STUDIES ON THE GROWTH, VIABILITY AND LIPIDS OF *STREPTOCOCCUS BOVIS*

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CHAPTER 1

INTRODUCTION

In their comprehensive review of rumen metabolism, Annison and Lewis (1959) emphasised the symbiotic relationship between the metabolic activities of the mixed population of anaerobic bacteria and ciliated protozoa, and the digestion of fodder consumed by the host. Prominent features of the microbial activity characterising the ruminant mode of digestion have been listed by Moir (1965) and may be summarised as follows:

(1) Cellulose is hydrolysed to monosaccharides by microbial cellulases and carbohydrates fermented to volatile fatty acids. While problems in the quantitative assessment of this volatile fatty acid production were reviewed by Warner (1964), it was also suggested that the amount produced in the rumen and absorbed directly into the blood stream, was sufficient to meet about 70 % of the host's energy requirements.

(2) Microbial protein is synthesised from both plant protein and inorganic nitrogen with the energy released during carbohydrate fermentation. Although the extent of this conversion has also proved difficult to quantitate, Phillipson (1964) has stated that the microbial synthesis of essential amino acids, not always present in the diet, makes the ruminant almost independent of the quality of dietary protein.

(3) As B vitamin deficiency has never been demonstrated in animals on a balanced intake of trace elements (Annison and Lewis 1959 p. 20) it would appear that the microbial population can synthesise the B vitamins.

A more extensive review of carbohydrate metabolism in the rumen can be found in the monograph by Oxford (1964), while nitrogen metabolism was covered thoroughly by Phillipson (1964) and Blackburn (1965).

To orientate investigations on the lipids of a rumen microorganism, Streptococcus bovis, current knowledge of the metabolism of lipids in the rumen is examined with special reference to the microbial activity responsible for modifying dietary lipid. Most attention will be directed towards features not discussed by Garton (1961, 1965) in his reviews of lipid metabolism in the rumen. The converse effect of dietary lipid in microbial activity is not considered, having been recently investigated and discussed by Robertson and Hawke (1964 a, b).

For further aspects of lipid digestion in the ruminant the reviews of Garton (1961, 1963, 1965) should be consulted.

LIPID METABOLISM IN THE RUMEN

After a brief examination of the constituents of dietary lipid, the breakdown and modification of lipid is reviewed in relation to microbial activity. The section concludes with a consideration of the lipids synthesised by rumen microbes.

(1) Lipids in the Diet of Ruminants

Based on an intake of 100 lb dry matter (D.M.) per day, Garton (1961) estimated the maximum lipid consumption of a grazing cow to be of the order of 500 g per day. However, the validity of this estimate is questionable, as Hutton, Hughes, Newth and Watanabe (1964) found the maximum intake of New Zealand Jersey cows was about 30 lb D.M. per day. In pasture plants the lipid content varies from 4 - 8 % (Shorland 1961, Garton 1961), giving a maximum lipid intake of about 1,000 g/day. This figure may be increased still further, by 100 g per day, if the pasture has been sprayed with emulsified oil to prevent bloat, as recommended by Reid and Johns (1957).

Data on the lipid composition of any plant species, let alone pasture plants, is still incomplete (Allen and Good 1965) but from the work of Shorland (1961) and Weenink (1959, 1961, 1962) it was found that over half of the lipid from a variety of pasture species was acetone-soluble; of which the galactolipids, mono- and di- galactosyl glycerol esters of linolenic were major components. For example in red clover, Weenink (1962) found that galactosyl glycerides amounted to 50 % of the total lipid while triglycerides, diglycerides, sterols, sterol esters and hydrocarbons together comprised less than 4 %. Virtually all of the galactosyl glycerides are thought to be present in plant chloroplasts (Benson 1964).

Known constituents of polar lipids in plants have been listed by Allen and Good (1965). These included the choline, ethanolamine, serine, glycerol and inositol esters of phosphatidic acid, the sulphonated sugar lipid (sulpholipid), the sphingolipids (including cerebrosides), and proteolipids. No estimate of the extent to which these constituents are present in the lipids of pasture plants appears to have been published.

Before reaching the small intestine, where absorption of long chain fatty acids occurs (Johnston 1963), the complex array of dietary lipids is subjected to the hydrolytic action of rumen microorganisms.

