PROPERTIES OF A FATTY ACID FORMING ORGANISM ISOLATED FROM THE RUMEN OF SHEEP

S. R. ELSDEN,[1] B. E. VOLCANI,[2] F. M. C. GILCHRIST,[3] AND D. LEWIS[4]

Division of Plant Nutrition, University of California, Berkeley, California and Agricultural Research Council Unit of Microbiology, Department of Microbiology, The University, Sheffield, England

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Three organisms, *Clostridium kluyveri* (Barker and Taha, 1942), *Rhodospirillum rubrum* (Kohlmiller and Gest, 1951) and an organism isolated from silage by Rosenberger (1952) and identified as *Clostridium scatologenes*, have been shown to synthesize fatty acids higher than *n*-butyrate, as end products of fermentative metabolism. In this paper we give an account of the isolation and properties of an anaerobic, gram negative organism which ferments certain carbohydrates and DL-lactic acid with the formation of hydrogen, carbon dioxide, acetate, propionate, *n*-butyrate, *n*-valerate, and *n*-hexanoate. The organism was isolated from the rumen contents of the sheep.

MATERIALS AND METHODS

Media. Except where otherwise stated the basal medium contained 0.4 per cent yeast extract (Difco), 0.05 per cent KH_2PO_4, 0.05 per cent NH_4Cl, 0.03 per cent $MgCl_2 \cdot 6H_2O$, 0.03 per cent thioglycolic acid in tapwater; the pH was adjusted to 7.4. Medium A consisted of the basal medium plus 2 per cent soluble starch. Medium B consisted of basal medium plus 2 vol per cent of 70 per cent DL-sodium lactate.

Rumen contents. The samples of rumen contents used were obtained from Scotch Blackface sheep fitted with rumen cannulae, and fed meadow hay and concentrates.

Cultural techniques. Liquid enrichment cultures were carried out in 60-ml glass-stoppered bottles completely filled with medium. Agar plates were incubated in desiccators filled with H_2 5 per cent CO_2 gas mixture and containing a capsule of palladiumized asbestos. Shake cultures were either incubated in desiccators prepared as above or else were protected from the atmosphere by covering the surface of the agar with a layer (1–2 cm thick) of a mixture of equal parts of 50 C paraffin wax and mineral oil. The incubation temperature was 38 C throughout.

Gas analysis. The gases produced during growth were analyzed by the Haldane method (Peters and Van Slyke, 1932). Carbon dioxide dissolved in the medium was determined by the manometric method described for urine by Peters and Van Slyke (1932).

Lactic acid. The method of Friedemann and Graeser (1933) was used. Prior to analysis the test solution was treated with the copper-lime reagent. To a 5-ml sample were added 1 ml of 20 per cent $CuSO_4\ 5H_2O$ (w/v) and 1 ml of a 10 per cent (w/v) suspension of $Ca(OH)_2$ and the mixture made up to 50 ml. After standing for 30 min the precipitate was filtered off and samples of the filtrate were taken for analysis.

Volatile fatty acids (VFA's). Total VFA's were determined by steam distillation in the apparatus of Markham (1942) and titration of the distillate with 0.02N NaOH in a stream of CO_2-free air. Qualitative analysis for VFA's were carried out by paper partition chromatography as described by Elsden and Lewis (1953).

Two methods were used for the quantitative analysis of the VFA's in the mixtures obtained by steam distillation. In the case of the fermentation of lactate the volatile acids were separated by the double distillation procedure of Friedemann (1938) and the mixture analyzed by the chromatographic method of Moyle, Scarisbrick, and Baldwin (1948). The Friedemann method served to remove traces of interfering acids such as formic, lactic, and pyruvic. The distillate so obtained was made alkaline to phenol red and taken to dryness on a sand bath. The fatty acids were then extracted with chloroform containing 5 per cent (v/v) *n*-butanol as described by Elsden

[1] Present address: A. R. C. Unit of Microbiology Department of Microbiology, The University, Sheffield, 10, England.

[2] Present address: Weizmann Institute of Science, Rehovoth, Israel.

[3] Present address: Onderstepoort Veterinary Research Laboratory, Pretoria, South Africa.

