The Biohydrogenation of α-Linolenic Acid and Oleic Acid by Rumen Micro-organisms

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1. α-[U-14C]Linolenic acid was incubated with the rumen contents of sheep and the metabolic products were characterized by thin-layer chromatography, gas-liquid chromatography and absorption spectroscopy in the ultraviolet and infrared. 2. A tentative scheme for the biohydrogenation route to stearic acid is presented. The main pathway is through diconjugated cis-cis-cis-octa decatrienoic acid, non-conjugated trans-cis (cis-trans)-octadecadienoic acid and trans-octa decenoic acid, but other pathways are apparent. 3. Washed rumen micro-organisms possessed only a limited capacity to hydrogenate α-linolenic acid and oleic acid but the rate was greatly stimulated by a factor(s) present in the supernatant rumen liquor. 4. Pure cultures of Clostridium perfringens, Streptococcus faecalis, Escherichia coli and a coliform organism isolated from sheep faeces possessed negligible ability to hydrogenate unsaturated fatty acids compared with a mixed population of rumen micro-organisms. Butyrivibrio fibrisolvens slowly converted linoleic acid into octadecenoic acid.

Biohydrogenation of unsaturated fatty acids is a characteristic reaction carried out by micro-organisms of the ruminant (Shorland, Weenink, Johns & McDonald, 1957; Garton, 1964; Ward, Scott & Dawson, 1964) and lower digestive tract (Ward et al. 1964). The main fatty acid in the natural pasture diet of the ruminant is cis-cis-cis-Δ5,12,15-octa decatrienoic acid (α-linolenic acid), which is esterified in a number of complex lipids; esterified linoleic acid and oleic acid are also present. Although it is possible that some of these esterified unsaturated fatty acids may be hydrogenated directly, it is likely that most are enzymically hydrolysed to the free acid (Garton, 1964) before hydrogenation takes place.

When linolenic acid is added to an artificial rumen it is hydrogenated to stearic acid via dienoic and monoenoic acid intermediates with no appreciable conversion into other metabolic products (Ward et al. 1964). Wood, Bell, Grainger & Teekell (1963) introduced radioactive linoleic acid into the intact rumen and later isolated most of the radioactivity from the rumen contents as C18 monoenoic acid and stearic acid.

In the present paper a study has been made of the initial metabolic products of the hydrogenation of α-linolenic acid and their place in the hydrogenation pathway. In addition, the capacity of whole and disrupted washed rumen micro-organisms to hydrogenate linolenic acid and oleic acid has been examined. The clear rumen-liquor supernatant has been shown to play an important role in the hydrogenation sequence.

EXPERIMENTAL

Rumen samples. Rumen contents for experiments in vitro were withdrawn from Clun Forest sheep with rumen fistulae fed on a hay-oats diet (5:1, w/w; 1200g./day). The fluid was strained through four layers of cotton gauze to remove coarse food particles.

Cultures of pure bacteria. The following bacteria were grown in pure culture: Butyrivibrio fibrisolvens (Dr P. N. Hobson, Rowett Research Station, Aberdeen); Escherichia coli B and Streptococcus faecalis Dunn (Dr G. S. Coleman, Institute of Animal Physiology, Babraham, Cambridge); a coliform organism (a motile lactose-fermenter) isolated from sheep faeces by Mr R. W. White; Clostridium perfringens S107 (Wellcome Research Laboratories, Beckenham, Kent). The organisms were grown anaerobically in 0-5% yeast-1% peptone broth (E. coli and S. faecalis), fortified casein hydrolysate (C. perfringens) or reinforced clostridial medium (B. fibrisolvens) and tested for their ability to hydrogenate unsaturated fatty acids towards the end of the exponential phase of growth.

