Isolation of a Rumen Bacterium that Hydrogenates Oleic Acid as well as Linoleic Acid and Linolenic Acid

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The conversion of dietary C_{18} polyunsaturated fatty acids in the rumen into stearic acid is a well-known phenomenon that involves considerable positional and geometric isomerization of the double bonds coupled with their successive hydrogenation (Shorland, Weenink, Johns & McDonald, 1957; Polan, McNeill & Tove, 1964; Wilde & Dawson, 1966; Kemp & Dawson, 1968). A number of individual rumen micro-organisms have been described that can hydrogenate linolenic acid and linoleic acid (Kepler, Hirons, McNeill & Tove, 1966; Kemp & White, 1968; Mills, Scott, Russell & Smith, 1969; Sachan & Davis, 1969), but none of these was capable of completing the hydrogenation beyond the octadecenoic acid stage or of converting oleic acid into stearic acid. Although washed rumen protozoa can hydrogenate oleic acid (Gutierrez, Williams, Davis & Warwick, 1962; Williams, Gutierrez & Davis, 1963; Chalupa & Kutches, 1968) it is known that these may contain viable bacteria (White, 1969). Further, the complete hydrogenation of linolenic acid and oleic acid to stearic acid by ciliate-free mixed micro-organisms obtained from a sheep whose rumen had been defaunated by treatment with a non-ionic detergent has been demonstrated (Dawson & Kemp, 1969a).

From the rumen of this defaunated sheep we have now isolated a bacterium in pure culture that can hydrogenate oleic acid to stearic acid. The diluted rumen fluid was seeded in roll bottles containing either the normal solid culture medium of Bryant & Robinson (1961) or the same medium that had been modified by substituting an equal weight of sodium L-lactate for glucose. Colonies from the roll bottles were subcultured in liquid medium (Bryant & Robinson, 1961) with CO_2 as the gas phase and tested for their abilities to hydrogenate $[1-^{14}C]$ oleic acid included in the culture medium. On being tested for labelled stearic acid (Kemp & Dawson, 1968) only one subculture from the original 'lactate'-medium roll culture possessed hydrogenating ability. This culture, which consisted entirely of Gram-negative bacilli, was separated into two components by further seeding into roll bottles and reculturing colony picks. Only one of these was able to hydrogenate $[1-^{14}C]$ oleic acid, and this activity

was present in all subsequent colonies obtained by subculturing the strain.

The organism is a small Gram-negative capsulated anaerobic bacillus, which is catalase-negative. It is motile by means of a flagellum, which appears to be localized midway between the two poles. The morphology varies with the substrate: generally the bacillus has slightly pointed ends, but is frequently filamentous and in the presence of yeast extract, which stimulates growth, some curved forms are seen.

The ability of the organism to hydrogenate unsaturated fatty acids was tested by culturing it in 5 ml of liquid medium (Bryant & Robinson, 1961) containing 10–20 μg of the ^{14}C -labelled acid (0.5 μCi). After 72 h of growth at 39°C the culture was poured into 120 ml of chloroform-methanol-6M-HCl (5:6:1, by vol.) and the labelled fatty acids were examined by radio-g.l.c. and t.l.c. on $AgNO_3$ -impregnated silica gel as described by Kemp & Dawson (1968).

The ability to hydrogenate oleic acid was always enhanced if the organisms were grown in a lactate-containing medium rather than one containing glucose, although this made little visible difference to the amount of growth. On the other hand yeast extract enhanced the growth but had little effect on the hydrogenation. About 80% of the oleic acid added to the lactate medium was converted into stearic acid; traces of *trans*-octadecenoic acid were also formed, together with a polar fatty acid whose methyl ester had the same retention time on an Apiezon L g.l.c. column as methyl hydroxystearate ($R_{methyl\ palmitate}$ 4.9) and the same R_F on t.l.c. [0.14 on silica gel H + 6% $AgNO_3$ with benzene-diethyl ether (99:1, v/v)]. Substantial hydrogenation was also observed if the oleic acid was added to a 72 h culture of the organism and the incubation continued for 2–4 h, but only if yeast extract was present in the original incubation medium.

$[1-^{14}C]$ Linoleic acid was also converted into stearic acid (80% in a 72 h culture). Traces of labelled octadecenoic acid and octadecadienoic acid are also present in the incubation medium, and from its properties on g.l.c. the latter appears to be conjugated.

Linolenic acid was not hydrogenated to stearic acid by the organism, but instead octadecenoic acids accumulated in the medium (80% conversion in 72h). These were predominantly *cis*-octadec-15-enoic acid (83%); the remainder were *trans*-octadec-15-enoic acid (12%), *trans*-octadec-14-enoic acid (4%) and *trans*-octadec-13-enoic acid (1%). Traces of a non-conjugated octadecadienoic acid were also present.

The above results show that the organism, as well as being able to hydrogenate oleic acid, is also able to hydrogenate the more unsaturated C₁₈ acids, but by a pathway that, at least for linolenic acid, is distinct from that of any of the other rumen micro-organisms so far examined. The production of *cis*-octadec-15-enoic acid from linolenic acids would explain our finding of this acid as a metabolic product when mixed rumen micro-organisms are incubated with [¹⁴C]linolenic acid (Dawson & Kemp, 1969*b*).

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