[4] Present address: A. R. C. Institute of Animal Physiology, Babraham Hall, Cambridge, England.

(1946). Samples of this solution were titrated with standard methanolic KOH as described by Moyle *et al.* (1948) to determine the total VFA'S present; further samples were analyzed on column IV of these authors using the solvent systems recommended. With this column the hexanoic acids pass through with the solvent front; the valeric, butyric, and propionic acids are separated quantitatively from one another with the appropriate sequence of chloroform *n*-butanol mixtures (there is no effective separation of isomers with this method). Acetic acid cannot be eluted from column IV and has to be determined by difference. Provided that the amount of hexanoic acid present is small as compared with the amount of valeric acid then hexanoic acid is reasonably well separated from valeric acid.

The gas liquid chromatogram of James and Martin (1952) was subsequently used in place of the method of Moyle *et al.* (1948). This method separates the isomers of butyric, valeric, and caproic acids and by measuring the retention volumes it is possible to identify the acids with a reasonable degree of certainty. The liquid phase was the stearic acid-phosphoric acid-silicone mixture recommended by James and Martin (1952). The extraction procedure used was that described by Annison[5] (1954).

Succinic acid. This compound was estimated by the method of Krebs (1937).

Glucose. Glucose was estimated by the fermentation method described by Winzler (1944).

RESULTS

Isolation and characterization of the organism. Our first isolate was obtained by chance during a study of the microflora of the sheep's rumen. Unfortunately, before we had time to complete our investigations the culture died, though not before we had identified the products of fermentation and made rough analyses of the growth medium for the individual VFA's. Some years passed before we managed to isolate new strains. The original isolate grew luxuriantly on media containing lactate as energy source, but when such media were inoculated with sheep rumen contents, only *Veillonella gazogenes* (Johns, 1951*a*) also known as *Micrococcus lactilyticus* (Foubert and Douglas, 1948) developed. However, during an investigation of the organisms developing on starch-containing media inoculated with sheep rumen contents we noticed that our organism appeared to multiply, though it never became dominant. This observation led to the development of a successful method for the isolation of the organism.

Isolation. Sterile 60-ml glass-stoppered bottles were filled with freshly autoclaved medium A, inoculated with 2–3 drops of rumen contents, and, after insertion of the stopper, incubated for 4 days at the end of which time there was a heavy growth. Microscopic examination showed the presence of a large gram negative organism in pairs and chains. Glass-stoppered bottles filled with medium B were inoculated with a drop of the first enrichment culture and incubated. Within 24 hr there was heavy growth and vigorous gas formation. Microscopic examination showed that the gram negative organism was dominant. Steam distillation of a sample of the medium showed the presence of considerable quantities of VFA's and paper chromatography of the distillate showed the presence of acetic, propionic, butyric, and valeric acids.

This culture was used for the isolation of pure cultures, using medium B solidified with 1.5 per cent (w/v) agar. The organism is very sensitive to oxygen and we obtained best results with the shake tube method despite the tendency of the agar to be split by the gas produced. After incubation for 24 hr there was growth in the first four tubes and by the fourth day a few colonies could be seen in the eighth tube, usually the highest dilution showing growth. The colonies were thin, disk shaped, and appeared honey colored; after 6 days the colonies in the higher dilutions were up to 4 mm in diameter.

A second series of shake tubes was prepared using for the inoculum a well-separated colony from the highest dilution showing growth; generally the third series of shake tubes gave a pure culture. Stock cultures were maintained in medium B containing 0.2 per cent agar; the cultures were incubated overnight at 38 C and were then stored in the refrigerator. It was essential to make transfers once every 2 weeks.

Properties of the organism. It is gram negative even in young (4-hr) cultures; it is nonmotile and spores have never been observed. The cells which are somewhat variable in both size and shape occur in pairs and chains of up to 16–20 cells. These chains give the impression of being made up

[5] The authors wish to thank Dr. Annison for informing them of his method prior to publication.

of pairs of cells. In stained preparations the adjacent sides of pairs of cells tend to be flattened but this is not quite so obvious in wet mounts when the cells appear to be more spherical. The greatest variation in both size and shape is to be found in the terminal cells of the chains which, particularly in old cultures, are frequently club shaped. Large, spherical cells are also observed, especially in old cultures. The dimensions of the living cells are 2.6 × 2.4μ. In stained preparations made from smears fixed by heating in the usual way the cells were 1.8–1.2 × 1.7μ indicating that considerable shrinkage had occurred.