(2) Hydrolysis of Lipid

The discovery of lipid hydrolysis in the rumen was made by Garton, Hobson and Lough (1958) who found that free fatty acids accounted for over half of the total lipid extracted from rumen contents. *In vitro* incubations of linseed oil with rumen contents released 75 % of the triglyceride as free fatty acids. Subsequent experiments by Garton, Lough and Vioque (1961) showed extreme variation in the extent of hydrolysis (*in vitro*) which may have been due to the extent of emulsification of the linseed oil. Thus cocoa butter, a very saturated fat and hence difficult to emulsify was less extensively hydrolysed than olive and linseed oils which are more readily emulsified. Further evidence for the hydrolysis of triglycerides was obtained by Hawke and Robertson (1964), who found mono- and di- glycerides in the rumen liquor of a dairy cow fed pasture and 500 g/day of linseed oil.

Besides triglycerides a wide variety of other lipid compounds are also hydrolysed. Thus lecithin and lysolecithin (Dawson 1959), ethyl esters of fatty acids (Hill, Saylor, Allen and Jacobson, 1960), monostearin, tributyrin, and Tween 80 (Wright 1961) and galactosyl glycerides, sterol esters and methyl esters (Garton 1965) have all been shown to undergo hydrolysis. However, attempts to isolate mono- and di- glycerides during the in vitro hydrolysis of galactosyl glycerides by rumen contents were unsuccessful (Hawke and Weenink unpublished. Cited by Hawke and Robertson 1964).

Consequent upon the hydrolysis of lipid ester linkages is the release of water soluble moieties such as glycerol, galactose and phosphatide bases. While galactose and glycerol are known to be fermented to volatile fatty acids (see Garton 1965) microorganisms can probably metabolise phosphatide bases such as choline and ethanolamine.

Hydrolytic Enzymes and Microbial Activity

Although enzyme preparations, capable of releasing the water soluble moieties from lipids, have been extracted from rumen bacteria and protozoa, attempts to demonstrate lipase activity in cell-free extracts have not been entirely successful. Thus Bailey (1962) obtained α- and β- galactosidase activity in cell-free extracts of rumen bacteria, but the preparations were inactive on intact galactosyl glycerides, depending upon their prior deacylation. Deacylation was demonstrated, however, with cell suspensions of the same bacteria, showing that bacteria could be responsible for total hydrolysis of the galactolipids.

Similarly Bailey and Howard (1963) and Howard (1963) reported the extraction of α- and β- galactosidase activity from four species of protozoa. While these enzymes were capable of hydrolysing the intact galactosyl glycerides no release of free fatty acids was observed.

The partial success in preparing cell-free lipase activity is typified by the reports of Dawson (1959) and Wright (1961). Butanol extracts of rumen bacteria prepared by Dawson (1959) gave complete hydrolysis of lysolecithin but were inactive against lecithin. Likewise Wright (1961) obtained extensive hydrolysis of tributyrin and trihexanoin with extracts of rumen bacteria or protozoa, but little activity was observed on esters of long chain fatty acids.

On the other hand Hobson and Mann (1961) repeatedly isolated an unidentified lipolytic bacterium from $^{1}/_{10}9$ dilutions of rumen fluid from a sheep fed hay and concentrates with and without linseed oil. Clear zones were observed around colonies on agar containing an emulsion of linseed oil, indicating that the organism may have secreted an extracellular lipase.

Further evidence to implicate bacteria as agents in the hydrolysis of lipids comes from studies with antibiotics. It is well known that some antibiotics are able to prevent the onset of bloat (Mangan, Johns and Bailey 1959) and some evidence for their mode of action accrues from the studies of Hill (1960 Cited by Garton 1965) and Wright (1961). Both of these workers showed that the lipase activity of rumen contents decreased when some antibiotics were fed and it was argued that reduced lipolysis would favour the continued antifoaming action of the intact lipids. However, Shellenberger (1964 Also cited by Garton 1965) was unable to confirm the earlier results.

