FORMATION OF METHYL ESTERS BY RAT MUCOSA IN METHANOL

M. YURKOWSKI and B. L. WALKER
Department of Nutrition, University of Guelph, Guelph, Ontario, Canada

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Methyl esters have been identified in the lipid extracts from several tissues [1–4]. While evidence has been presented for their occurrence as natural constituents of the tissue in question [1,2] in many cases they are believed to be artefacts arising during extraction and isolation of the lipids [3,4]. In a recent study on the lipids of rat intestinal mucosa [5], a fraction isolated from the chloroform–methanol extract of this tissue was identified as fatty acid methyl esters on the basis of its behaviour on thin-layer and gas-liquid chromatography. Further investigation revealed that these compounds were probably produced by enzymic esterification of free fatty acids during an initial extraction of the mucosa with absolute methanol.

In the original study, mucosal samples were collected from 15 rats and added to ice cold absolute methanol. Consequently, the tissue taken from the rats processed first was in contact with the methanol for a considerable period of time (approx. 1½ hours). In order to determine whether the methyl esters were formed during this initial extraction period, mucosae from individual rats were subjected to different extraction procedures. One sample was extracted for 60 min in 10 ml of absolute methanol at 0°, and for a further 30 min after the addition of 20 ml of chloroform. A second sample was extracted directly with 30 ml of chloroform–methanol (2:1 v/v) for 90 min at 0°, and a third sample was stored in a dry ice bath for 60 min prior to extraction with chloroform–methanol (2:1 v/v). After extraction and isolation, 200 μl aliquots of the lipids were chromatographed on thin layers of silica gel G, using light petroleum–ether–acetic acid (90:10:1) as the developing solvent. Only in the case of the sample extracted in cold methanol for 60 min were methyl esters detected. Increasing the period of incubation in methanol prior to the addition of chloroform increased the yield of methyl esters (estimated from the relative areas of the peaks obtained by densitometry of the thin-layer plate). Homogenizing the mucosa in methanol, using a high speed blender, also increased ester production.

Mucosae were isolated from the intestine of a single animal and suspended in 10 ml of physiological saline at 0°. The suspension was divided into equal portions and one part was heated in a boiling water bath for 15 min while the other was kept at 0°. After centrifuging and removing the supernatant, the mucosae were extracted with ice-cold methanol for 60 min, chloroform was then added and the lipids isolated after an additional 30 min extraction. Methyl esters were detected in mucosa incubated at 0° prior to extraction, but not in the heated sample, thus indicating that a heat-labile agent in the mucosa was responsible for the formation of esters in methanol. There was an increase in the free fatty acid content of the lipid extracts when the mucosae were stored in saline before extraction with chloroform–methanol, indicating that lipolysis was quite active in the saline suspension.

Substitution of ethanol, propanol or butanol for methanol in the extraction procedure resulted in the formation of the corresponding fatty acid esters, although propanol appeared to be less readily esterified than the other alcohols. Endogenous free fatty acids appeared to be the source of the methyl esters produced, since the fatty acid composition of the methyl esters isolated from mucosal lipids was very

similar to that of the free fatty acid fraction of the mucosa. Moreover, the addition of an excess (100 mg) of linseed oil free fatty acids to the methanolic mucosal suspension resulted in the formation of esters containing appreciable amounts of linolenic acid. When linseed oil triglycerides were added to the mucosal suspension, linolenic acid was incorporated to a lesser degree. Addition of ethanolamine or choline glycerophosphatides containing characteristic polyunsaturated fatty acids failed to result in the incorporation of these acids into the methyl ester fraction isolated from mucosa.

Preliminary experiments indicate that ester formation is mediated primarily by the supernatant fraction (isolated at 105,000 *g*) of the mucosal cells, although some activity was observed in the nuclear, mitochondrial and microsomal cell fractions. In all cases exogenous free fatty acid was added as substrate.

Newsome and Rattray [6] reported on the enzymic esterification of fatty acids and ethanol by pancreatin. The alcohol was present in relatively low concentration (10%). In the present experiment, the tissue was added to absolute methanol. Rat intestinal mucosa thus contains an enzyme capable of esterifying endogenous (or exogenous) free fatty acids in concentrated methanol solutions at 0°. Consideration should be given to this fact when choosing extraction procedures for this tissue, and contact of the mucosa with alcohols in the absence of other solvents should be avoided.

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