Staining with Loeffler's methylene blue or crystal violet is irregular in cultures more than 16 hr old. Some cells, especially the large spherical ones, stain intensely and uniformly while others scarcely take up the stain at all. The majority, however, contain bright, deeply stained granules which show up very clearly against a pale blue background (figure 1). Similar granules are also present when the cells are stained with Neisser's stain (figure 2), or by the Leybourn modification of Albert's stain.

When grown under an atmosphere of H_2 plus 5 per cent CO_2 on plates of either medium B or basal medium plus 1 per cent glucose solidified with agar, the colonies are slightly raised, circular with an entire edge. The colonies on medium B were up to 2 mm in diameter after 4 days at 38 C; whereas on the glucose medium they were smaller, 0.5–1.0 mm. When grown in shake tubes in medium B the colonies were thin, disk shaped and up to 4 mm in diameter. The color varied with the strain and the medium. Strains LC-1 and LC-2 were greenish gray on medium B and honey colored on glucose plates; on the other hand, strain LC-3 was honey colored on both media. Thick suspensions of the organsim in phosphate buffer pH 6.5 are a greenish yellow but when such suspensions are kept under H_2 the color becomes much lighter and may even approach a dirty white. Peel (1955) has found that the organism contains very large amounts of flavin and this color change may represent the reduction of the flavin compounds. The colonies glisten, but when viewed under the dissecting microscope have a slightly roughened appearance. Colonies from plates of medium B were of a buttery consistency and emulsified readily, but those from plates of basal medium plus glucose could be lifted off the agar with a needle and, when touched gently with a needle, slid over the surface. Cultures grown in tubes of medium B plus 0.2 per cent agar have a characteristic granular appearance; growth is re-

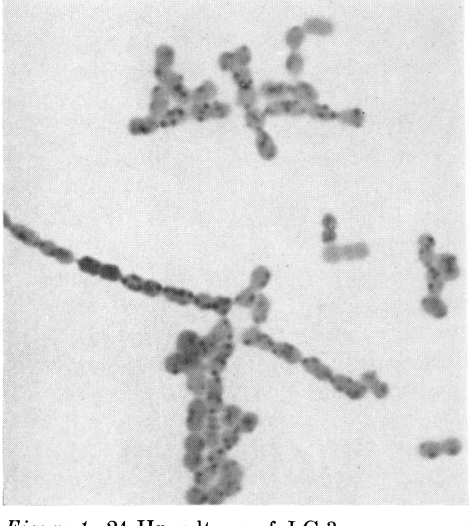

Figure 1. 24-Hr culture of LC-3 grown on medium B and stained with Loeffler's methylene blue; × 1840.

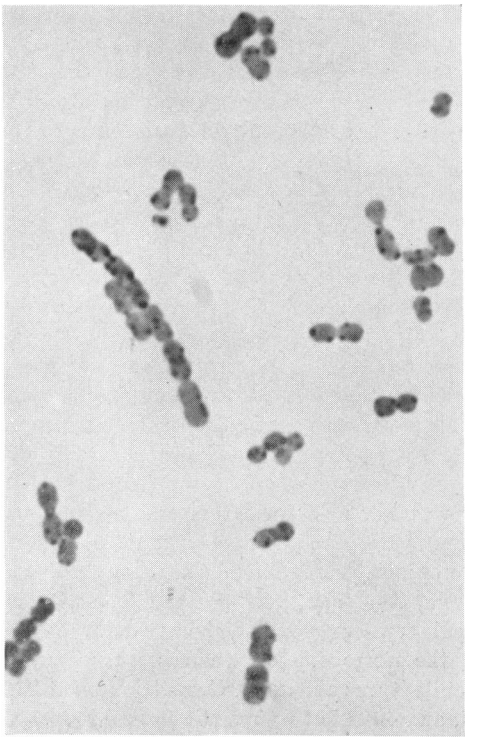

Figure 2. 24-Hr culture of LC-3 grown on medium B and stained with Neisser's stain; × 1840.

stricted to the line of inoculation and the surface of the medium is capped with a collar of froth due to the vigorous gas formation.