Washed rumen micro-organisms. The strained rumen contents were centrifuged at 23500g for 30min. in a Servall centrifuge at 4°. The supernatant (rumen liquor) was decanted and the sediment suspended in the same volume of a reducing buffered saline, pH7-0, prepared as
follows: NaCl (8.5g) and 4.1 ml of conc. HCl were added to 11. of water and the solution was boiled. After cooling, 6.06g. of tris and 200 mg of cystine hydrochloride in 1.2 ml of 0.1-NaOH were added and the whole was covered with a layer of liquid paraffin. The suspension was centrifuged as before and the sediment rewashed a further two times. The washed micro-organisms were suspended in the original volume of buffered saline for incubation.

**Disruption of micro-organisms.** Washed micro-organisms were disintegrated either by 20 min. irradiation at 0° with ultrasound (MSF 60 w disintegrator) or in a Mickle shaker for 30 min. at 4° with 50% (by vol.) of Ballotini no. 13 glass beads and no air space.

**Microbial incubation with radioactive fatty acid.** The radioactive fatty acid was dissolved in 0.05-0.1 ml of methanol in a 50 ml pear-shaped flask and an equal volume of buffered saline was added. The emulsion was evaporated under vacuum to remove most of the methanol and 2-10 ml of the rumen preparations was added. Incubation was carried out at 38° with continuous gassing with water-saturated N2+CO2 (85:5) to stir and maintain anaerobic conditions in and above the preparation.

**Extraction of fatty acids from the incubation mixture.** The incubation mixture (10 ml) was shaken with 100 ml of chloroform-methanol-conc. HCl (50:50:1, by vol.) containing 2.6-di-tert.-butyl-4-methylphenol (20 mg) as an antioxidant. After standing 10 min. the suspension was filtered and the filtrate shaken with 20 ml of water; the lower layer was removed and evaporated to dryness on a rotary evaporator. The residue was extracted with 20 ml of ether, the ethereal solution evaporated to dryness and 5 ml of 6% (w/v) KOH in ethanol-water (19:1, v/v) added. After refluxing for 1 hr., 5 ml of water was added and the mixture was extracted three times with equal volumes of light petroleum (b.p. 40-60°). The aqueous phase was acidified with 5 N HCl and extracted twice with equal volumes of light petroleum (b.p. 40-60°). The light-petroleum extracts, containing the fatty acids, were evaporated to dryness.

**Gas-liquid chromatography.** Gas-liquid chromatography was carried out as described by Ward et al. (1964) and collection of samples from preparative columns by the procedures of Scott, Ward & Dawson (1964). Radioactive scanning of the gas-liquid-chromatogram effluent was performed with an apparatus similar to that of James & al. (1960). and whose construction was described by Scott et al. (1964). Fatty acids were methylated with diazomethane in methanol-ether (1:9, v/v) (Schlenk & Gellerman, 1960).

**Thin-layer chromatography.** The methyl esters of the geometric isomers of C18 mono-, di- and tri-enoic acids were separated on thin-layer plates of silica gel G (E. Merck A.-G., Darmstadt, Germany) impregnated with 5% (w/w) AgNO3 (de Vries, 1962, 1963; Ward et al. 1964). Benzene-ether (1:1, v/v) was used to separate monoenoic acid and stearic acid, and benzene-ether (99:1, v/v) was used to examine the dienoic acids. The fatty acid methyl esters were detected either by spraying with 0-2% (w/v) 2,7-dichlorofluorescein in ethanol and examination under u.v. light or by spraying with aq. 50% (v/v) H2SO4 and then heating at 100°. Radioactive spots were located either by scanning with a mica-window Geiger-Müller counter (Ward et al. 1964) or by radioautography with Kodak 0-800 glass photographic plates. If the methyl esters were not being recovered, the silica gel was sprayed with Neatan varnish (Merck) before radioautography to facilitate handling. For recovery of the methyl ester spots, the silica gel corresponding to the radioactive area was sucked up into a small sintered-glass column (Ward et al. 1964) and then eluted with ether.