Although Oxford (1958 a) observed the ingestion of chloroplasts by a protozoal species it is not clear to what extent the hydrolysis of lipids may occur as an intra- or extra- cellular process. Thus studies to define the location and extent of lipid hydrolysis merit high priority, especially if it should be shown that the presence of intact lipid in the rumen liqour is necessary for the prevention of bloat in cattle.

(3) Hydrogenation of Unsaturated Fatty Acids

Although the composition of the body fat of monogastrics reflects the nature of the dietary fatty acids, ruminant fats retain a highly saturated fatty acid composition in spite of the ingestion of predominantly linoleic and linolenic acids from plant lipids (Garton 1963). The studies of Reiser (1951) pointed to the occurrence of hydrogenation in the rumen to account for this phenomena, as a reduction in linolenic acid concentration was observed in incubations with rumen contents. Further evidence for the hydrogenation of plant fatty acids was obtained by Shorland, Weenink and Johns (1955) who showed that the high proportions of linolenic and linoleic acids present in herbage were not reflected in the fatty acid composition of rumen contents.

Hydrogenation and Microbial Activity

The hydrogenating activity of protozoa was indicated by Wright (1959) who incubated linseed oil, sodium linoleate and chloroplast lipids with washed suspensions of protozoa in the presence of antibiotics to minimise the activity of any bacteria also present.

Analysis of the fatty acids after incubation showed a decrease in iodine numbers or changes in fatty acid composition consistent with the occurrence of hydrogenation. More recently Gutierrez, Williams, Davis and Warwick (1962) have demonstrated the uptake of 1-C^{14} oleic acid by washed suspensions of two ciliate protozoal species and its conversion to stearic acid.

Although initial attempts to demonstrate that rumen bacteria could also hydrogenate fatty acids were unsuccessful, it was later found by Wright (1960 a) that glucose and rumen fluid were necessary for hydrogenation to occur and were possibly needed for the fermentative activity of the bacterial suspensions. Recently, Polan, McNeill and Tove (1964) have developed an assay system for measuring hydrogenation activity of washed suspensions of bacteria in the presence of boiled rumen fluid and 0.25 - 4.0 mg/ml of 1-C^{14} linoleic acid as substrate. In view of the high concentration of substrate, compared with the levels of fatty acids found in rumen contents by Hawke and Robertson (1964), the claim by Polan et.al. (1964) that carbon dioxide inhibited hydrogenation is not necessarily valid; especially as Ward, Scott, and Dawson (1964) obtained extensive hydrogenation with a gaseous phase of carbon dioxide/nitrogen in an artificial rumen, where microorganisms were supplied with a diet of hay and oats, and only 0.001 - 0.28 mg/ml of a C^{14} - labelled unsaturated acid employed as substrate. These levels approximated to the concentration in rumen contents reported by Hawke and Robertson (1964). Polan et.al. (1964) also found it necessary to gas their system with hydrogen in order to effect measurable rates of hydrogenation.

A survey of rumen bacterial species by Polan et. al. (1964) for hydrogenation activity indicated that only Butyrioibrio fibrisolvens was active, but further studies revealed a distinct loss of activity with aged cultures of this species. In the light of this finding it is disturbing that the survey of bacterial species was not repeated using suspensions known to be viable or capable of fermentative activity. These authors also found that suspensions of mixed species of bacteria were capable of hydrogenation whereas pure species, presumably of a similar age were inactive. Attempts to define the nature of this symbiosis were inconclusive but made no allowance for the possibility that the death or lysis of one species was providing fermentable substrate for another.

It was also reported by Polan et. al. (1964) that glucose, formate or amino acids were unable to replace rumen fluid and thereby act as hydrogen donors. However, as Wright (1960 a) found that both rumen fluid and glucose were needed for the hydrogenating activity of washed suspensions of bacteria, the experiments of Polan et. al. (1964) probably failed to provide conditions necessary to test their hypothesis viz.; that glucose, formate or amino acids served as hydrogen donors.

Evidence for the location of hydrogenation on or within the cell wall was sought by Polan et. al. (1964) by measuring the extent of hydrogenation in the precipitate and supernatant fractions of their incubation system. As identical activity was noted in each fraction their claim that hydrogenation occurred on or within the cell wall presupposed that acids were released into the supernatant as rapidly as they were hydrogenated. No evidence for

the validity of this assumption was considered and consequently the results fail to support their claim.

