacterial species of the rumen". M.J. Wolin. (b) "Interrelationship between rumen ciliate protozoa and bacteria". G.S. Coleman. (c) "Microbial interactions with the diet and the host animal". M.M. Schwartz and F.M.C. Gilchrist.
6. "Microbial ecology of the rumen". (1980). R.A. Prins and R.T.J. Clarke. In The Proceedings of the Vth International Symposium on Ruminant Physiology, Clermont-Ferrand, France.

7. "The rumen fermentation: a model for microbial interactions in an anaerobic ecosystem". (1979). Wolin, M.J. Adv. Microb. Ecol., 3. 49. Ed. M. Alexander, Plenum Press, New York.

8. "Microbe-animal interaction in the rumen". (1982). Orpin, C.G. In Experimental Microbial Ecology. Ed. R.G. Burns and J.H. Slater, p. 501.

9. "Interactions of microbial populations in cellulose fermentation". (1983). Wolin, M.J. and Millar, T.L. Fed. Proc., 109.

10. "Rumen microbes and digestion of plant cell walls". (1981). D.I. Demyer, Agriculture and Environment, 6, 295.

C. Synthetic processes

1 "Quantitative aspects of microbial protein: synthesis in the rumen". (1970). J.P. Hogan & R.H. Weston. In Physiology of digestion and metabolism in the ruminant. Oriel Press Ltd.

2. "Tracer studies on non-protein nitrogen for ruminants". I(1972), II(1975) and III(1976). Proceedings of a panel organised by Int. Atomic Energy Agency, Vienna.

3. "Energetic aspects of the growth of micro-organisms". (1977). A.M. Stouthammer, Symp. Soc. Gen. Microbiol., 28, 285.

4. "Reassessment of efficiency of synthesis of microbial matter in the rumen". (1978). J.W. Czerkawski. J. Dairy Sci., 61, 1261.

5. "Methods for determining and factors affecting rumen microbial protein synthesis". (1979). M.D. Stern & W.M. Hoover. J. Anim. Sci., 49, 1590.

6. "Protein nutrition in ruminants". (1982). E.R Orskov, Academic Press, London, New York.

7. "Protein requirements for cattle: symposium". (1982) Ed F.N. Owens. Okalhoma State University.

8. "Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications". (1983). F.N. Owens and W.G. Bergern. J. Anim. Sci., 57, 498.

9. "Protein metabolism and nutrition". (1983). Proceedings IV International Symposium, INRA, Clermont-Ferrand.

D. Fate of metabolic hydrogen

1. "Microbial formation of methane". (1971). R.S. Wolfe. Adv. Microbial Physiol., 6, 107.

2. "Fate of metabolic hydrogen in the rumen". (1972). J.W. Czerkawski. Proc. Nat. Soc., 31, 141.

3. "Methanogenesis, an integrated part of carbohydrate fermentation, and its control". (1975). D.I. Demeyer & C.J. Van Nevel. In Digestion and Metabolism in the Ruminant. Ed. I.W. McDonald & A.C.I. Warner. New England Publishing Unit, Armidale, N.S.W., Australia.

4. "Transfer of metabolic hydrogen in the rumen". (1978). J.W. Czerkawski. The Hannah Research Institute 1928-1978. Ed. J.H. Moore and J.A.F. Rook.

5. "The bioenergetics of methanogenesis". (1984). L. Daniels, R. Sparling and G.D. Sprott. Biochim. Biophys. Acta, 768, 113.

6. "Ionophores: their effect on production efficiency and mode of action". (1984). W.G. Bergen and D.B. Bates. J. Anim. Sci., 58, 1465.