**Oxidation of fatty acids.** It was found that the procedure of Rudloff (1956) for oxidation at the double bond, though satisfactory for monoenoic acid, gave extensive over-oxidation with dienoic and trienoic acids. Consequently these were oxidized by the alkaline-permanganate method of Layworth & Mottram (1925) suitably scaled down. This gave much better yields of the expected di- and mono-carboxylic acids from known linolenic acid and linoleic acid samples. After oxidation the acidified solutions were exhaustively extracted with light petroleum, and the oxidation products were methylated and examined by gas-liquid chromatography on a polyethylene glycol aliphatic column isothermally at 150°. Complete separation of the mono- and di-carboxylic acids was obtained.

**Radioactive counting and materials.** Methods were as described by Ward et al. (1964). The all-cis-[U-14C]- and [1-14C]-fatty acids were obtained from The Radiochemical Centre, Amersham, Bucks. [1-14C]Elaic acid was obtained by the Se-catalysed isomerization of [1-14C]oleic acid (Swern & Scanlan, 1953). The α-[U-14C]linolenic acid showed no radioactive impurities on gas-liquid chromatography on a polyethylene glycol aliphatic column with radioactive scanning. Thin-layer chromatography on silica gel 5% AgNO3 plates also failed to show any radioactive component other than α-linolenic acid.

**RESULTS**

**Biohydrogenation of α-linolenic acid by whole rumen contents.** Rumen contents were incubated with α-[U-14C]linolenic acid under anaerobic conditions, and thin-layer chromatograms on silica gel-silver nitrate plates were prepared of the fatty acids isolated from the mixture. Radioautographs showed that in 2 hr. the α-linolenic acid had been substantially converted into stearic acid, although some C18 trans-monoenoic acid was also present. With shorter periods of incubation radioautography revealed a complex pattern of radioactive degradation products (Fig. 1) with the transient accumulation of C18 dienoic acid intermediates that had been observed by Ward et al. (1964). No hydrogenation occurred when the α-linolenic acid was incubated under similar conditions with 0-9% sodium chloride in place of rumen contents. When incubations were carried out for different time intervals it became apparent from radioautography of the thin-layer plates that there were well-defined sequential changes in the metabolite products formed (Fig. 2). By choosing suitable incubation times it was possible to obtain a sufficient accumulation of the radioactive intermediates to isolate them by preparative thin-layer chromatography. This allowed their gas-liquid chromatographic properties to be investigated: the radioactivity in the effluent from the columns was continually
incubating cx-linolenic spectra. of the possible was on layer plates of silica Component A. Gas-liquid chromatography tents.

rumen contents. in plus 10mg. acid rumen of 1. produced on incubation of a-[U-14C]linolenic acid was incubated at 37° for 30 min. with 4 ml. of strained rumen contents. The fatty acids were isolated and after methylation run as a strip on a thin-layer-chromatographic plate in benzene-ether (99:1, v/v). Radioautography was carried out for 18 hr. The trace radioactive component E is not usually seen.

scanned in a proportional counter after oxidation of any emerging radioactive fatty acid to $^{14}$CO$_2$. It became obvious that some of the apparent constituents that ran as single spots on thin-layer chromatography were resolved into two or more components on gas-liquid chromatography (Table 1). For clarity these components have been labelled according to: (1) their running on thin-layer plates of silica gel–silver nitrate; (2) their resolution on gas-liquid chromatography (see Fig. 1 and Table 1). By using large-scale incubations of rumen contents (200 ml) with a-[U-14C]linolenic acid plus 10 mg of unlabelled α-linolenic acid it was possible to isolate sufficient quantities of some of the intermediates to examine their infrared and ultraviolet spectra.