Although the nature of the hydrogenation reaction remains obscure, Shorland, Weenink, Johns and McDonald (1957) and Ward, Scott and Dawson (1964) have both made an intensive examination of the reaction products. Shorland et. al. (1957) incubated relatively large amounts of oleic, linoleic and linolenic acids with rumen contents and using classical fractionation procedures demonstrated the formation of trans - unsaturated acids and positional isomers of mono- and di- enoic acids among the products of hydrogenation, indicating migration of the double bond during the reaction. On the other hand Ward et. al. (1964) employed radioactive substrates in an artificial rumen and drew conclusions from the radioactivity recovered in fractions obtained from thin-layer and gas-liquid chromatographic separations. Unsaturated acids were oxidised and the products examined for radioactivity. As well as substantiating the results obtained by Shorland et. al. (1957) further evidence was obtained for the formation of a conjugated dienoic acid from linolenic acid and the migration of the C-15 double bond toward the methyl group in non-conjugated acids. Monoenoic acids formed were predominantly of the trans configuration with double bond mainly at C-13 and C-14.

Both Shorland et. al. (1957) and Ward et. al. (1964) have noted the resemblence between the products of hydrogenation in the rumen and the products of catalytic hydrogenation of natural oils. As the reducing potential of the rumen is only -0.35 V (Hungate 1963) at a temperature of 39°C, little if any, catalytic hydrogenation would be expected and the presence of enzymes is implied. Polan et. al. (1964) obtained some evidence for the presence of iron and sulphydryl groups in a

hypothetical enzyme as both cyanide and arsenite at 0.1 M concentration inhibited hydrogenation whereas 0.1 M azide had no effect. It was also found that B. fibrisolvens, while capable of converting linoleic to a monoenoic acid, was unable to form the saturated acid, indicating the possible existence of specific enzymes for each stage of the reaction.

The relationship between hydrolysis and hydrogenation has yet to be fully clarified, but both Garton, Lough and Vioque (1961) and Hawke and Robertson (1964) have obtained evidence for the preferential hydrogenation of free fatty acids over triglycerides, as higher proportions of saturated acids were found in the free fatty acids released during hydrolysis, than in the unhydrolysed triglycerides. However the unhydrolysed triglycerides also contained a higher proportion of saturated acids than the linseed oil employed as substrate, suggesting that either some hydrogenation of intact triglycerides occurred or that preferential hydrolysis of more unsaturated triglyceride species was taking place. Transesterification reactions could also be invoked to explain these results but no consideration to their occurrence in the rumen has been given.

(4) Degradation of Fatty Acids

An evaluation of the extent of degradation of long chain fatty acids is hampered by a paucity of experimental data and only the studies of Wood, Bell, Grainger and Teekell (1963) give positive indication of the occurrence of breakdown. These workers added 1-C^{14} linoleic acid to the rumen contents of sheep, then recovered steam volatile and long chain fatty acids after 48 hours. With the reticulo-omasal orifice ligated, loss of digesta from the rumen was prevented and 90 % of the radioactivity added was recovered as long chain acids. Less than 1 %

of the original radioactivity was present in steam volatile fatty acids from the rumen, while further small amounts of labelled volatile and long chain fatty acids were found in the jugular blood, having been absorbed from the rumen. Thus only minor proportions of long chain fatty acids are thought to be broken down in the rumen and no evidence is available to implicate microorganisms in this capacity.

The ω-oxidation of hydrocarbons to fatty acids observed by McCarthy (1964) in ruminants does not appear to be undertaken by rumen microorganisms as following the administration of C^{14} - hexadecane or C^{14} - octadecane no labelled fatty acids were found in rumen contents.

Thus through the hydrolytic and hydrogenating reactions occurring in the rumen, substantial changes to dietary lipids are made prior to their absorption from the small intestine. However, as microbial growth proceeds with fermentation (Hungate 1963) lipids are synthesised by microorganisms for cell membranes, or cell walls (Kates 1964). With the outflow of digesta and microbes from the rumen, a wide variety of lipids of both dietary and microbial origin are subjected to further hydrolysis by pancreatic lipases and absorbed from the small intestine (Borgstrom 1960).