The organism is catalase and indole negative; gelatin is not liquefied and nitrate is not reduced to nitrite. H_2S is produced. Fermentation tests were carried out in a medium consisting of 0.4 per cent (w/v) yeast extract (Difco), 0.03 per cent (w/v) thioglycolic acid and 1 per cent (w/v) substrate; the pH was adjusted to 7.4. The tubes, which contained Durham tubes, were incubated at 38 C under H_2 containing 5 per cent CO_2. Under these conditions good growth and vigorous gas formation occurred with DL-lactate, glucose, and fructose. There was slight growth and gas formation with maltose, sorbitol, and mannitol, but the significance of this is doubtful. There was neither growth nor acid formation nor gas production from galactose, mannose, xylose, arabinose, sucrose, lactose, raffinose, salicin, dextrin, starch, inulin, rhamnose, and glycerol. The pH of the medium after growth on glucose and fructose was in the range 4.5–5.0 whereas after growth on lactate it was about 8.0. Growth occurs at 25, 30, 37, and 40 C but no growth occurred at 45 C.

Analysis of the VFA's by paper partition chromatography showed that growth on lactate gave rise to acetate, propionate, butyrate, and valerate; while on glucose and fructose the products were acetate, butyrate, and hexanoate but no propionate and only relatively small amounts of valerate. H_2 and CO_2 were produced from all substrates.

Metabolism of Growing Cultures

Fermentation products from lactate. A quantitative study was made of the products formed when the organism was grown upon the basal medium plus an amount of lactate such that volume of gas evolved could be measured in a 100-ml gas burette. The fermentation vessel was a round-bottom flask with a long neck on which was etched a calibration mark; the volume to the mark was 130 ml. The flask was sterilized by dry heat and then filled to the mark with freshly autoclaved medium. A sample of the medium was set aside for estimation of the initial concentration of lactate. The flask was inoculated with 1 drop of a vigorously growing culture, of strain LC-S, the plug pushed down into the neck, and a rubber bung bearing a capillary tube bent at 90° inserted. The flask plus contents was quickly warmed to 38 C and attached to a gas burette by pressure tubing and a glass-to-glass joint. The burette was filled with mercury from a leveling bulb. The apparatus was placed in a constant temperature room at 38 C and after thermal equilibrium had been attained the pressure was adjusted to atmospheric with the leveling bulb and the burette was read. Vigorous gas formation and good growth occurred within 24 hr. At the end of 3 days, by which time gas formation had ceased, the final volume of gas in the burette was measured, the tap of the gas burette then closed, and the burette disconnected from the flask. Samples of the gas were analyzed in the Haldane apparatus for H_2 and CO_2, and samples of the fermentation fluid were analyzed for dissolved CO_2, VFA's (Moyle *et al.*, 1948), and residual lactate. The results of the analysis are set out in table 1. The carbon recovery is not unreasonable but, in view of the complexity of the end products the very good agreement between the observed redox ratio and the theoretical is probably fortuitous.

Analysis, by paper partition chromatography, of the mixture of fatty acids produced during growth showed that, when glucose and fructose were the substrates, the products were mainly those acids containing an even number of carbon atoms, whereas when lactate was fermented large amounts of propionate and valerate were formed, but no hexanoate. Table 2 shows the results of an analysis of the mixture of acids produced by growing cultures from glucose, fructose, and DL-lactate. Three tubes, each containing 20 ml of medium consisting of 0.4 per cent (w/v) yeast extract (Difco) and 0.03 per cent (w/v) $Na_2S \cdot 9H_2O$ (sterilized separately) and containing 0.5 per cent (w/v) glucose, 0.5 per cent (w/v) fructose, and 1 per cent (v/v) 70 per cent DL-sodium lactate solution, were inoculated with 1 drop each of a suspension of washed cells and incubated under H_2 containing 5 per cent CO_2 for 7 days at 38 C. At the end of the incubation period the pH was measured with the glass electrode and samples analyzed for fatty acids by the method of James and Martin (1952). In this experiment distillation over mercuric sulfate was omitted. The amounts of the individual VFA's are expressed as per cent total volatile acid produced. In general the results confirm the impression obtained by paper partition chromatography. From the retention volumes it was established that the VFA's