Table 1

Concentration of important carbohydrates and other constituents of ruminant food (% DM). Mean values based on the data compiled in Tables 9.2 9.3 and general references.*

	Legumes	Grasses	Grains	Brassica	Straw
Non-structural carbohydrates					
Simple sugars	8	5	2	-	-
Fructosans	-	8	-	-	-
Starch	7	1	64	-	-
Total	15	15	66	(33)	7
Structural carbohydrates					
Cellulose	14	24	8	10	32
Hemicellulose	7	20	4	5	31
Pectin	6	2	-	12	3
Total	27	46	12	27	66
Total carbohydrate	42	61	78	60	73
Other components					
Crude protein	24	14	12	17	4
Lipids	6	4	4	3	2
Organic acids	8	4	1	5	-
Tannins etc.	6	3	1	2	2
Lignin	5	7	2	4	10
Ash	7	7	3	6	6
Total	56	39	21	37	24
Total accounted for	98	100	99	97	97

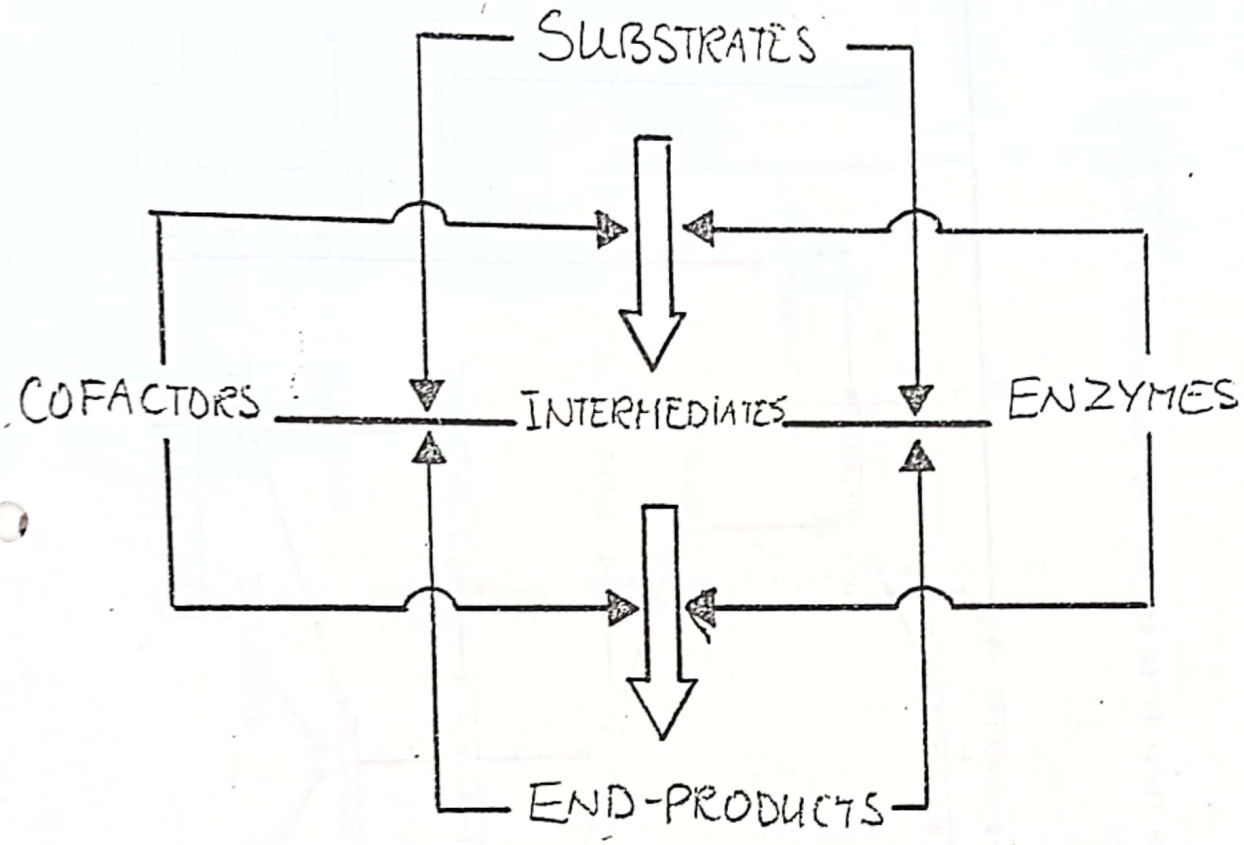


Fig. 1. Importance of enzymes and cofactors in the conversion of substrates to end-products.