Identity of radioactive components formed on incubating α-linolenic acid with whole rumen contents. Component A. Gas-liquid chromatography (Table 1) indicated that this was the original α-linolenic acid. Zero-time incubations (Fig. 2) showed that it contained little contaminating material. The radioactive material remaining at the zero-time origin (Fig. 2) was probably autoxidized fatty acid (see Nichols & Morris, 1964).

Component Ba. Catalytic hydrogenation (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959) yielded stearic acid as the sole product. The fatty acid possessed a strong absorption peak in ethanol at 233nm, which is characteristic of a conjugated dienoic acid (Holman, 1957). However, the gas-liquid-chromatographic (Table 1) and thin-layer-chromatographic (Fig. 1) properties were different from the expected behaviour of a conjugated octadecadienoic acid (Ward et al., 1964; de Vries, 1962, 1963). Alkaline isomerization (Holman, 1957) produced a main absorption peak at 268 nm, characteristic of a triconjugated trienoic acid. This indicated that the fatty acid is a trienoic acid in which two of the double bonds are conjugated. Oxidation of the acid gave a gas-liquid-chromatographic picture that was very similar to that from α-linolenic acid, i.e. propionic acid and azelaic acid were the main products. The fatty acid is therefore probably Δ$^9,11,15$, or Δ$^9,13,15$, octadecatrienoic acid. In the

Table 1. Gas-liquid chromatography of methyl esters of fatty acids formed from α-linolenic acid

<table>
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<tr>
<th>Spots of thin-layer chromatogram (Fig. 1)</th>
<th>Retention volume relative to methyl palmitate</th>
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<td>On 10% polyethylene glycol adipate (180°)</td>
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<td>Experiment</td>
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<td>Whole rumen contents</td>
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<td>B (0-30)</td>
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<td>C (0-39)</td>
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<td>D (0-56)</td>
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<td>G (0-82)</td>
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Fig. 1. Thin-layer chromatogram of the fatty acids produced on incubation of α-[U-14C]linolenic acid with rumen contents. A 0.5 mg sample (6 μC) of α-[U-14C]linolenic acid was incubated at 37° for 30 min. with 4 ml. of strained rumen contents. The fatty acids were isolated and after methylation run as a strip on a thin-layer-chromatographic plate in benzene-ether (99:1, v/v). Radioautography was carried out for 18 hr. The trace radioactive component E is not usually seen.
infrared no distinct trans peak was visible although a weak peak at 978 cm\(^{-1}\) was apparent, so that the fatty acid probably has a cis–cis–cis configuration.

Component Bb. This fatty acid occurred as a minor transient intermediate in the biohydrogenation process. Thin-layer chromatography with light petroleum–ether (99:1, v/v) solvent did not separate it appreciably from component Ba but a slightly better differentiation was apparent in benzene–ether (9:1, v/v). Its gas–liquid-chromatographic retention volumes and its \(R_p\) on thin-layer chromatography indicated that the acid is a cis–cis–octadecadienoic acid. In a previous investigation this was noted as an intermediate in the ruminal hydrogenation of linolenic acid (Ward et al. 1964).

Component Bc. This fatty acid was never more than a very minor component of the incubation medium and was not always found. Its gas–liquid-chromatographic retention volumes and behaviour on thin-layer chromatography suggest that it is a geometric isomer of non-conjugated octadecatrienoic acid. As small amounts of a fatty acid of identical chromatographic properties were formed when \(\alpha\)-linolenic acid was incubated with 0·9% sodium chloride at 37\(^\circ\) under anaerobic conditions, it is probably not a true intermediate in the metabolic pathway of \(\alpha\)-linolenic acid hydrogenation in the rumen.

Component Ca. The gas–liquid-chromatographic retention volumes of this acid (Table 1) indicate that it is a non-conjugated octadecadienoic acid. Its infrared spectrum in carbon disulphide showed a strong peak at 969 cm\(^{-1}\), indicative of the trans configuration. This and its behaviour on thin-layer chromatography indicates that it is a non-conjugated cis–trans (trans–cis)–octadecadienoic acid.