(5) Lipids of Rumen Microorganisms

Apart from studies on the formation of branched chain fatty acids, lipid metabolism by rumen microorganisms has received scant attention. However Kates (1964) in a general review of bacterial lipids, their distribution in the cell, and metabolism emphasised the similarities of lipid composition shown by closely related species. Although Bryant (1959) has indicated that many of the bacterial species isolated from the rumen are unique to this environment, it is expected that most aspects of their lipid metabolism will resemble that discovered in other

anaerobic bacteria. In this review attention is confined to three aspects of microbial fatty acid synthesis -

(a) The pathway of fatty acid synthesis in anaerobic bacteria.

(b) The incorporation of free fatty acids.

(c) The synthesis of branched chain fatty acids and aldehydes by rumen bacteria.

(a) Fatty Acid Synthesis by Bacteria

Early evidence for the bacterial synthesis of fatty acids from acetate was obtained by Stephenson and Whetham (1922) who showed that the lipid content of Mycobacterium phlei was increased by the addition of acetate to the growth medium. A similar effect of acetate on Escherichia coli lipid was also observed by Dagley and Johnson (1953). More recently, Goldfine and Bloch (1961) working with Clostridinum butyricum, and Thorne and Kodicek (1962 b) working with Lactobacillus casei, have shown that labelled acetate was incorporated into long chain fatty acids.

For many years fatty acid synthesis from acetyl - CoA was thought to occur by reversal of the pathway of β-oxidation (O'Leary 1962). However Wakil (1958) showed that fatty acid synthesis in pigeon liver proceeded via carboxylation of acetyl-CoA to form malonyl-CoA. Subsequently Wakil and Ganguly (1959) found the enzyme which catalysed the synthesis of fatty acids by coupling the decarboxylation of malonyl-CoA with the elongation of the acyl chain. A similar enzyme was found in yeast by Lynen (1961) who also carried out an extensive investigation of this, the condensation reaction.

Recently Vagelos and co-workers have extracted soluble enzymes for the condensation reaction from Clostridium kluyveri and E. coli.

Fractionation of these enzymes yielded a heat stable protein which was purified and found to contain one sulphydryl group. As this enzyme was shown to bind the acyl group during condensation with successive two-carbon fragments, it has been called the acyl carrier protein (ACP) (Vagelos 1964). The following reactions were catalysed by the complete enzyme system;

$$\text{Acetyl-S-CoA} + \text{ACP-SH} \longrightarrow \text{acetyl-S-ACP} + \text{CoASH}$$

$$\text{Acetyl-S-ACP} + \text{malonyl-CoA} \longrightarrow \text{acetoacetyl-S-ACP} + CO_2 + \text{CoASH}$$

$$\text{acetoacetyl-S-ACP} + 2\text{NADPH} + 2H^+ \longrightarrow \text{butyryl-S-ACP} + 2\text{NADP}^+ + H_2O$$

and shown to be intermediates in the formation of fatty acids from malonyl-CoA (Alberts, Goldman and Vagelos 1963, Goldman, Alberts and Vagelos 1963 a, 1963 b, Goldman 1964). These results constitute strong evidence for the bacterial synthesis of fatty acids by the malonyl-CoA pathway similar to that found in yeast (Lynen 1961) and pigeon liver (Wakil 1961).

On the other hand biosynthesis of unsaturated fatty acids in anaerobic bacteria proceeds by a different pathway from the aerobic desaturation of fatty acids common to the actinomycetes, yeasts and higher organisms (Bloch 1962). Current knowledge of the anaerobic pathway, which involves elongation of β, γ- decenoic acid to cis-vaccenic acid, has been reviewed by Vagelos (1964) who also presents evidence for the occurrence of this pathway in facultative as well as obligate anaerobes.

(b) Incorporation of Free Fatty Acids

In addition to the synthesis of fatty acids, Goldfine and Bloch (1961) and Thorne and Kodicek (1962 c) have shown that bacteria can incorporate preformed fatty acids from the media. In view of the existence of long chain fatty acids in rumen fluid (Garton, Lough and Vioque 1961, Hawke and Robertson 1964), it is obvious that the fatty acids found in bacteria

harvested from the rumen, are not necessarily those synthesised from short chain precursors.