TABLE 1

Fermentation of DL-*lactate by strain LC-S**

	Substrate used	Products	Substrate-C	Products-C	Reduction value	Oxidation value
	m moles/100 ml	*m moles/100 ml*	*m. atoms C/100 ml*	*m.atoms C/100 ml*		
Lactic acid	12.9	—	38.7	—	0	0
Acetic acid	—	2.04		4.08	0	0
Propionic acid	—	2.43		7.29	2.43	
Butyric acid	—	2.04		8.16	4.08	
Valeric acid	—	2.58		12.90	7.74	
Caproic acid	—	0.15		0.90	0.6	
Carbon dioxide	—	7.45		7.45	—	14.9
Hydrogen	—	0.47			0.47	
			38.7	40.78	15.32	14.9

* Basal medium and lactate, total vol 130 ml inoculated with LC-S and incubated at 38 C for 3 days.
Carbon recovered, 105%.
O/R ratio 0.98; theoretical = 1.0.

TABLE 2

*Acids produced by strain LC-3 growing on glucose, fructose and lactate**

Substrate	Fructose	Glucose	DL-Lactate
Formate	1.4	4.0	0
Acetate	7.4	7.2	18.7
Propionate	2.57	1.8	32
Butyrate	20.1	14.3	21.6
Valerate	13.4	11.1	27.7
Caproate	55.2	61.6	0
Final pH	4.48	5.1	7.84

* Results expressed m moles fatty acids per 100 m moles total fatty acid formed.

Medium consisted of 0.4 per cent yeast extract (Difco) plus 0.03 per cent $Na_2S \cdot 9H_2O$, sterilized separately as a 1 per cent (w/v) solution plus 0.5 per cent (w/v) of the sugars or 1 per cent (v/v) of a 70 per cent DL-sodium lactate. The sugars were sterilized separately as 20 per cent (w/v) solutions; lactate sterilized with the medium. Total volume of medium = 20 ml in each experiment in 6- x 1-in. pyrex tubes. Each medium inoculated with 1 drop of a washed, overnight, culture of stain LC-3 and inoculated for 7 days at 38 C under H_2 5 per cent CO_2 gas mixture. Fatty acid analyses by the method of James and Martin (1952).

were all of the *n* series. The distillates from both the glucose and the fructose cultures contained small amounts of an acid which had the same retention volume as formic acid; this acid was not found in the lactate cultures. Whether the formic acid was produced from the substrate or whether it was derived from other constituents of the medium is not clear. In the case of the glucose and fructose cultures 5-ml samples of medium were analyzed, whereas in the case of the lactate 0.5 ml was used. This was due to the fact that growth on carbohydrate medium stops after about 2–3 m moles per 100 ml have been utilized (table 3). Consequently, if formic acid was present in the culture in quantities comparable to those found in the glucose and fructose fermentations, the amount of formic acid in the sample used would be too small to be detected. The results shown in table 2 were obtained with strain LC-3. Similar results were also obtained with strains LC-S, LC-1, and LC-2; and the preliminary results with the original strain, isolated in Berkeley, California, from sheep rumen contents obtained from the local abbatoir, followed the same general pattern, at least insofar as lactate and glucose were concerned, although the analytical techniques then available were not so refined as those at present in use.

Succinate and propionate formation. Johns (1951*b*) has shown that *V. gazogenes* decarboxylates succinate with the formation of propionate and carbon dioxide. Since this organism, like *V. gazogenes*, ferments lactate with the formation of acetate, propionate, hydrogen, and carbon dioxide, the ability of LC to decarboxylate succinate was tested. Under no conditions did washed

TABLE 3
Effect of succinate on the fermentation of glucose by Strain LC-3

	Experiment 1	Experiment 2
Initial glucose (m moles/100 ml)	2.96	2.95
Final glucose (m moles/100 ml)	0.13	0.00
Glucose used (m moles/100 ml)	2.83	2.95
Initial succinate (m moles/100 ml)	0.103	1.59
Final succinate (m moles/100 ml)	0.09	1.53
Total volatile acids formed (mEq/100 ml)	2.34	2.60
mEq volatile acid/m mole glucose used	0.83	0.88