Component Da. The gas–liquid-chromatographic retention volumes again indicate that, like component Ca, this is a non-conjugated octadecadienoic acid. The infrared spectrum in carbon disulphide showed a trans peak at 969 cm\(^{-1}\). From its behaviour on thin-layer chromatography a trans–trans–octadecadienoic acid is indicated, or a positional isomer of component Ca [i.e. a non-conjugated cis–trans (trans–cis)–octadecadienoic acid]. From the ready interconversion of components Da and Ca and the intensity of the trans peak the latter identification is more likely.

Component Fa. The gas–liquid-chromatographic retention volumes did not correspond to any recorded C\(_{18}\) fatty acid and not enough was present to isolate sufficient for studies of its absorption spectra. Its specific radioactivity was extremely high compared with the other fatty acid intermediates and this suggests that it may not be a normal product of \(\alpha\)-linolenic acid hydrogenation in the rumen.

Component Fb. The gas–liquid-chromatographic retention volumes and thin-layer-chromatographic properties are consistent with this being cis-octadecenoic acid.

Component G. The gas–liquid-chromatographic properties indicate this is a trans–octadecenoic acid. In previous studies this was identified as an intermediate of the ruminal metabolism of \(\alpha\)-linolenic acid (Ward et al. 1964).

Component H. This fatty acid behaves identically with stearic acid on gas–liquid and thin-layer chromatography.

**Sequence of metabolic changes during hydrogenation of \(\alpha\)-linolenic acid.** Thin-layer chromatography (Fig. 2) and gas–liquid chromatography of serial samples from an incubation mixture indicated that the cis–cis–cis–octadecatrienoic acid with two of the double bonds conjugated (component Ba) was the initial product of \(\alpha\)-linolenic acid metabolism in the rumen. This was followed by hydrogenation at the conjugated double bond, this process being associated with a considerable cis–trans isomerization of the remaining double bonds.
The main products were \( \text{cis}-\text{trans} \) (trans-cis)-octadecadienoic acid (component \( \text{Ca} \)) and to a smaller extent \( \text{cis}-\text{cis} \)-octadecadienoic acid (component \( \text{Bb} \)) (Fig. 2). Also, an isolated B fraction from a thin-layer chromatogram gave predominantly \( \text{cis}-\text{trans} \) (trans-cis)-octadecadienoic acid (fraction C) on incubation for 30 min. with rumen contents (Fig. 3). The \( \text{cis}-\text{trans} \) (trans-cis)-octadecadienoic acid (component \( \text{Ca} \)) could be slowly isomerized to the minor unknown octadecadienoic acid (component \( \text{Da} \)) (Figs. 2 and 3), the reaction being reversible (Fig. 3). The \( \text{cis}-\text{trans} \) (trans-cis)-octadecadienoic acid was then hydrogenated, giving mainly trans-octadecenoic acid (Figs. 2 and 3) and probably a small amount of the cis isomer (see Ward et al. 1964). The \( \text{C}_{18} \) monoenoic acids were then rapidly hydrogenated to stearic acid.

**Biohydrogenation of \( \alpha \)-linolenic acid by washed rumen micro-organisms.** On centrifuging rumen contents and examining the micro-organisms and supernatant separately, it became clear that the capacity to hydrogenate was solely confined to the micro-organisms. After the complete removal of the supernatant liquor from the micro-organisms, incubation with \( \alpha-[U-\text{14C}] \)-linolenic acid for 2 hr. resulted in the slow accumulation of metabolic products, which, on thin-layer chromatography on silica gel-silver nitrate plates, exactly corresponded to spots B, C and D (see Fig. 1).

However, in this case, in spite of the long period of incubation, only limited amounts of monoenoic acid and stearic acid had been formed. In addition, on isolation of the spots from the thin-layer chromatograms and examination by gas-liquid chromatography it became apparent (Table 1) that, though fraction B contained the products formed by whole rumen contents, fractions C and D were far more complex and somewhat variable in composition.