For example, using gas liquid chromatography, Keeney, Katz and Allison (1962), Erwin, Sterner and Marco (1963) and Tweedie (1965) have all found polyenoic acids in bacteria harvested from the rumen, yet despite intensive investigation no polyenoic acids have been found to be synthesised by bacteria (Bloch, Baronowski, Goldfine, Lennarz, Light, Norris and Scheuerbrandt 1961, Kates 1964, Erwin, Hulanicka and Bloch 1964).

To overcome this problem fatty acid metabolism in rumen microorganisms can only be studied with radioactive precursors or in media known to be free of long chain fatty acids.

Similar arguments also apply to the long chain fatty acid metabolism of rumen protozoa, but, to date no studies of the fatty acids synthesised by comparable species of anaerobic protozoa, let alone rumen species, appear to have been reported. However, Gutierrez, Williams, Davies and Warwick (1962) showed that palmitic, stearic, oleic and linoleic acids were taken up from the media by washed suspensions of two species of rumen protozoa.

(c) Branched Chain Fatty Acids and Aldehydes

Evidence for the occurrence of iso- and anteiso- long chain acids in butterfat was reviewed by Shorland and Hansen (1957). With the discovery of branched chain fatty acids in the lipids of bacteria harvested from the rumen by Keeney, Katz and Allison (1962), it was realised that these bacteria were the probable origin of branched chain acids in butterfat. Similarly Katz and Keeney (1964) have suggested that the branched chain fatty aldehydes of complex plasmalogen lipids in ruminant tissues are synthesised by rumen bacteria. These authors isolated fatty aldehydes from rumen bacterial lipids and by gas-liquid chromatography of the

reduced aldehydes showed that 45 % of the total aldehyde fraction had a branched chain structure.

Tweedie (1965) has reviewed the occurrence of branched chain fatty acids in bacterial lipids and the metabolism of branched chain volatile fatty acids which are considered to be precursors of the higher branched chain acids. Several studies claiming to demonstrate the incorporation of branched volatile fatty acids into their higher homologies have been reported. Thus Allison, Bryant, Katz and Keeney (1962) found that Ruminococcus flavefaciens incorporated 1-C^{14} isovalerate into long chain fatty acids and aldehydes while R. albus, another cellulolytic bacterium, incorporated 1-C^{14} isobutyrate. Similarly Wegner and Foster (1963) found that both 1-C^{14} valerate and 1-C^{14} isobutyrate were incorporated into the long chain fatty acid and aldehyde moieties of an ethanolamine plasmalogen, in the cellulolytic Bacteroides fibrisolvens. Tweedie (1965) using mixed cultures of rumen bacteria demonstrated the incorporation of 1-C^{14} isobutyrate into bacterial lipids. These workers then used preparative gas-liquid chromatography to separate the methyl esters of the fatty acids. In each case the highest activity was associated with fractions tentatively identified as the homologous branched chain fatty acids. However, no evidence for the purity of the fractions collected was quoted in the literature, nor was evidence other than retention volumes on gas-liquid chromatography obtained for the chemical structure. Despite these deficiencies the results conform to the theory of branched chain fatty acid biosynthesis. This visualises the elongation of a branched volatile fatty acid with two-carbon units derived from malonyl-CoA (Kates 1964).

The fatty acid composition of a non-cellulolytic rumen microorganism, Streptococcus bovis was studied by Tweedie (1965) using gas-liquid

chromatography (Table 1). Unlike the cellulolytic bacteria mentioned above, S. bovis did not incorporate isobutyrate into long chain fatty acids, nor could the formation of any iso- acid be induced by the presence of isobutyrate.

As Tweedie (1965) had found lipids to constitute 7 % of the dry weight of S. bovis, the present investigations were commenced with the long-term objective of assessing the importance of lipids as endogenous reserves. To keep this aim in perspective, the importance of S. bovis as a member of the rumen population is deduced from current knowledge of rumen microbiology and metabolism. Subsequent sections of this review cover the general biochemistry of S. bovis, lipid metabolism in the lactic acid bacteria and evidence for the function of lipids in bacteria.