Experiments carried out in 6- x 1-in. pyrex tubes, each containing 20 ml freshly autoclaved medium. Medium consisted of 0.4 per cent (w/v) yeast extract (Difco), glucose (sterilized separately) and, in the case of experiment 2, added sodium succinate. Each tube inoculated with 1 drop of an overnight culture of LC-3 and incubated under H_2 containing 5 per cent CO_2 for 7 days at 38 C.

suspensions of any strain of LC produce carbon dioxide from succinate; neither were fumarate nor DL-malate attacked. Propionibacteria decarboxylate succinate very slowly (Delwiche, 1948; Johns 1951c) but Johns found that if succinate was added to cultures of *Propionibacterium shermanii* growing in a glucose-containing medium then, provided that the medium was allowed to become acid, thd added succinate was quantitatively decarboxylated to propionate. The medium consisted of 0.4 per cent yeast plus glucose and succinate. The succinate was autoclaved with the medium and the glucose autoclaved separately. A medium-containing glucose but no succinate served as the control. Samples were removed from both media prior to inoculation for the estimation of the initial concentrations of glucose and succinate. The cultures were incubated under H_2 containing 5 per cent CO_2 for 7 days at 38 C and the media then analyzed. The results for LC-3 are given in table 3. It will be seen that, within experimental error, no succinate disappeared, the same amount of glucose used in each case and the total volatile acids produced was approximately the same. Paper partition chromatography showed that propionate was produced neither in the presence nor absence of succinate, though a small amount of valerate was present in both cases.

DISCUSSION

The fact that this organism is a gram negative coccus suggests that it might be placed in the genus *Neisseria* for anaerobic species of this genus have been described. According to *Bergey's Manual* (Breed *et al.*, 1948) members of this genus do not form chains, although the definition of Miles and Wilson (1946) does not rigidly exclude the possibility of chain formation. Both in size and in morphology it shows certain similarities to *Moraxella*, as shown by the photomicrographs published by Murray and Truant (1954); but, while old cultures show considerable pleomorphism, vermiform cells such as described by Murray and Truant have never been observed. Further, species of *Moraxella* are said to have an absolute requirement for oxygen (Breed *et al.*, 1948).

Thus, while it has features in common with both *Neisseria* and with *Moraxella* it does not fit readily into either genus. Any attempt to assign it to one or the other genus would involve a critical examination of the criteria of both with particular emphasis on the significance of chain formation as a characteristic feature of the genus *Neisseria* and of an absolute requirement for oxygen in the case of the genus *Moraxella*. The possibility that it is a member of a new genus cannot be dismissed. These considerations, coupled with the fact that only a relatively small number of strains have been isolated, all from one source, namely the rumen of the sheep, make it seem prudent to defer further consideration of its taxonomic position until such time as more strains have been isolated and studied from the taxonomic point of view. We propose for the time being, therefore, to refer to it as rumen organism LC. Cultures of it have been deposited in the National Collection of Industrial Bacteria, Chemical Research Laboratory, Teddington, Middlesex, England.

The fact that it was isolated from sheep rumen contents obtained in Berkeley, California, and in Sheffield, England, suggests that it is a common inhabitant of the sheep's rumen. Its characteristic morphology makes it relatively easy to identify in wet mounts of rumen contents and we have so observed it. We have, however, no information on the numbers present in the rumen but it is possible that it is in part responsible for the propio-

nate and valerate found in the rumen (El-Shazly, 1952). Gray, Pilgrim, Rodda, and Weller (1952) reported that addition of carboxyl-labeled propionate to the rumen leads to the formation of labeled valerate, a reaction carried out by washed suspensions of LC (Elsden and Lewis, 1953).

