the isolation and fractionation of human anti-dextran antibodies (Schlossman & Kabat, 1962). The affinity of the desired protein towards the dextran gel is exploited in both cases. Subsequent displacement by low-molecular-weight competitive inhibitors (haptens) yields a purified protein.

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Cifonelli, J. A., Montgomery, R. & Smith, F. (1956). *J. Amer. chem. Soc.* **78**, 2485.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). *Analyt. Chem.* **28**, 350.

Flodin, P. (1962). *Dextran Gels and their Applications in Gel Filtration*. Uppsala: Pharmacia.

Goldstein, I. J., Hollerman, C. E. & Merrick, J. M. (1963). *Fed. Proc.* **22**, 297.

Goldstein, I. J., Hollerman, C. E. & Merrick, J. M. (1965a). *Biochim. biophys. Acta*, **97**, 68.

Goldstein, I. J., Hollerman, C. E. & Smith, E. E. (1965b). *Biochemistry*, **4**, 876.

Pharmacia (1963). *Sephadex in Gel Filtration*, p. 2. New York: Pharmacia Fine Chemicals.

Schlossman, S. F. & Kabat, E. A. (1962). *J. exp. Med.* **116**, 535.

So, L. L. & Goldstein, I. J. (1965). *Abstr. Pap. 149th Meet. Amer. chem. Soc., Detroit*, p. 12c.

Sumner, J. B. (1919). *J. biol. Chem.* **37**, 137.

Sumner, J. B. & Howell, S. F. (1936). *J. Bact.* **32**, 227.

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Octadecenoic Acids in Sheep Rumen

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It is now well established that the rumen microflora is capable of efficient hydrogenation of unsaturated fatty acids (Reiser & Reddy, 1956; Shorland, Weenink, Johns & McDonald, 1957; Garton, Lough & Vioque, 1961). Though the rumen is the principal site of hydrogenation, there is evidence that hydrogenation takes place to a limited extent in other parts of the ovine digestive tract (Ward, Scott & Dawson, 1964). The dietary C_{18} polyenoic acids occur almost exclusively in the *cis* form, and although they are converted efficiently into stearic acid, the presence and possible accumulation of the *trans*-acids implies isomerization during hydrogenation (Garton, 1959). Kepler, Hirons, McNeill & Tove (1965) have reported that the rumen micro-organism *Butyrivibrio fibrisolvens* converts linoleic acid into a conjugated *trans* intermediate and then to elaidic acid *in vitro*. Our findings, made incidental to studies of the effects of fatty acids on the energy metabolism of the sheep and on the fermentation in their rumens, support these observations (Czerkawski, Blaxter & Wainman, 1965).

After control periods lasting for at least 3 weeks in which no infusions were made, emulsions of fatty acids were infused continuously into the rumens of sheep for similar periods. Samples of rumen fluid were withdrawn at the end of the control and infusion periods. Total lipids were extracted

with chloroform-methanol according to the method of Folch, Lees & Sloane-Stanley (1957); they were saponified, and the isolated fatty acids esterified with dry methanol in the presence of HCl (Kates, 1964). The methyl esters were separated on the Pye Argon Chromatograph with Apiezon L (Edwards High Vacuum Ltd., Crawley, Sussex) or polyethylene glycol adipate as the stationary phase (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959). The amounts of various components were estimated from the areas under the peaks.

The *cis*- C_{18} monoethenoic acid found in the rumen was identified as elaidic acid in the following way. It had exactly the same retention volume as oleic acid on the polyethylene glycol adipate column but emerged between oleic acid and stearic acid on the Apiezon column (retention volume 0.890 compared with 0.855 for oleic acid, assuming that stearic acid has a retention volume of 1.0). Its peak was eliminated by bromination; hydrogenation also led to its disappearance with a concomitant stoichiometric increase in the amount of stearic acid. The compound migrated between stearic acid and oleic acid on thin-layer plates of silicic acid impregnated with $AgNO_3$, and had the same R_F value as the reference sample of elaidic acid (Morris, 1964). Consequently in many instances, particularly when the proportions of oleic acid and elaidic acid were such as to give poor

resolution of these two compounds on gas-liquid chromatography, the samples were separated and estimated on thin-layer plates. The spots were eluted and estimated colorimetrically as esters, or re-run on the gas-liquid chromatograph.

The oleic acid and linoleic acid used for infusion were commercial products (Hopkin and Williams Ltd., Chadwell Heath, Essex) containing at least 80% of the principal component. The linolenic acid preparation (Price's, Bromborough, Cheshire) contained about 70% of the trienoic acid. The linseed-oil acids (marketed by British Drug Houses Ltd., Poole, Dorset, as 'linoleic acid, so called') contained nearly 60% of linolenic acid. Neither the basal diet of dried grass nor the fatty acids infused contained any measurable amounts of elaidic acid.

The results of the effect of infusion of the various acids on the fatty acid concentrations in the rumen fluid are summarized in Table 1. They show that, under normal circumstances when no fatty acids were infused and the only acids entering the rumen were derived from dried grass, the predominant monoenoic acid was in the *trans* form. The relative amounts of the major fatty acids in grass lipids change during storage, but the mean composition (palmitic acid, 15%; oleic acid, 4%; linoleic acid, 13%; linolenic acid, 56%) is similar to that of linseed-oil acids (palmitic acid, 6%; oleic acid, 16%; linoleic acid, 14%; linolenic acid, 60%). The infusion of a small amount of oleic acid, which introduced several times as much fatty acid as

was derived from grass, led to an accumulation of the *cis* form, together with somewhat smaller amounts of the *trans* form. There was also an increase in stearic acid, which indicates that hydrogenation to full saturation continued. On subsequent infusion of larger amounts of oleic acid (60 g./day) no accumulation of oleic acid was observed but a further increase in elaidic acid took place. Presumably this is an example of bacterial adaptation, so common in the rumen.