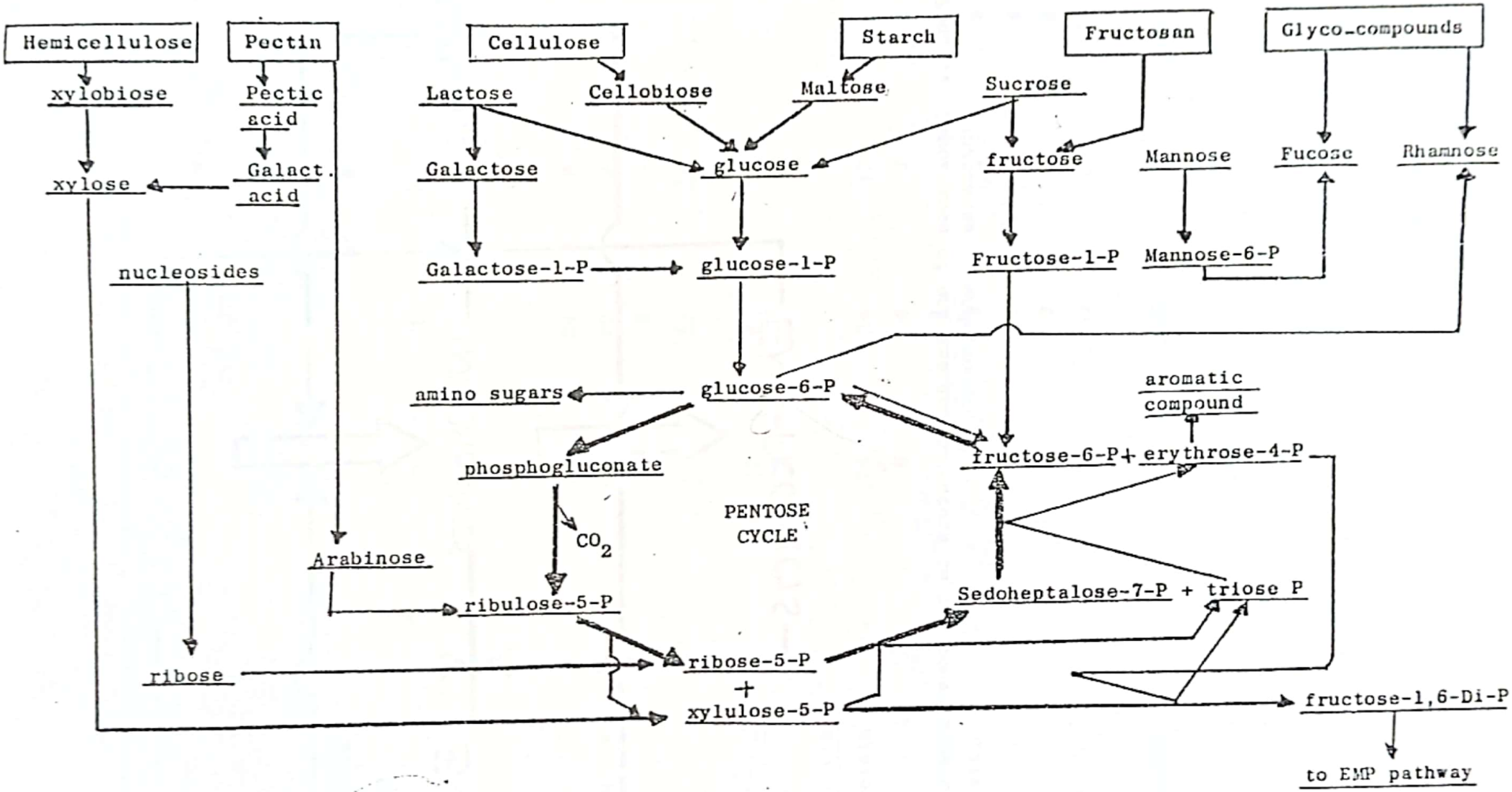


Fig. 2. Important carbohydrates in ruminant diet; their degradation and the interconversion of sugars.