S. BOVIS IN THE RUMEN

Bryant (1959) and Hungate (1963) have both made comprehensive reviews of the variety of bacterial species found in sufficient numbers to account for some aspect of rumen metabolism. One of these species, S. bovis, has been isolated from rumen contents by MacPherson (1953), Mann, Masson and Oxford (1954), Perry, Wilson, Newland and Briggs (1955), Hungate (1957), Bailey and Oxford (1958 a), Krogh (1963) and Clarke (1964). All of these authors obtained positive evidence for the identification of their isolates and many were made from dilutions of greater than $^{1}/_{10}6$, while Gall and Huhtanen (1951), Hungate, Dougherty, Bryant and Cello (1952), Higginbottom and Wheater (1954) and Krogh (1959, 1960, 1961) have also found more than a million streptococci per ml of rumen contents. Problems encountered in making repeatable counts of rumen bacteria were discussed by Bryant (1959), but the results obtained indicate that sufficient streptococci are present to have some role in the overall metabolism in the rumen.

Isolates have been predominantly facultative anaerobes, but Hungate (1957) claimed that most of his isolates showed an obligate requirement for anaerobiosis.

The characteristic fermentation pattern for S. bovis is the formation of lactate from hexose (Annison and Lewis 1959), but only when diets rich in soluble carbohydrate are fed does the concentration of lactic acid in rumen fluid reach a measurable concentration (Balch and Rowland 1957). Furthermore, studies on the turnover of lactic acid by Jayasuriya and Hungate (1959) demonstrated that lactic acid formation accounted for less than 1 % of the total fermentation of a hay diet, but could reach 8 % of the total on grain feeding. Thus the metabolic activity of S. bovis is normally of minor importance to fermentation in the rumen.

However, a sudden excessive intake of soluble carbohydrate causes an acute indigestion accompanied by the accumulation of lactic acid in the rumen (Hungate et. al. 1952). This was also associated with a sharp increase in the numbers of viable streptococci as the pH of the rumen fell to 5.0. Below pH 5.0, Krogh (1959, 1960, 1961) found that lactobacilli predominated, suggesting that these species were more tolerant of acidity than the streptococci and had taken over the role of lactic acid production. Death of the animal was commonly observed as the result of indigestion.

If however, a gradual change was made to a diet rich in soluble carbohydrate, no symptoms of indigestion occurred and it is currently thought (Annison and Lewis 1959 p. 166) that the increased lactic acid production is matched by an increase in the numbers of lactic acid fermenting bacteria. Thus Jayasuriya and Hungate (1959) have demonstrated the conversion of 2-C^{14} lactate to a mixture of acetic, propionic and butyric acids in rumen contents, while a number of lactate fermenting bacteria have been isolated from the rumen. (see Annison and Lewis 1959 p. 48). On the other hand, evidence for the slow absorption of lactate

from the rumen has been presented by Williams and Mackenzie (1965).

From the data at present available, it seems as though S. bovis is of minor importance during normal metabolism in the rumen, however, its capacity for extremely rapid growth (Hungate 1963) places it at an obvious advantage when given access to the soluble carbohydrates it is capable of fermenting.

BIOCHEMISTRY OF S. BOVIS

Strain I of S. bovis, which was cultured throughout the present investigation, was isolated by Bailey and Oxford (1958 a). According to "Bergey's Manual of Determinative Bacteriology" (Breed, Murray and Smith 1957), S. bovis is a member of the "viridans" group within the Group D streptococci. All Streptococcus species are members of the Lactobacteriaceae family within the order Eubacteriales.

To introduce a study on the lipids of S. bovis, current knowledge of the biochemistry of the species, the lipids of other lactic acid bacteria and the function of lipids in bacteria will be reviewed.

In previous studies of S. bovis most attention was directed toward the synthesis and degradation of polysaccharides or the ability of some strains to utilise ammonia as the sole source of nitrogen. Although other aspects of S. bovis metabolism have received scant attention, the biochemistry of S. faecalis has been extensively investigated and was recently reviewed by Deibel (1964) with particular emphasis on the similarities and differences between the various members of the Group D streptococci. Therefore in a review of the biochemistry of S. bovis under the headings indicated below, it is relevant to discuss literature dealing with related streptococci and lactobacilli as many aspects of their metabolism appear to be similar.