METABOLISM OF 4-HYDROXY FATTY ACIDS BY RAT LIVER SLICES*

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4-Hydroxybutyrate or its lactone (γ-butyrolactone) exhibits anaesthetic properties when intravenously injected into rats.[1,2] Furthermore it has been shown that the depressive action of 1,4-butanediol on the central nervous system is mediated through its metabolite, 4-hydroxybutyrate.[3] The metabolism of labelled 4-hydroxybutyric acid has previously been studied by Walkenstein *et al.*[4] and Roth and Giarman,[5] who reported that it is converted to labelled carbon dioxide by the rat. However, no oxidation of 4-hydroxybutyrate to succinate could be demonstrated. Walkenstein *et al.*[4] suggested that direct β-oxidation of 4-hydroxybutyrate occurs.

In the present study direct evidence of an important alternative pathway in the metabolic degradation of 4-hydroxy fatty acids is presented.

MATERIAL AND METHODS

The sodium salts of 4-hydroxybutyric acid, 4-hydroxy-n-valeric acid, 4-hydroxy-isocaproic acid and 4-hydroxypent-2-enoic acid were prepared by adjusting the pH of an aqueous solution of the corresponding γ-lactones to a pH>13. These solutions were left overnight and adjusted to pH 7·4 just before use.

Male albino rats of the Wistar strain (250 G) were killed by decapitation, and slices were prepared from the livers as described by Umbreit *et al.*[6] Slices (200 mg.) were incubated in Krebs-Ringer phosphate buffer solution[7] (5 ml., pH 7·4), containing glucose (20 mM) and the sodium salts of the 4-hydroxy acids (200 μg.). Incubations were carried out in oxygen at 37°C for 30 min. Sodium hydroxide (0·5 ml., 1·0 N) was added and the mixtures were heated at 90°C for 20 minutes to hydrolyse any formed esters. These solutions were then centrifuged and extracted with ether. The ethereal solutions were discarded and the aqueous layers acidified by the addition of 1 N hydrochloric acid. It was then finally extracted with 3 portions of 20 ml. ether and the extracts were dried on sodium sulphate. Controls, in which the substrates were omitted, were prepared in the same way.

After evaporation of the solvent the residues were either dissolved in a little chloroform and chromatographed on a thin-layer chromatoplate of silica gel G, using light petroleum-diethyl ether (9 : 1 v/v) as mobile phase and spraying with 0·4% bromo-cresol green indica-

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tor reagent, or esterified for gas-liquid chromatographical analyses. For this purpose the residues were heated under reflux in a boiling waterbath for 20 minutes with 10 ml. 5% perchloric acid in methanol. The mixtures were then extracted with three 10-ml. portions of a mixture of n-hexane-diethyl ether (1 : 1 v/v) and the ethereal layers subsequently washed with sodium carbonate solution and dried on anhydrous sodium sulphate. Chlorobenzene (20 μg.) in ether was added as internal standard. These extracts were concentrated at 40°C and submitted to gas-liquid chromatography, which was carried out in a Pye Series 105 Model 15 chromatograph fitted with a glass column (180 cm. long, 4·0 mm. internal diameter) packed with silanized acid-washed Chromosorb W (60 - 80 mesh) coated with 10% FFAP (Varian Aerograph, Calif.). The carrier gas flow (nitrogen) was 60 ml./min. and the operating temperature 100°C.