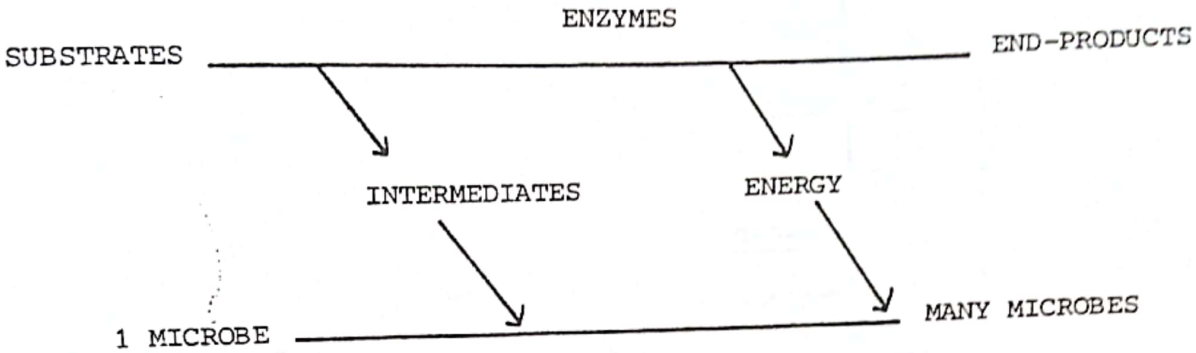


Fig. 3. Relationship between degradation of substrates and microbial growth.

