Effects of alkali-metal ions on phospholipid and triglyceride synthesis in rat liver slices

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SUMMARY The influence of alkali-metal ions on the incorporation of labeled precursors into phospholipids and triglycerides was studied in rat liver slices. By use of acetate-1-C14 and palmitate-1-C14 as lipid precursors, a comparison was made of the pathway from acetate to phospholipid and the pathway from palmitate to phospholipid. While the rate of incorporation of palmitate-1-C14 into phospholipids and triglyceride fatty acids was virtually independent of the alkali-metal ion present in the medium, the rate of incorporation of acetate-1-C14 into the same compounds was strongly influenced by the alkali-metal ions, the highest incorporations being obtained with potassium and rubidium. Under the same conditions lithium appears to have an inhibitory effect. The effects of the alkali-metal ions on phospholipid and triglyceride synthesis in liver are located on the pathway between acetate and long-chain fatty acids.

KEY WORDS fatty acid biosynthesis · phospholipid biosynthesis · triglyceride biosynthesis · rat · liver slices · alkali-metal ions

Some alkali-metal ions are known to affect lipid metabolism in several organs. Both lithium and potassium ions have been shown to influence fatty acid oxidation in liver slices (1, 2). Cholesterol synthesis, as studied by acetate incorporation in rat liver slices, also appears to be greatly depressed when lithium is present in the incubation medium (3). On the other hand, Curran and Clute (4) demonstrated that choledogenesis from acetate by rat liver is increased by the addition of potassium to the incubation medium and Ashmore, Weber, and Landau (5) found that the same ion stimulated fatty acid synthesis from glucose in liver slices. Nevertheless, Kline and DeLuca (6) were unable to obtain a consistent effect of potassium ion on the rate of incorporation of acetate into phospholipids. More recently, Minard and Davis (7) found a stimulatory effect of potassium on lipogenesis from acetate in rat liver slices and Yoshida and Nukada (8) showed that the incorporation of orthophosphate-P32 into the phospholipids of brain slices, but not of liver slices, was increased by potassium ion, provided that sodium was also present in the incubation medium.

In the present work the effects exerted by the group of elements known as alkali metals on phospholipid and triglyceride synthesis in rat liver are studied and a first attempt is made to localize their action in the scheme leading to phospholipid and triglyceride synthesis, by comparing their effects on the incorporations of acetate-1-C14 and palmitate-1-C14 into the phospholipids and glyceride fatty acids of rat liver slices.

It has been established that the alkali-metal ions affect the incorporation of acetate into long-chain fatty acids.

MATERIALS AND METHODS

Animals
Adult female rats of the hooded Norwegian strain aged about 5–6 months and weighing 180–200 g were fed ad lib. on diet 41 of Bruce and Parkes (9). The animals were killed by cervical dislocation and the livers were rapidly removed, rinsed, and placed in ice-cold isotonic sucrose.

Incubation Procedure
Slices approximately 0.4 mm thick were cut from the livers with a Stadie-Riggs (10) microtome and 500 mg
of them were suspended in 5 ml of a modified Krebs-Ringer phosphate buffer (11), pH 7.4, in which sodium and potassium chlorides had been replaced by the chloride of the alkali metal to be investigated. The solution contained either acetate-1-C\textsuperscript{14} or albumin-bound palmitate-1-C\textsuperscript{14} at a final concentration of 0.2 µc/ml. The acetate had a specific activity of 12.1 µc/mmole and the palmitate, 30.8 µc/mmole. Both substrates were obtained from the Radiochemical Centre, Amersham, U.K. No carrier was added. The palmitic acid-1-C\textsuperscript{14} was complexed with crystallized bovine albumin (British Drug Houses, Ltd., Poole, Dorset, U. K.) by the technique described by Glenn et al. (12).

The flasks were gassed with oxygen and were incubated at 37° with shaking. The time of incubation varied from 30 min to 3 hr.