Huhtanen and Gall (1953) have described briefly an organism (RO-C8) isolated from the rumen of cattle which, from the photomicrograph in their paper, appears to have certain morphological features in common with LC. This organism is said to be gram positive but quickly reverts to gram negative, whereas LC is, in our hands, gram negative at all stages of growth. Metabolically the two organisms also appear to have certain similarities. Thus both are vigorous gas producers and both utilize lactate, though it is not clear from the paper whether RO-C8 will grow upon lactate as sole source of carbon and energy. In addition to butyrate and propionate, fatty acids containing more than 4 carbon atoms are said to be produced from lactate, but no quantitative data were given; indeed the methods used for the detection and estimation of these compounds were not described.

LC grows best upon a lactate-containing medium, and Dr. J. L. Peel of the Sheffield Laboratory has found that corn steep liquor is an excellent growth factor supplement. The organism contains large amounts of flavin compounds, up to 1.5 μmoles total flavin/g dry weight (Peel, 1955), and there are indications that riboflavin is an essential constituent of the medium.

The method of isolation developed is empirical. Since LC grows upon lactate it was expected that lactate would be a suitable carbon source for enrichment cultures, but whenever this was tried only *V. gazogenes* grew. Since LC does not ferment starch the function of this compound in the enrichment medium is puzzling. When a starch-containing enrichment medium is inoculated with rumen contents the dominant organism after 24 hr incubation at 38 C is, in our experience, *Streptococcus bovis* (see also Hungate *et al.*, 1952). Lactate is the main end product of starch fermentation by this organism and it may well be that the growth of *S. bovis* not only serves to convert starch into a substrate which can be utilized by LC but also in some way reduces the numbers of *V. gazogenes* present to such an extent that when transfers to the lactate medium are made LC is able to grow unimpeded. The fact that LC does not appear to multiply when the lactate medium is inoculated with rumen contents suggests that *V. gazogenes* in some way suppresses its growth, whether by the formation of inhibitory substance (s) or by multiplying more rapidly than LC is not known.

The fermentation is complex. The experiments of Elsden and Lewis (1953) with washed suspensions suggest that *n*-butyrate, *n*-valerate, and *n*-hexanoate are produced by reactions similar to those found by Barker and his colleagues in *C. kluyveri*. The mechanism of propionate formation however remains obscure. The present work shows that growing cultures do not metabolize succinic acid added to the medium; and further, that washed suspensions do not decarboxylate succinate. More recently, Lewis and Elsden (1955) have found that both washed suspensions and acetone powders of LC reduce acrylate to propionate with hydrogen. Working with acetone powders, indirect evidence was obtained for the occurrence of a dismutation of acrylate according to the following equation:

$$3\ CH_2{=}CHCOOH + 2\ H_2O \rightarrow 2\ CH_3\ CH_2COOH + CH_3COOH + CO_2$$

In view of these findings Lewis and Elsden suggested that the mechanism of propionate formation might be similar to that proposed by Johns (1952) for *C. propionicum* with the modification that the reactants might be CoA derivatives rather than free acids; in this case the reaction sequence would be: lactyl CoA → acrylyl CoA → propionyl CoA → propionate. This scheme has the advantage that it provides an explanation for the fact that during growth on lactate, propionate and *n*-valerate are produced in large amounts, whereas with glucose and fructose the fatty acids with an even number of C atoms predominate. The similarity between this scheme and that suggested by Stadtman (1953) for the conversion of β-hydroxybutyrate to butyrate by *C. kluyveri* is obvious.

Lewis and Elsden (1955) observed that L-serine, L-threonine, and L-cysteine were fermented by washed suspensions of LC and that formate was one of the end products; on the other hand the amount of formate produced from both pyruvate and acrylate was insignificant. The production of formate from these three amino acids might well explain the appearance of traces of formate in the culture medium, as recorded in table 2.

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SUMMARY

The isolation and properties of a gram negative, anaerobic organism are described. It does not seem advisable at this time to try to classify the few available strains; however, the organism is closely related to members of the genera *Neisseria* and *Moraxella*. This organism ferments DL-lactate, glucose, and fructose. The fermentation products from DL-lactate are hydrogen, carbon dioxide, acetate, propionate, *n*-butyrate, and *n*-valerate. The products from glucose and fructose are acetate, *n*-butyrate, small amounts of *n*-valerate and *n*-hexanoate, hydrogen, and carbon dioxide. Growing cultures do not decarboxylate added succinate.

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