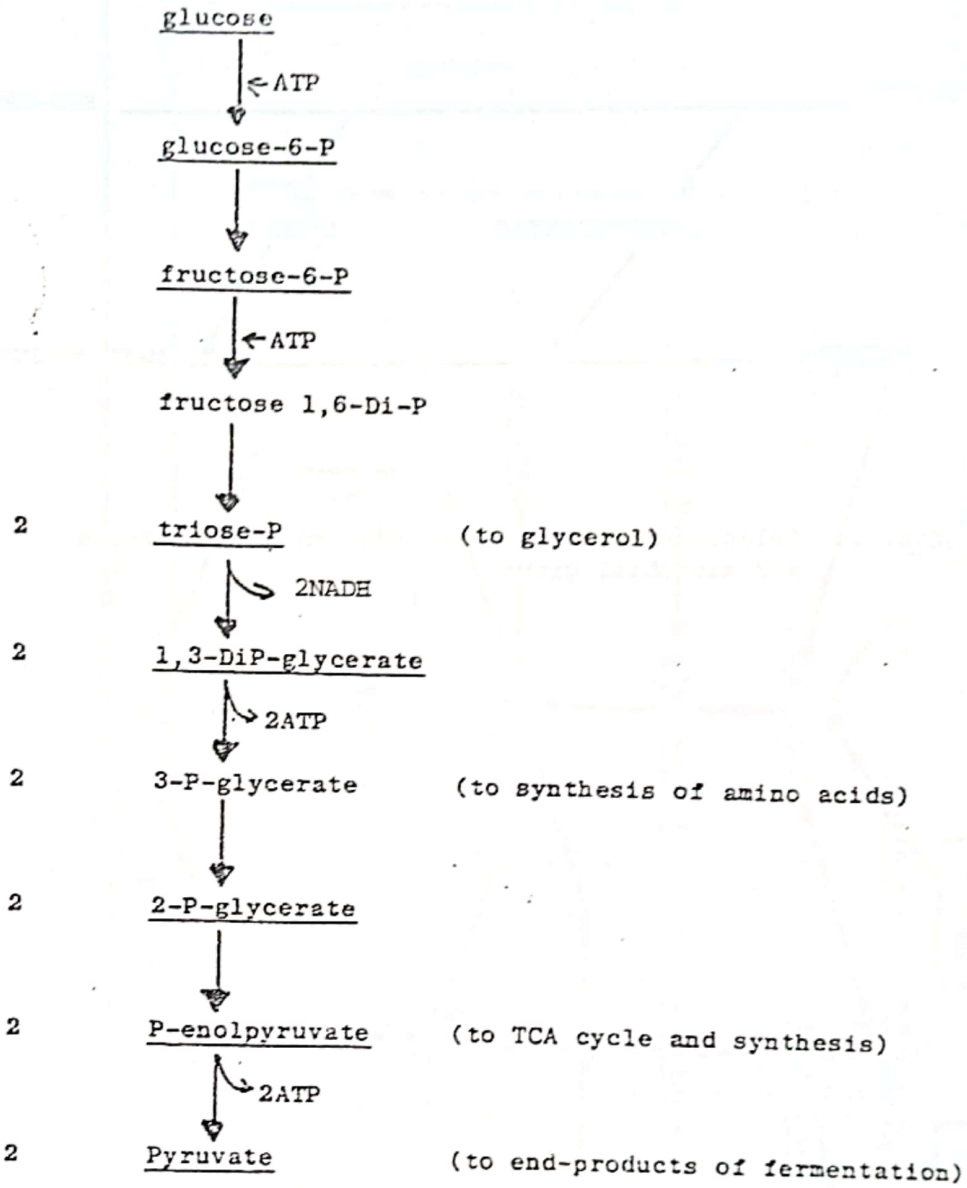


Fig. 4. Empden-Meyerhof-Parnas scheme of glycolysis (Overall reaction: glucose → 2 pyruvate + 2 (H_2) + 2 ATP).

