Preliminary Notes

PN 1252

Characterization of hydroxy acids in depot fat after feeding of ricinoleic acid

The increase in hydroxy value of rat epididymal adipose tissue after administration of ricinoleic acid has been reported[1-3]. However, the deposited hydroxy acid has not yet been characterized, although it has generally been assumed to be ricinoleic acid.

Recently we have shown[4] that some strains of *Escherichia coli* convert ricinoleic acid to 10-hydroxyhexadec-7-enoic, 8-hydroxytetradec-5-enoic and 6-hydroxydodec-3-enoic acids. The latter two acids were excreted as final products and accumulated in the culture medium in a good yield. Furthermore, evidence was obtained[5] showing that a certain fungus produced several intermediate hydroxy acids from ricinoleic acid.

For these reasons, the occurrence of hydroxy acids other than ricinoleic acid in animal tissue was to be expected. The present study was undertaken to characterize the hydroxy acids in adipose tissue of the rat after feeding of ricinoleic acid.

Adult male rats were given daily 1 g of emulsified ricinoleic acid (average diameter of droplet was 2 μ) for 4 weeks. Lipids were extracted from fat tissue followed by hydrolysis to yield fatty acid mixture.

The mixture was separated by chromatography on silicic acid. Less polar fatty acids were removed by hexane–ether (9:1). Hydroxy acids were eluted by hexane–ether (7:3). A thin-layer chromatogram of each fraction indicated the effective separation of hydroxy acids from other fatty acids (Fig. 1). A gas–liquid chromatogram of the latter fraction methylated with diazomethane is shown in Fig. 2.

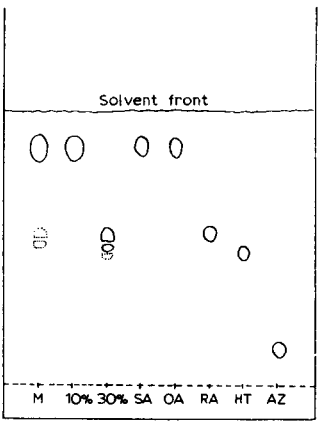

Fig. 1. Thin-layer silica-gel chromatogram of fatty acids. Solvent was hexane–ethyl acetate–acetic acid (70:30:1)[4]. M, fatty acid mixture prepared from rat adipose tissue after feeding of ricinoleic acid; 10%, 10% ether in hexane fraction fractionated by silicic acid column; 30%, 30% ether in hexane fraction. Abbreviations: SA, stearic acid; OA, oleic acid; RA, ricinoleic acid; HT, 8-hydroxytetradecenoic acid[4]; AZ, azelaic acid. Indicator: 2′,7′-dichlorofluorescein.

The identification of each component was performed by comparing the retention time with those of metabolites of ricinoleic acid which were obtained in previous work[4].

Table I indicates the occurrence of appreciable amounts of hydroxy acids shorter than ricinoleic acid. The accumulation of these hydroxy acids in adipose tissue has not previously been reported. This may be due to the difficulty in the separation of these acids from the normally occurring fatty acids, such as linoleic or arachidonic acids, by means of gas–liquid chromatography (*cf.* Fig. 2).

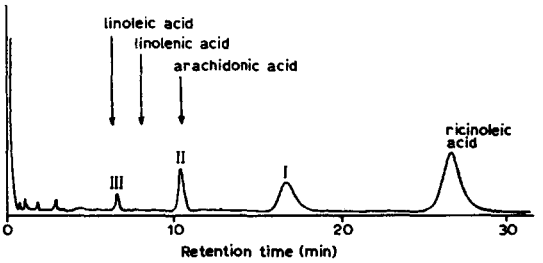

Fig. 2. Gas–liquid chromatogram of methyl esters of hydroxy acids separated by silicic acid column chromatography. Rats were fed 1 g of ricinoleic acid for 4 weeks. Fatty acid mixture was obtained from adipose tissue. Hydroxy acids were eluted with hexane–ether (7:3). The retention times of known methyl esters are indicated. Column conditions: 4 mm × 3 m column; 20% diethyleneglycol succinate polyester on 100 mesh Celite-545; operated at 200°; flow rate, 60 ml of He per min. Peak I, 10-hydroxyhexadecenoic acid; Peak II, 8-hydroxytetradecenoic acid; Peak III, 6-hydroxydodecenoic acid.