Extraction and Purification of Lipids

At the end of the incubation period the slices were collected by centrifugation, washed twice with ice-cold 0.1 m sodium phosphate buffer, pH 7.0, and then suspended and homogenized in 7 ml of cold 10% trichloroacetic acid in an all-glass Potter-Elvehjem (13) homogenizer. The suspension was centrifuged and the residue reextracted with 7 ml of cold 5% trichloroacetic acid. The trichloroacetic acid extracts were rejected. The solid residue was extracted once with 5 ml of warm 80% ethanol, twice with 4-ml portions of absolute ethanol, and finally once with 4 ml of warm ether. The alcohol and ether extracts were combined, the solvents were removed by evaporation in vacuo, and the residue was extracted three times with 4-ml portions of light petroleum (bp 60–80°). From the combined extracts the phospholipids were isolated by precipitation as their magnesium complexes from acetone solution, purified by redissolving in 0.5 ml of chloroform and reprecipitating by the addition of acetone, and counted at infinite thinness in a windowless gas-flow counter, as described previously (14).

Saponification and Radioactivity Measurements

The nonphospholipids remaining in the acetone solution were saponified in 4 N aqueous potassium hydroxide for 3 hr at 80°. After extraction of the unsaponified fraction by light petroleum the aqueous layer was acidified with 5 N H\textsubscript{2}SO\textsubscript{4} and fatty acids were extracted with light petroleum. These fatty acids are derived mainly from triglycerides and are referred to as “triglyceride fatty acids” in this paper.

In the experiments on the incorporation of albumin-bound palmitate into triglyceride fatty acids, before the saponification procedure, the phospholipid-free extract was evaporated to dryness in vacuo, the residue was dissolved in petroleum ether (bp 60–80°) and shaken in a separating funnel with cold aqueous 0.02 N potassium hydroxide solution. This procedure was repeated twice and appeared to remove adequately any free palmitic acid, as tested in experiments where palmitic acid-1-C\textsuperscript{14} was added to unlabeled liver triglycerides and the triglyceride fraction tested for radioactivity. Samples of fatty acids were plated at infinite thinness on nickel planchets and weighed, and the radioactivity was measured in a 20th Century Electronics WF2 windowless gas-flow counter with a conventional scaler.

RESULTS AND DISCUSSION

The results showing the incorporation of carboxyl-labeled acetate into the liver phospholipids after various periods of incubation are given in Table 1. It may be seen that the rate of incorporation of the precursor used into the phospholipid molecule is strongly influenced by the alkali-metal ions, the highest activity being obtained when potassium is present in the incubation medium. Lithium, on the other hand, appears to have an inhibitory effect on phospholipid synthesis from acetate. It can also be seen that cesium resembles sodium closely in its influence on the rate of incorporation of the precursor used into the phospholipid fraction. Under the same conditions, rubidium appears to be slightly less

<table>
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<th>Rubidium</th>
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</table>

Conditions of incubation were as described in Table 1.
The main reactions in the synthesis of long-chain fatty acids from acetate are the activation of acetate to acetyl CoA, the carboxylation of acetyl CoA to malonyl CoA, and the reaction of acetyl CoA with 7 moles of malonyl CoA to form palmitate. From the data presented here, however, it is not possible to conclude at which of these steps the pathway of fatty acid synthesis is affected by the alkali-metal ions.

It must also be emphasized that the effect of the alkali-metal ions on lipogenesis may not be a direct one: the possibility exists that they can influence fatty acid synthesis indirectly by their action on carbohydrate metabolism. Potassium ion, for instance, is known to be involved in glucose metabolism in rat liver slices (16) and homogenates (17), and it has also been shown to affect glycogen synthesis in liver (18), which would tend to influence lipid metabolism.

It is also worthy of mention that a similar effect of the alkali-metal ions, on another synthetic pathway, has been reported. Walwick and Main have found (19) a marked effect of the alkali metals on nucleic acid synthesis in a rat thymus system, and the order of increasing effectiveness of the monovalent cations on DNA synthesis was the same as reported here for phospholipid and triglyceride synthesis from acetate in rat liver slices.

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REFERENCES