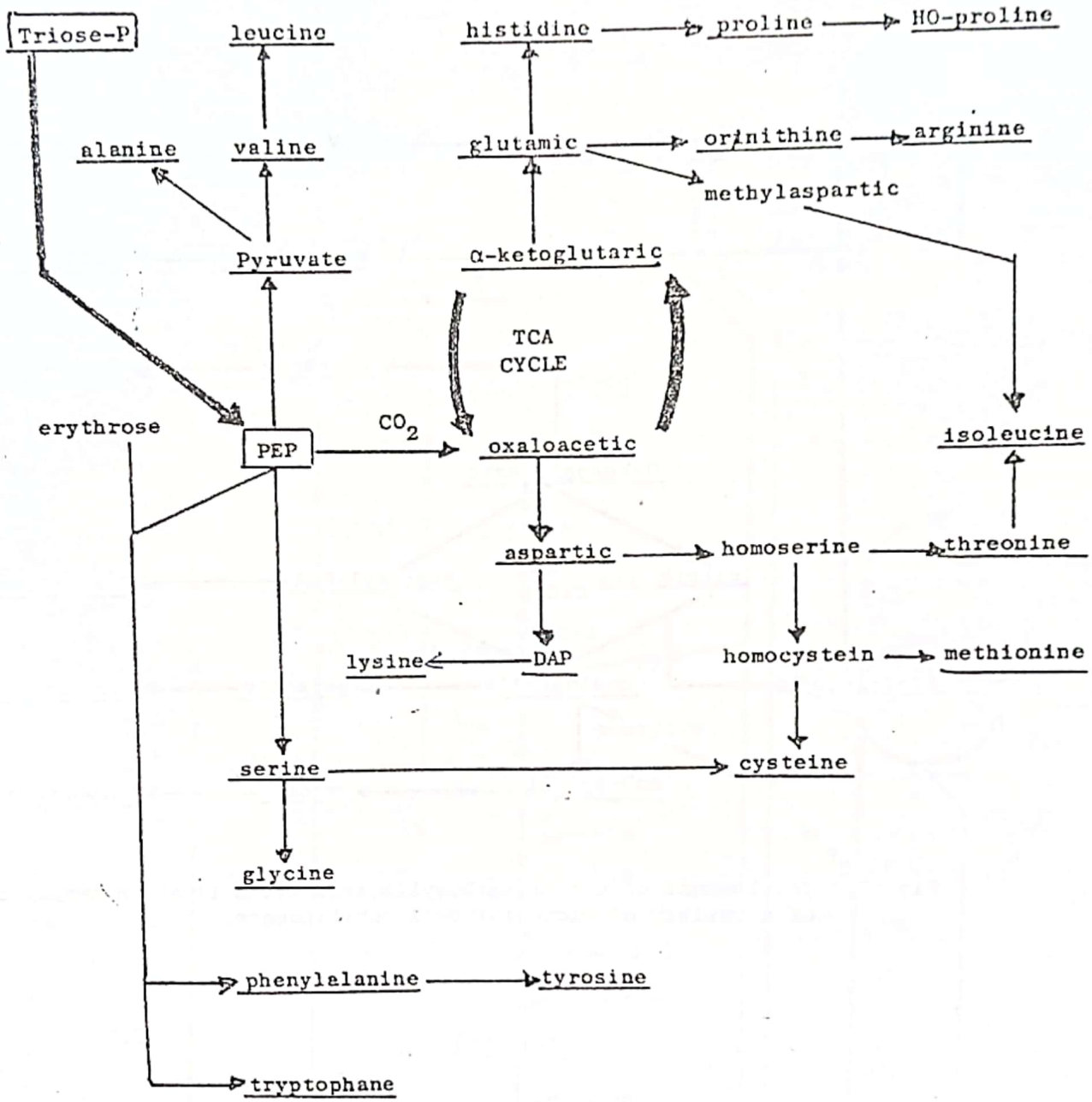


Fig. 5. Involvement of phosphoenolpyruvic acid (PEP) and the tricarboxylic acid cycle (TCA) in the pathways of synthesis of amino acids and hence protein.