The infusion of linoleic acid resulted in an increase in all the C₁₈ acids (Table 1), the proportional increase being most pronounced in the di- and tri-enoic acids. In agreement with the results of Kepler *et al.* (1965), elaidic acid increased, but an even greater increase in the concentration of oleic acid occurred.

The infusion of linolenic acid gave an increase in all the C₁₈ acids, and the relative distribution was similar in both the control and the infusion periods. The presence of a small amount of an unidentified substance with a retention volume between those of oleic acid and elaidic acid was found and made precise interpretation difficult. When infused in moderate amounts the linseed-oil acids did not lead to marked changes in fatty acid distribution. When very large quantities were given, however, both oleic acid and elaidic acid accumulated and there was a decrease in stearic acid formation. It is significant that, in spite of the accumulation of monoenoic acids, there was no

Table 1. *Distribution of C₁₈ acids in the rumen fluid of sheep in control experiments and when unsaturated fatty acids and linseed-oil acids were infused into the rumen*

Details are given in the text. 'Trace' indicates less than 5% of monoenoic acids or less than 1% of total fatty acids.

Treatment	Nominal amount infused (g./24 hr.)	Concn. of C ₁₈ acids (mg./l. of rumen fluid)			
		Stearic acid	Oleic acid	Elaidic acid	Linoleic acid + linolenic acid
Oleic acid	None	171	Trace	75	20
	30	348	190	152	29
	60	952	Trace	161	Trace
	None	902	Trace	310	75
Linoleic acid	None	225	Trace	85	37
	30	359	34	107	67
	60	551	163	198	455
	None	296	Trace	108	35
Linolenic acid	None	237	30	88	30
	30	256	37	109	34
	60	583	75	104	102
	None	382	Trace	148	33
Linseed-oil acids	None	508	Trace	179	58
	44	930	145	312	195
	128	182	250	1175	135
	None	435	52	250	28

pronounced change in the ratio of the *trans* to the *cis* forms, with the concentration of the former always being several times greater than that of the latter.

There was little evidence of double-bond migration under normal conditions. The conjugated linoleic acid and linolenic acid have retention volumes greater than those of the normal compounds and could be separated by gas-liquid chromatography (Daniels & Richmond, 1960). No conjugated linoleic acid and linolenic acid could be detected either during control periods or during periods in which oleic acid was infused.

Conjugates accounted for 5–10% of the polyenoic acids during infusion of small amounts of non-conjugated linoleic acid and linolenic acid and 15–25% during infusion of larger amounts. The conjugated polyenoic acids are hydrogenated with difficulty (Garton, 1959) and would be expected to accumulate to a much greater extent than the non-conjugated acids.

The above results offer no support for the hypothesis that the process of hydrogenation involves appreciable bond migration and accumulation of conjugated acids (Shorland *et al.* 1957; Kepler *et al.* 1965), but they are consistent with the

hypothesis that elaidic acid, and not oleic acid, is an intermediate in the hydrogenation of the polyenoic acids in the rumen.

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- Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W. (1965). *Proc. Nutr. Soc.* **24**, (in the Press).
Daniels, N. W. R. & Richmond, J. W. (1960). *Nature, Lond.*, **187**, 55.
Farquhar, J. W., Insull, W., Rosen, P., Stoffel, W. & Ahrens, E. H. (1959). *Nutr. Rev.* **17**, 30.
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Garton, G. A. (1959). *Proc. Nutr. Soc.* **18**, 112.
Garton, G. A., Lough, A. K. & Vioque, E. (1961). *J. gen. Microbiol.* **25**, 215.
Kates, M. (1964). *J. Lipid Res.* **5**, 132.
Kepler, C. R., Hiron, K. P., McNeill, J. J. & Tove, S. B. (1965). *Fed. Proc.* **24**, 290.
Morris, L. J. (1964). *Lab. Practice*, **13**, 284.
Reiser, R. & Reddy, R. H. G. (1956). *J. Amer. Oil Chem. Soc.* **33**, 155.
Shorland, F. B., Weenink, R. O., Johns, A. T. & McDonald, I. R. C. (1957). *Biochem. J.* **67**, 328.
Ward, P. F. V., Scott, T. W. & Dawson, R. M. C. (1964). *Biochem. J.* **92**, 60.

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The Cytochemical Localization of Alkaline Phosphatase in *Escherichia coli* at the Electron-Microscope Level

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Malamy & Horecker (1961, 1964a,b) discovered that alkaline phosphatase (EC 3.1.3.1) of high specific activity is obtained in quantitative yield when *Escherichia coli* cells containing the enzyme are converted into spheroplasts by the action of EDTA and lysosyme. Their observations showed that the enzyme, which is synthesized after the release of an inorganic phosphate repressor mechanism, assumes a remarkably well-defined localization. In intact cells this enzyme behaves as though it were contained in a compartment separated from the external medium and not in complete equilibrium with it. Malamy & Horecker (1964a) suggested that the alkaline phosphatase lies in a compartment between the cell wall and

cell membrane. In this communication we report the demonstration by an electron-microscope technique that the enzyme is located in an intermediate layer of the cell wall, between the outer membrane and the plasma membrane.

Cells of *E. coli* strain W 3747, kindly supplied by Dr Frank Rothman, were cultured in the medium described by Levinthal, Signer & Fetherolf (1962). Here, de-repression occurs after an initial period of growth that is limited by the small amount of phosphate present. KH_2PO_4 (3.2 mm) was added to a 'control' culture to repress enzyme synthesis.

After 18 hr. the cultures were centrifuged at 8000g (10000 rev./min. in an International centrifuge). We used a modification of the method of