The Stimulation of Cholesterol Synthesis by Citrate and α-Ketoglutarate in High Concentrations

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It is well known that fatty acid synthesis is controlled by certain tricarboxylic acid cycle intermediates.[1] The regulation of cholesterol biosynthesis by these intermediates has also been investigated in some laboratories,[2-4] but the results are not consistent. Masoro and co-workers [2] incubated rat liver slices with 4—20 mM pyruvate, citrate, succinate or α-ketoglutarate, but they were unable to detect any effect on the conversion of acetate to cholesterol. Foster and Bloom [3] found a two- to threefold increase in fatty acid synthesis and a fall in cholesterol concentration of approximately the same magnitude when 0.1 mM citrate and liver slices were used, and a five- to sevenfold stimulation of fatty acid synthesis accompanied by a fivefold decrease in cholesterol concentration in a microsomal-supernatant system with a medium containing 0.2 mM citrate. Ichihara and co-workers [4] reported a stimulatory effect of citrate on cholesterol synthesis in dispersed rat liver cells, but they did not mention the concentration of citrate used.

In order to investigate the effect of some Krebs cycle intermediates on the synthesis of cholesterol, we incubated rat liver homogenates with ^{14}C-acetate in the presence of various acids and found that the synthesis was stimulated strongly by citrate in concentrations between 5 and 100 mM, but to a much smaller extent by α-ketoglutarate. Pyruvate, isocitrate, succinate, fumarate, and malate cannot replace citrate.

Livers of female albino rats weighing over 200 g were used. The animals were fed *ad libitum* on laboratory rat pellets. After removal and chilling, each liver was homogenized in a Potter-Elvehjem tissue grinder with 25 ml of a medium described by Bucher and McGarrahan.[5] The homogenate was centrifuged at 1500 *g* (0—4°C) for 10 min to remove tissue fragments.

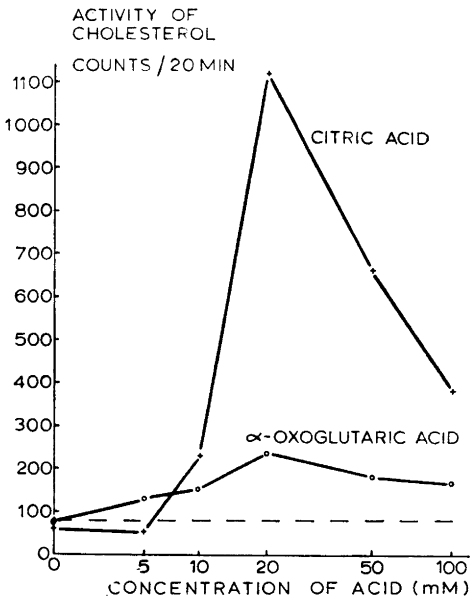

Fig. 1. The effect of citrate and α-ketoglutarate on the incorporation of 1-^{14}C-acetate into cholesterol in rat-liver homogenate.

2 ml of this homogenate were used with 12 mM potassium acetate, 0.8 mM NAD, 7.5 μC of sodium 1-^{14}C-acetate, and a concentration of the acids from 5 to 100 mM in a total volume of 2.875 ml (pH 7.4). The mixtures were incubated in wide-mouthed Erlenmeyer flasks with continual shaking for 2 h at 37°C. After the incubation, the lipids were extracted by the method of Nieminen.[6] The extract was evaporated to dryness, after addition of 50 μC of cholesterol as carrier, and saponified with 4 % potassium hydroxide in 66 % ethanol under nitrogen overnight at 60°C.[6] After extraction, the free cholesterol was isolated by thin-layer chromatography and localized by the method of Mangold.[7] Cholesterol was eluted from the adsorbent with a 2:1 chloroform-methanol mixture. The incorporated ^{14}C was counted in a low-beta counter with an efficiency of 4.4 %. The amount of cholesterol recovered was determined by adding *ca.* 50μC of ^{14}C-cholesterol to a liver homogenate and analysing as described. The standard deviation of the analytical procedure calculated from the results of 11 determinations was 11 %.

The data in Fig. 1 indicate that there was an about fourteenfold stimulation of cholesterol synthesis by 20 mM citrate and a fourfold stimulation by 20 mM α-ketoglutarate. Under the same conditions, pyruvate, isocitrate, succinate, fumarate, or malate had little effect on the cholesterol synthesis. On the activity level of the control experiments (*ca.* 80 counts/20 min) the incorporation of acetate into cholesterol was 5×10^{-4} %. This low activity may be related to the fact that our incubation medium contained neither ATP nor NADPH.

Some results of the earlier studies [2,3] are not consistent with our results, but the discrepancy may be due to the different material and to the considerably higher concentration of citrate we used. Our new and unexpected results suggest that the regulating action of citrate and α-ketoglutarate on cholesterol synthesis may be more complex also in physiological conditions.

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Comments on Some Contradictory Results of Polarimetric and NMR Studies of the Configurational Inversion of Several Biphenyl Derivatives

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There are apparently very few examples in the literature of suitable optically active molecules whose configurational inversion process has been studied by both polarimetric and NMR kinetic methods. Lüttringhaus and Rosenbaum [1] investigated 4,5-6,7-dibenzo-1,2-dithia-cyclooctadiene (I) by both methods, but were unable to obtain a rate constant with NMR since the large enthalpy of activation ($\Delta H^{\ddagger} = 26.6$ kcal/mole by polarimetry) made the molecule inappropriate for study on the NMR time scale.

Ollis and Sutherland [2] studied the temperature dependence of the NMR spectrum of tri-*o*-thymotide (II) and compared their results with those available from an earlier polarimetric investigation by Newman and Powell.[3] However, the paper by Ollis and Sutherland [2] is in the form of a preliminary communication, and it is stated that "...it has yet to be established that the exchange process and the racemization process involve the same conformational changes." [2]

Ōki and Iwamura [4] have recently reported a NMR kinetic study of the AB system provided by the $-CH_2-$ protons of the dioxepin III, using line width measurements to estimate the rate of exchange of proton environments both above and below the coalescence temperature. Their activation parameters are in agreement with those obtained polarimetrically (from kinetic data at two temperatures) by Mislow *et al.*[5]

Other data from the literature may be combined to yield some strikingly anomalous and contradictory conclusions about the optical stability of several biphenyl derivatives and related compounds. An example is afforded by a comparison of the results of Iffland and Siegel [6] (polari-