When the mixture of fatty acids formed by incubating washed rumen micro-organisms with radioactive \( \alpha \)-linolenic acid were isolated and incubated with rumen-liquor supernatant, there was no conversion into monoenoic acid or stearic acid. However, the addition of the supernatant liquor to the washed rumen micro-organisms completely restored their original hydrogenation capacity towards \( \alpha \)-linolenic acid. Clearly therefore rumen liquor contains some factor that assists the micro-organisms to hydrogenate \( \alpha \)-linolenic acid completely.

This essential factor(s) in rumen liquor was only partially destroyed by boiling for 5 min. On treating the liquor with 8 vol. of ethanol, it was concentrated in the ethanol-insoluble fraction. It could not be replaced by NADH\(_2\), NADPH\(_2\) (1.4 \( \mu \)moles/ml.), succinate, lactate or glucose.

Disruption of the washed rumen micro-organisms with ultrasound (15 min. in the MSE 60 w disintegrator) resulted in loss of ability to carry out hydrogenation, although some conversion into the diconjugated octadecatrienoic acid was observed.

**Biohydrogenation of oleic acid.** When \([U-\text{14C}]\)-oleic acid was incubated with rumen contents, radioautographs of the thin-layer chromatograms of the resultant fatty acids indicated rapid conversion into \( \text{trans} \)-monoenoic acid and stearic acid. Neither the whole washed micro-organisms nor homogenates of these produced on disintegration with ultrasound, butanol treatment or in the Mickle shaker could hydrogenate oleic acid or elaidic acid. By itself rumen liquor was incapable of hydrogenating oleic acid, but on the addition of this to washed rumen micro-organisms their ability to convert oleic acid into \( \text{trans} \)-monoenoic acid and stearic acid was fully restored. Boiling the rumen liquor for 5 min. partly destroyed this capacity and its action could not be replaced by NADH\(_2\) and NADPH\(_2\).

**Ability of pure cultures of bacteria to carry out biohydrogenation of unsaturated fatty acids.** A number of pure cultures of anaerobic or facultative anaerobic bacteria found in the intestinal tract.

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**Fig. 3.** Incubation of the isolated intermediates in \( \alpha \)-linolenic acid hydrogenation with rumen contents. Intermediary fractions B, C and D were isolated from a 30 min. incubation of \( \alpha-[U-\text{14C}] \)-linolenic acid with rumen contents by preparative thin-layer chromatography of the fatty acid methyl esters. After saponification the free fatty acid fractions obtained (B, C and D) were again incubated individually for 30 min. with rumen contents. The fatty acids were extracted, methylated and subjected to thin-layer chromatography and radioautography.
were tested to determine whether they possessed any ability to hydrogenate unsaturated fatty acids. These included *C. perfringens* S107, *S. faecalis* Dunn, *E. coli* B and *B. fibrisolvens*. The actively growing pure cultures were incubated anaerobically for 2–3 hr. with the radioactive fatty acid, and the fatty acids were extracted and examined by thin-layer chromatography as before. *S. faecalis* did not metabolize [U-14C]linolenic acid. The *C. perfringens* culture completely metabolized added [1-14C]linoleic acid but only a very little stearic acid was formed. The main product ran much slower than linoleic acid on thin-layer silica gel-silver nitrate plates and was possibly octadecatrienoic acid. Similarly, when [1-14C]oleic acid was incubated with *C. perfringens* the main metabolic product was a dienoic acid or trans-trienoic acid, with only minimal amounts of stearic acid being formed (cf. Harris & James, 1965). When *E. coli* cultures were incubated with [U-14C]linolenic acid a very slow conversion into stearic acid was observed. On recovering the organisms by centrifugation and washing them with reducing saline the ability to metabolize linolenic acid was lost. A coliform organism isolated from sheep faeces possessed no ability to hydrogenate α-[U-14C]linolenic acid. *B. fibrisolvens* grown in the presence of linoleic acid for 21 hr. partially converted it into octadecenoic acid. However, when organisms in the exponential phase of growth were incubated for 2 hr. with linoleic acid under hydrogen, little hydrogenation of the fatty acid occurred.