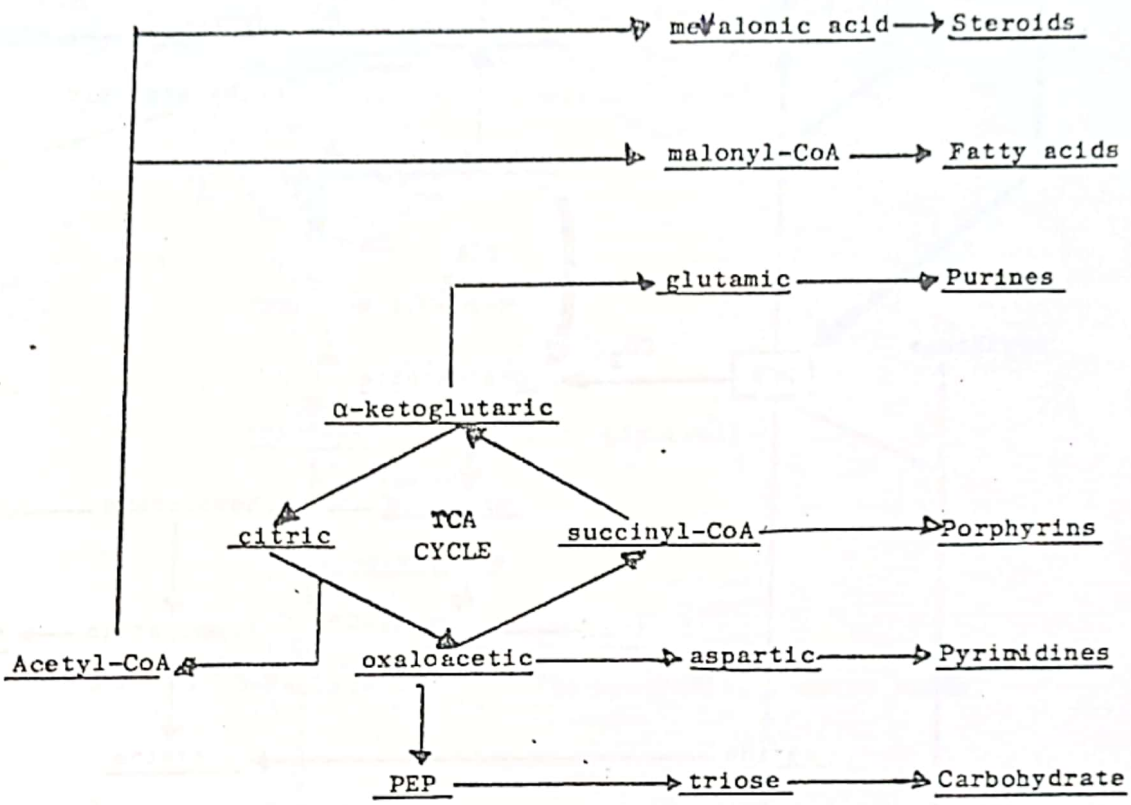


Fig. 6. Involvement of the tricarboxylic acid cycle (TCA) in synthesis of a variety of microbial cell constituents.