TABLE I

ACCUMULATION OF HYDROXY ACIDS IN RAT FAT TISSUE AFTER FEEDING OF RICINOLEIC ACID

Daily dose of ricinoleic acid was 20 ml (5% emulsion). 5 g of adipose tissue were used for fatty acid analysis. Expt. 1: administration period, 7 days; total fatty acids, 3.444 g. Expt. 2: administration period, 27 days; total fatty acids, 3.055 g.

Hydroxy acids	*Expt. 1*		*Expt. 2*	
	Weight (mg)	*% of total fatty acids*	*Weight (mg)*	*% of total fatty acids*
Ricinoleic	17.6	0.51	117.0	3.85
10-Hydroxyhexadecenoic	20.6	0.60	10.1	0.33
8-Hydroxytetradecenoic	1.0	0.03	2.5	0.08
6-Hydroxydodecenoic	—	—	0.8	0.03
Unidentified	—	—	4.2	0.14
Total	39.4	1.14	134.5	4.25

The formation of these acids can be accounted for by degradation of ricinoleic acid in the tissues or in the intestinal tract. WATSON AND GORDON[3] described that ricinoleic acid deposited in adipose tissue disappeared rapidly in a manner which suggested metabolic degradation rather than excretion after withdrawal of ricinoleic acid from diet. So it is likely that the intermediate hydroxy acids were produced by intestinal bacteria. Oxidation of ricinoleic acid with hepatic enzymes is now under investigation.

Institute of Pharmacy, Tohoku University School of Medicine, Sendai (Japan)

MITSURU UCHIYAMA
REIKO SATO
MICHINAO MIZUGAKI

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PN 1251

Intermediates of the oxidative breakdown of ricinoleic acid by Candida genus

In our previous paper[1] it was reported that a certain soil bacterium and some strains of ***Escherichia coli*** converted ricinoleic acid to three specific metabolites, which were chemically characterized as 10-hydroxyhexadec-*cis*-7-enoic (I), 8-hydroxytetradec-*cis*-5-enoic (II), and 6-hydroxydodec-*cis*-3-enoic (III) acids. The latter two acids were excreted into the culture medium and accumulated as final products. These hydroxy acids were also isolated from adipose tissue of rats after feeding of ricinoleic acid[2].

In the present communication evidence was found that Candida metabolizes ricinoleic acid in a special manner. It is converted to intermediate hydroxy acids, which finally disappear from the culture fluid. Under appropriate culture conditions these intermediates could be isolated from the medium.

All strains of Candida examined, such as *Candida albicans*, *C. parakrusei*, *C. guilliermondi*, *C. stellatoidea*, *C. tropicalis*, *C. pseudotropicalis*, and *C. krusei* which were grown in the medium containing 2 % meat extract and 0.3 % ricinoleic acid, were able to oxidize ricinoleic acid. The metabolites did not accumulated as the final products, but the amount of each intermediate varied with the duration of cultivation as illustrated in Fig. 1.

Fig. 2 shows a gas–liquid chromatogram of hydroxy acids which were extracted

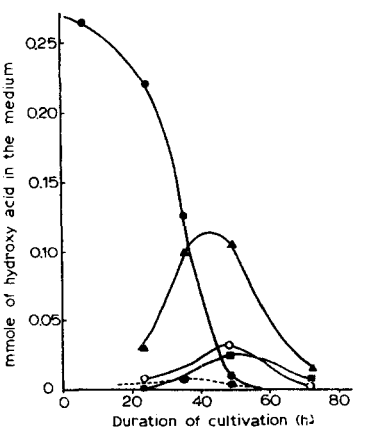

Fig. 1. Amounts of hydroxy acids isolated from the culture medium of *Candida guilliermondi*. ●—●, ricinoleic acid; ▲—▲, decanoic acid-γ-lactone; O—O, 6-hydroxydodecenoic acid; ■—■, 2-hydroxyoctanoic acid; ●---●, 8-hydroxytetradecenoic acid.