Short Communications

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The esterification of cholesterol by rat-liver preparations*

Several reports have been published concerning an enzyme present in mammalian liver which catalyzes the conversion of cholesterol to cholesterol ester[1-6]. Recently, two groups of workers have reported that the enzymic conversion of cholesterol to cholesterol ester in liver requires ATP and CoA or, alternatively, that the esterifying fatty acid be present as its thioester with CoA[7,8].

CLOMSET *et al.*[9,10], studying the esterification of cholesterol *in vitro* in human and rat plasma, concluded that, in the absence of ATP and CoA, ^{14}C-labelled free fatty acid present in the medium was not incorporated into cholesterol esters. However, if lecithins or triglycerides containing ^{14}C-labelled fatty acids were present in the reaction medium, these labelled fatty acids were transferred to cholesterol to form cholesterol esters.

It was of interest to determine whether a similar fatty acid-transferring enzyme was also present in rat liver. Attempts were therefore made to demonstrate a transfer of ^{14}C-labelled linoleic acid from the β-position of lecithins to cholesterol, using a mitochondrial and microsomal preparation of rat liver.

Livers were excised from male Sprague–Dawley rats and 10 g was homogenized with 40 ml of 0.44 M sucrose plus 0.0018 M citric acid. The homogenate was diluted to 100 ml with 0.44 M sucrose and was lightly centrifuged at 700 × *g* for 20 min.

TABLE I

ESTERIFICATION OF CHOLESTEROL

Reaction mixtures 1 and 3: 500 μmoles ATP disodium salt tetrahydrate, 5 μmoles CoA, 5 μmoles [1-^{14}C]linoleic acid, 2 μmoles cholesterol (added in 1.0 ml of acetone), 2 mmoles potassium phosphate buffer (pH 7.4), 300 mg egg albumin, liver enzyme preparation equivalent to 5.0 g of fresh tissue; total volume 32 ml. *Reaction mixtures 2 and 4*: as 1 and 3 but ATP and CoA omitted. *Reaction mixture 5*: as 1 and 3 but contained 12 instead of 2 μmoles cholesterol, and 12 μmoles of β-[1-^{14}C]linoleyl lecithins instead of 5 μmoles [1-^{14}C]linoleic acid. *Reaction mixture 6*: 6.4 μmoles cholesterol, 6.4 μmoles [1-^{14}C]linoleic acid, 5 mg sodium taurocholate, 1 mmole potassium phosphate buffer (pH 6.0), liver enzyme preparation equivalent to 5.0 g of fresh tissue; total volume 20.0 ml. *Reaction mixture 7*: as 6 but contained 12 instead of 6.4 μmoles cholesterol, and 12 μmoles of β-[1-^{14}C]linoleyl lecithins instead of 6.4 μmoles of linoleic acid.

Expt. No.	*Reaction time (h)*	*ATP and CoA present*	*Radioactivity recovered (% of supplied)*	*Distribution of radioactivity (% of total recovered)*			
				Free fatty acids	*Lecithins*	*Triglycerides*	*Cholesterol esters*
1	1	+	63	67	12.2	12.6	8.2
2	1	—	78	100	—	—	—
3	1	+	67	65.2	18.3	8.1	8.4
4	1	—	88	100	—	—	—
5	1	+	91	—	100	—	—
6	18.5	—	83	100	—	—	—
7	18.5	—	92	—	100	—	—

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The supernatant was centrifuged at 144000 × *g* for 2.5 h and the sedimented fraction of mitochondria and microsomes was homogenized with 10 ml of 0.44 M sucrose. All operations were carried out at 5°.

The β-[1-^{14}C]linoleyl lecithins were prepared by the procedure of LANDS[11], and had a specific activity of 1005 counts/min/mg. The specific activity of the linoleic acid used was 1875 counts/min/mg.

After incubation at 37.5°, the mixtures at pH 7.4 were acidified with 1.5 ml of 1.0 N HCl (no HCl was added to mixtures at pH 6.0). The total lipids were then extracted by the method of BLIGH AND DYER[12]. Portions of the lipid extracts were chromatographed on silicic acid-impregnated papers with *n*-heptane–diisobutyl ketone (96:6, v/v) and with diisobutyl ketone–acetic acid–water (40:25:5, v/v)[13]. The chromatograms were stained with Rhodamine 6G and the distribution of radioactivity in the major lipid classes was determined by means of a 2π-chromatogram scanner. In order to determine the recovery of radioactivity, aliquots of the total lipid extracts were plated onto planchets and counted.

The results (Table I) of Expts. 1–4 support the conclusions of MUKHERJEE *et al.*[7] and DEYKIN AND GOODMAN[8], *i.e.*, that the esterification of cholesterol with free fatty acid appears to require ATP and CoA.

Expt. 5, in conjunction with Expts. 1–4, indicated that β-[1-^{14}C]linoleyl lecithins did not act as a source of linoleic acid for the esterification of cholesterol under conditions conducive to the formation of cholesterol esters from cholesterol and free fatty acid.

Expts. 6 and 7 indicated that even after prolonged incubation of the liver preparation, in the absence of ATP and CoA, with either cholesterol and [1-^{14}C]linoleic acid or cholesterol and β-[1-^{14}C]linoleyl lecithins, no labelled cholesterol esters were detected. While it is unlikely that spots containing less than 1 % of the total radioactivity applied to the chromatogram would be detected by the chromatogram scanner, the results clearly show that with a rat-liver microsomal and mitochondrial preparation, cholesteryl esters are preferentially formed from cholesterol and free fatty acid rather than cholesterol and the β-fatty acid of lecithin.

Division of Applied Biology, National Research Council, Ottawa (Canada) J. R. BENNETT

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