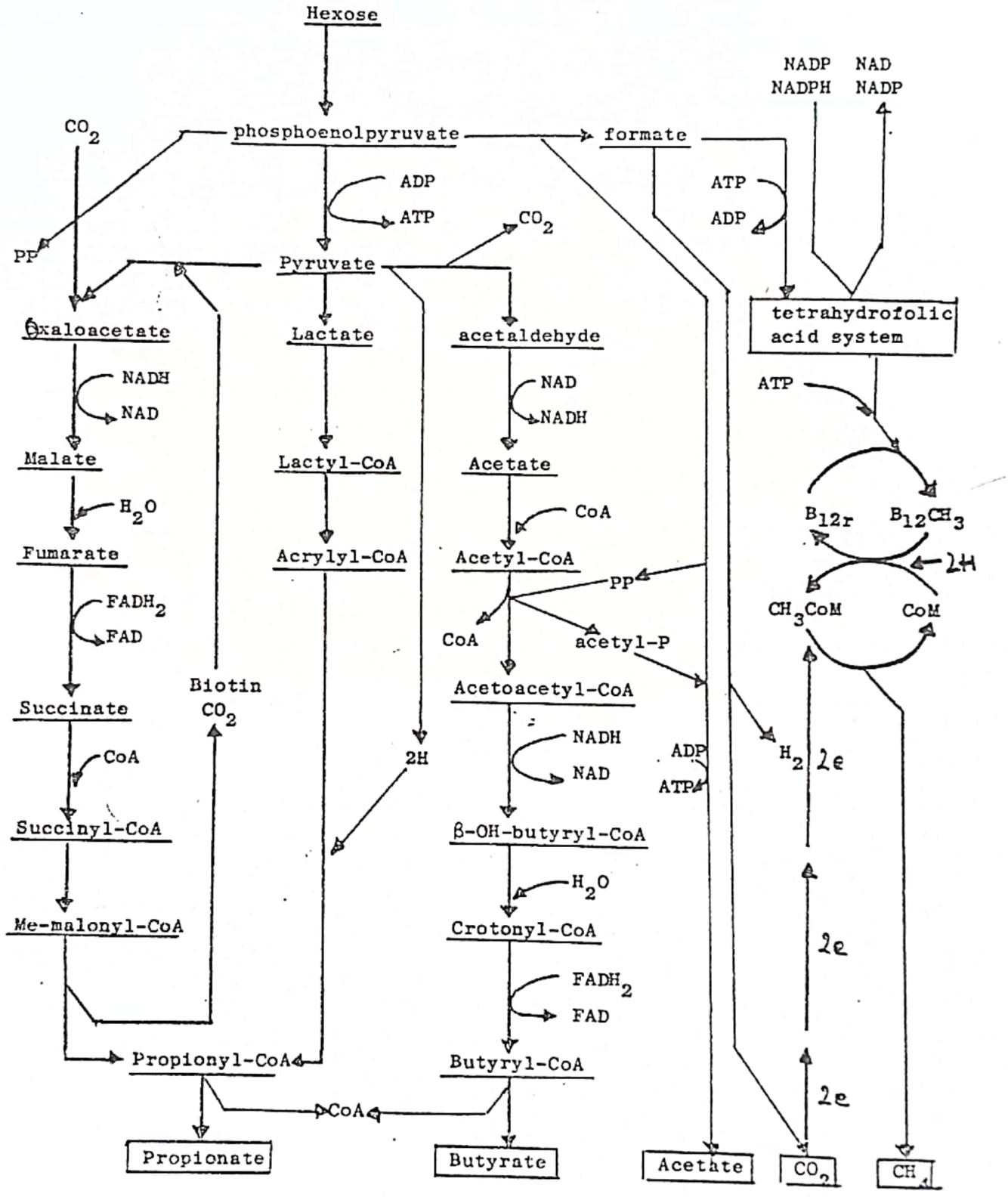


Fig. 7. Conversion of C_3-products of glycolysis to the main end-products of fermentation in the rumen.

Table 2

Net production of reducing power and ATP during conversion of glucose to the end-products of fermentation (mol/mol product).

Product (molar proportion)	Reducing power				ATP			
	NADH	$FADH_2$	H_2	Total	SLP	ETP	U	Total
Acetate (a)	+1	-	+1	+2	2	-	-	2
Propionate (b)	-	-1	-	-1	1	2	-	3
Butyrate (c)	+1	-1	+2	+2	4	-	2	2
Valerate (d)	-1	-1	+1	-1	3	2	2	3
Lactate (e)	0	0	0	0	1	-	-	1
$CO_2 \rightarrow CH_4$	-1	-1	-2	-4	-	3	2	1

SLP Substrate level phosphorylation

ETP Electron transport phosphorylation

U Used in conversion of C_2 and C_3 to C_4 or C_5 etc.

Total = SLP + ETP - U

The main pathways in the degradation of carbohydrates in the rumen are summarized in Fig. 2, which also shows two important points. Firstly, the hydrolysis of polymers gives rise to a variety of simple sugars including hexoses and pentoses and these have to be 'activated' by converting them to phospho-derivatives before they could be processed further. Secondly, the existence of the pentose cycle and its enzymes ensures that all these sugars can be interconverted to each other and particularly to glucose. Therefore, henceforth we can assume that the hydrolysis of carbohydrate polymers will ultimately lead to production of glucose and only the degradation of this compound should be considered in detail.

Proteins are hydrolysed to polypeptides and eventually to amino acids which are the building blocks for the synthesis of microbial protein. However, many rumen microorganisms can synthesise their own amino acids and hence protein from NH_3 and suitable carbon precursors. The reductive deamination of amino acids derived from feed protein predominates in the rumen.

The predominant lipids in ruminant feed are triglycerides, glycolipids and phospholipids. They are readily hydrolysed to fatty acids, glycerol and galactose.

(b) Cleavage and transformation of molecules

The microorganisms require energy to be able to grow and to maintain themselves. There is no store of energy, instead the synthetic reactions are linked to catabolic reaction which are accompanied by a release of useful energy (see Fig. 3). Most of these reactions result in the cleavage and transformation of chemical compounds. An important series of reactions of this type is shown in Fig. 4, in which 1 mole