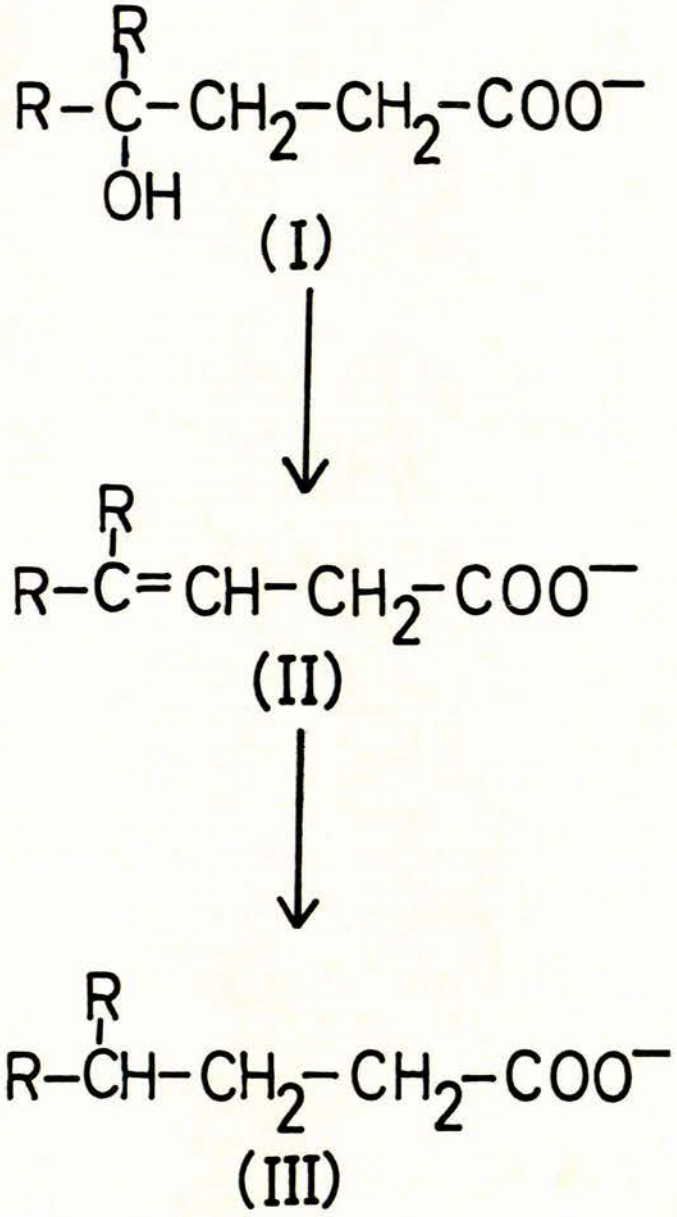

Fig. 1. Postulated alternative pathway in the metabolic degradation of 4-hydroxy fatty acids.

RESULTS AND DISCUSSION

The formed fatty acids were identified by comparison of the retention times and R_F values of these compounds with those of authentic samples as well as by co-chromatography (Table I). The quantitative determination of the fatty acids has limited value, since liver utilizes n-butyric acid and n-valeric acid fairly easily.[8] It was, however, estimated that the fatty acids detected in the mixtures amount to approximately 10% of the initial concentrations of the 4-hydroxy fatty acids.

TABLE I. RETENTION TIMES AND R_F VALUES OF FATTY ACIDS FORMED FROM CORRESPONDING 4-HYDROXY FATTY ACIDS BY RAT LIVER SLICES

4-hydroxy fatty acid	*Fatty acid detected*	*Peak retention time of methyl esters (min.)*	R_F
4-hydroxy-n-butyrate	n-butyric acid	3·5	0·43
4-hydroxy-n-valerate	n-valeric acid	6·3	0·54
4-hydroxy-isocaproate	Isocaproic acid	10·1	0·61
4-hydroxy-pent-2-enoate	None	—	—

In the controls no low-molecular-weight fatty acids (up to C6) were detected. When the sodium salt of 4-hydroxy-pent-2-enoic acid was used as substrate no metabolite was detected by either thin-layer or gas-liquid chromatography. This suggests that this metabolic reaction of the 4-hydroxy fatty acids (I) probably proceeds through successive dehydration to a 3,4-unsaturated acid (II) followed by reduction of the resulting double bond to yield a saturated fatty acid (III) (Fig. 1). It is reasonable to expect that such a pathway would be blocked by the introduction of a double bond in the 2 : 3 position of the hydroxy acid.

Furthermore, such a pathway could account for the formation of 4-(3′-pyridyl)-butyric acid and 3-pyridyl-acetic acid in the further metabolism of the nicotine metabolite, 4-(3′-pyridyl)-4-oxobutyric acid by the dog and rat.[9]

SUMMARY

The metabolic degradation of 4-hydroxy fatty acids, including 4-hydroxybutyric acid, a compound with anaesthetic properties, was studied. By incubating the sodium salts of 4-hydroxybutyric acid, 4-hydroxy-n-valeric acid and 4-hydroxy-isocaproic acid with liver slices it was shown by means of thin-layer chromatography and gas-liquid chromatography that the corresponding fatty acids (butyric, valeric and isocaproic acid) were formed.

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