FORMATION OF LONG-CHAIN ALCOHOLS IN THE GASTRO-INTESTINAL TRACT OF THE RAT

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1. Introduction

Some thirty years ago, it was shown that both oxidation of hexadecyl alcohol to palmitic acid, and reduction of palmitic acid to hexadecyl alcohol occur in the intestine of the rat [1]. Long-chain alcohols have been found in human intestine [2] as well as in animal faeces [2] and in human stools [2–5]; however, their composition has not been determined.

We have recently suggested that, in the rat, saturated and mono-unsaturated, but not polyunsaturated fatty acids, or their esters, are reduced to alcohols [6]. This assumption was based on the observation that the alkyl and alk-1-enyl moieties in the alkoxy-lipids of tissues from animals fed a 'normal diet' were exclusively saturated and mono-unsaturated, whereas in the tissue lipids of animals fed a di-unsaturated long-chain alcohol, di-unsaturated alkoxy groups could be found [6].

The present communication provides more direct evidence for the reduction to alcohols of both saturated and mono-unsaturated fatty acids or esters, in the gastro-intestinal tract of the rat. Moreover, additional information is presented to support the assumption that di-unsaturated and tri-unsaturated acids or esters are either not at all, or not as readily reduced.

Rats were fed a normal diet, 'Purina Laboratory Chow'. The constituent fatty acids of the lipids found in this diet included over 30% linoleic and over 6% linolenic acids, i.e., almost 40% polyunsaturated fatty acids. Long-chain alcohols were isolated from the small intestine and the faeces of the animals. They were found to consist exclusively of saturated and mono-unsaturated compounds having the same chain lengths as the constituent fatty acids of the dietary lipids. Fractions which might represent polyunsaturated alcohols were detected only in traces.

Small amounts of wax esters were found in lipid extracts of both the small intestine and the faeces; in addition, methyl esters were detected in the faeces.

2. Materials and methods

2.1. *Animals and their diet*

Six 4 to 5 week old rats of the Sprague-Dawley strain were fed for 14 days, ad libitum, a diet consisting of 'Purina Laboratory Chow', and water.

2.2. *Lipids*

Model mixtures of various lipid classes and reference mixtures of methyl esters were purchased from The Hormel Institute Lipids Preparation Laboratory. The methyl esters were converted to alkyl acetates by reaction with lithium aluminium hydride followed by

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acetylating decomposition of the lithium alumino complexes with acetic anhydride [7].

2.3. *Lipid analysis*

The lipids contained in the rats' diet were extracted with chloroform–methanol (2:1, v/v) and purified following established procedures [8]. The total lipids were reacted with methanol–sulfuric acid to yield methyl esters of fatty acids [9]. The methyl esters were purified by thin-layer chromatography on Silica Gel G with the solvent hexane–diethyl ether (90:10, v/v) and recovered by extraction with water-saturated diethyl ether. After evaporation of the solvent, the methyl esters were analyzed by gas chromatography, in a Beckman GC-2A instrument having a flame ionization detector. The instrument was equipped with a column, 6 ft × 1/8 inch I.D., packed with 20% diethyleneglycol succinate (DEGS) on Anakrom A, 100/110 mesh, the column temperature was 175°, helium served as the carrier gas at a pressure of 25 psi.

The faeces of the animals were collected on the 12th, 13th and 14th day. On the 15th day, the rats were sacrificed, the small intestine was excised and rinsed with saline solution, and the lipids of intestine and faeces were each extracted and purified [8].

The lipid extracts of diet, small intestine, and faeces were fractionated into classes of compounds by thin-layer chromatography. Silica Gel G served as absorbent and hexane–diethyl ether–acetic acid (60:40:1 v/v/v) as developing solvent; lipid fractions were detected in U.V. light (260 nm) after spraying the layers with a 0.2% solution of 2′, 7′-dichlorofluoresein in ethanol [10]. The fractions of long-chain alcohols which were detected on thin-layer chromatograms of lipid extracts of intestine and faeces, were eluted with water-saturated diethyl ether. The solvent was evaporated and the alcohols were acetylated. The resulting alkyl acetates were purified by thin layer chromatography on Silica Gel G using hexane–diethyl ether (95:5, v/v) as developing solvent, and analyzed by gas chromatography at 170°, on a column, 6 ft × 1/8 inch I.D., filled with 20% diethyleneglycol succinate on Anakrom A, 80/100 mesh.

3. Results and discussion

The lipids present in Purina Laboratory Chow were found to consist of triglycerides and fatty acids; long-chain alcohols could not be detected. The fatty acid composition of the total lipids from this diet is given in fig. 1.

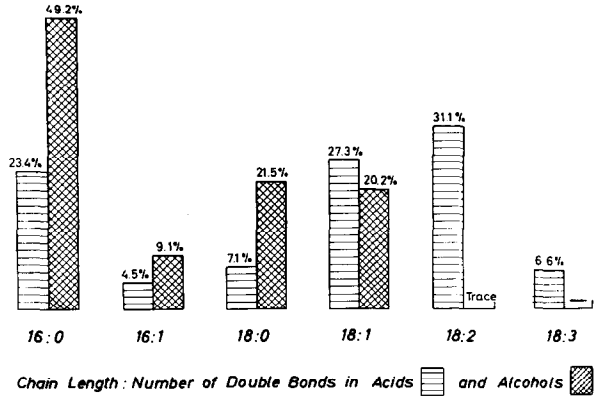

Fig. 1. A histogram facilitating comparison of the fatty acid composition of dietary lipids with the composition of the long-chain alcohols found in the faeces of rats that ate those lipids.

Long chain alcohols were detected in the lipid extracts of small intestine and of faeces; they were exclusively saturated and mono-unsaturated. The composition of the alcohols isolated from faeces is given in fig. 1.

This histogram facilitates a comparison of the composition of the fatty acids in lipids of the diet, with that of the long-chain alcohols in the faeces of rats which had lived on this diet. It is not clear whether the alcohols were formed from dietary or endogenous fatty acids, or both. However, it is obvious that the various fatty acids or their esters, were reduced at different rates: the relative concentrations of hexadecyl and octadecyl alcohol in the faeces are more than double those of the corresponding fatty acids in the diet. Similarly, the proportion of hexadecenyl alcohol is twice as high as that of palmitoleic (16:1) acid; however, that of octadecenyl alcohol is less than that of oleic (18:1) acid. The polyunsaturated alcohols corresponding to linoleic (18:2) and linolenic (18:3) acids occur as a trace constituent or not at all.

Obviously, the enzyme or enzymes catalyzing the formation of alcohol from fatty acids or esters are more active with substrates having saturated chains of 16 and 18 carbon atoms, than with the correspon-

ding mono-unsaturated compounds, and they do not act readily upon polyunsaturated compounds.

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