**DISCUSSION**

Biohydrogenation of α-linolenic acid by rumen micro-organisms is clearly a very complex chemical process and in this respect is equivalent to its chemical hydrogenation catalysed by metals (Allen & Kies, 1955; Scholfield *et al.* 1960).

Though certain fatty acids show a transient accumulation during the metabolic hydrogenation, it must be emphasized that these may not be the only intermediate products and in fact could be merely those whose further metabolism is a rate-limiting step. With this proviso in mind, the present results coupled with previous studies (Ward *et al.* 1964) allow a tentative metabolic scheme for the hydrogenation of α-linolenic acid to stearic acid to be formulated (Scheme 1). It must, however, be emphasized that this scheme will be additionally complicated by the considerable positional migration of the double bonds that can occur during hydrogenation (Shorland *et al.* 1957; Ward *et al.* 1964). The techniques used produce poor separation of the positional isomers of a given geometric fatty acid isomer and information about them is only likely to be obtained by a detailed study of the oxidation products.

It is clear from the present experiments that the full hydrogenation capacity of rumen micro-organisms towards α-linolenic acid and oleic acid requires an essential cofactor(s) that is present in the rumen-liquor supernatant. This is in agreement with the results of Polan, McNeill & Tove (1964), who reported that boiled rumen fluid stimulated

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**Scheme 1.** Pathways of hydrogenation of α-linolenic acid by rumen micro-organisms. The letters in parentheses refer to the fractions described in Table 1 and in the text.
the hydrogenation of linoleic acid and oleic acid by rumen micro-organisms from a steer. The identity of this factor is unknown, but it is possible that it could be a substrate for a dehydrogenase that would supply reduced nicotinamide nucleotides. However, lactate, succinate and glucose were ineffective as substitutes.

Washed rumen micro-organisms on their own have only a limited capacity for hydrogenation, and fatty acids accumulate that are not normally found with whole rumen contents. Some of these may be conjugated. Shorland et al. (1957) observed the accumulation of conjugated dienoic acids when linoleic acid was incubated with rumen contents under non-physiological conditions, but none was formed from linolenic acid.

It is possible that these additional acids are on subsidiary pathways and only accumulate when the main pathway of hydrogenation is blocked by adverse physiological conditions, such as the absence of rumen liquor cofactor(s). Alternatively, they could be intermediates in the main hydrogenation pathway that are normally metabolized very quickly so that no appreciable concentration ever forms.

Our experiments with a limited number of pure cultures of bacteria typically found in the alimentary tract show that the ability to biohydrogenate rapidly linolenic acid to stearic acid is not possessed by all bacteria. A strain of E. coli showed some ability to hydrogenate linolenic acid to stearic acid, but the rate was very low compared with a mixed population of rumen micro-organisms. Polan et al. (1964) found that among 20 pure cultures of rumen bacteria only certain strains of B. fibrisolvens could hydrogenate linolenic acid, and then only to an octadecenoic acid, not stearic acid. We have confirmed this observation but in our hands the rate of hydrogenation was much lower and its extent more limited than that carried out by the mixed population of rumen micro-organisms. Presumably other species must exist in the rumen that are more efficient at hydrogenation and can convert the octadecenoic acid into stearic acid.

We are extremely grateful to Dr G. S. Coleman and Mr R. W. White, who prepared cultures of pure bacteria, and to Mr David Lander, who rendered valuable technical assistance. Dr P. N. Hobson and Dr G. S. Coleman are thanked for supplying slopes of various pure bacteria.

REFERENCES