in the adduct. The identification of the peaks is somewhat tentative.)

Full experimental details of this work will be published later.

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Enhanced Synthesis of Myristic Acid by Rat Liver Homogenates after Addition of Citrate

HEINZ J. M. HANSEN and LIS G. HANSEN

The Danish Atomic Energy Commission Research Establishment Risö, Denmark and The Finsen Laboratory, København Ø, Denmark

The stimulating effect of citrate on lipogenesis was first shown by Brady and Gurin.[1] Later Brady, Mamoon and Stadtman [2] showed that this effect was due partly to citrate generating reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) and Martin and Vagelos [3] and Abraham, Lorch and Chaikoff [4] among others showed that citrate specifically stimulated the acetyl-coenzyme A carboxylase reaction. Many workers have since added citrate to their incubation mediums. Recently Lorch, Abraham and Chaikoff [5] have compared the synthetic patterns of long chain fatty acids formed from ^{14}C-acetate by rat liver slices with the corresponding patterns from synthesis by rat liver homogenates. They show that while synthesis by the slices results in 5 % myristic acid and 53 % palmitic acid, synthesis by citrate stimulated homogenates results in 25 % myristic acid and 33 % palmitic acid when acetate or acetyl-CoA are used as precursors. With malonyl-CoA as the precursor, synthesis by the citrate stimulated homogenates results in nearly the same fatty acid patterns as from the non-stimulated slices (7 % myristic acid and 51 % palmitic acid). These differences are not commented on in relation to the addition of citrate since the experiments include no homogenate incorporations without citrate stimulation. Earlier work by Porter and Tietz [6] with citrate stimulated pigeon liver homogenates and acetate as the precursor resulted in 23 % myristic acid and 64 % palmitic acid. Bhaduri and Srere [7] also base their work on synthesis from acetate by pigeon liver homogenates. They find 24 % myristic acid and 46 % palmitic acid independent of whether they add citrate or not. However, synthesis without citrate is so low that the results seem uncertain.

In our own work we have compared (a) synthesis from ^{14}C-acetate by rat liver slices with synthesis from acetate by nuclei-free reconstructed rat liver homogenates incubated (b) without added citrate as described by Bucher and McGarrahan,[8] (c) with citrate stimulation as described by Catravas and Anker,[9] and (d) with citrate substituted by glucose-6-phosphate and glucose-6-phosphate dehydrogenase in the Catravas and Anker medium. The results from the incorporations by liver slices have been taken from a previous investigation.[10] The total amounts of radioactivity and the relative distributions of these activities among the individual fatty acids were determined by paper chromatography as previously described,[10] except that the assay of the fatty acids from the homogenates in addition also included a more detailed analysis based on extraction and hydrogenation of single fatty acid spots. In a separate experiment we tested the ability of the added glucose-6-

Table 1. Fatty acids synthesized from ^{14}C-acetate by rat liver preparations. (Means ± S.E. of means).

Reaction system	Number of animals	Specific activity after incorp. (μC/g f.a.)	Percentage of incorporated ^{14}C in product fatty acids					
			Myristic	Palmitic	Stearic	Palmitoleic	Oleic	Unsaturated $>C_{18}$
Slices [a]	10	17.6 ± 6.0	4 ± 1	72 ± 4	4 ± 1	4 ± 2	8 ± 2	8 ± 1
Homogenates acc. to Bucher and McGarrahan [b]	6	2.4 ± 1.0	11 ± 3	43 ± 6	16 ± 4	6 ± 2	12 ± 3	11 ± 3
Homogenates acc. to Catravas and Anker [c]	4	14.0 ± 5.3	45 ± 3	25 ± 7	9 ± 2	4 ± 3	3 ± 1	11 ± 4
Homogenates as above with citrate substituted by glucosephosphate [d]	2	0.8 ± 0.5	3 ± 1	14 ± 5	32 ± 9	−1 ± 3	13 ± 0	34 ± 10

Incubation media: [a] Krebs-Ringer phosphate buffer with 2×10^{-7} M (2-^{14}C)-acetate (10 C/mole);
[b] 0.07 M K−PO_4, 0.002 M $MgCl_2$, 0.02 M nicotinamide, 0.2 M sucrose, 0.001 M NADH or NADPH or both, (in a single case 0.005 M ATP) and 7×10^{-8} M (1-^{14}C)-acetate (29.0 C/mole);
[c] 0.04 M K−PO_4, 0.01 M $MgCl_2$, 0.02 M nicotinamide, 0.1 M sucrose, 0.02 M citrate, 0.001 M NADH or NADPH or both, (in a single case 0.005 M ATP) and 7×10^{-8} M (1-^{14}C)-acetate (29.0 C/mole);
[d] 0.04 M K−PO_4, 0.005 M $MgCl_2$, 0.02 M nicotinamide, 0.1 M sucrose, 0.02 M glucose-6-phosphate, 2−20 units glucose-6-phosphate dehydrogenase, 0.001 M NADP, 0.001 M NADPH, 0.005 M ATP and 7×10^{-8} M (1-^{14}C)-acetate (29.0 C/mole).

phosphate dehydrogenase to generate NADPH. This was done by incubating with and without added enzyme under the same conditions as (d) and measuring partly the amount of NADPH formed during an incubation period, partly whether the enzyme could continue to form NADPH after the primary incubation. NADPH concentrations were determined by spectrophotometry at $\lambda = 338$ mμ.

Results are shown in Table 1. Apparently addition of citrate to the incubation medium not only stimulates total synthesis but also specifically enhances the synthesis of myristic acid. Compared with the results from synthesis without citrate stimulation (b), the percentage of total incorporated ^{14}C activity found in myristic acid is 34 % higher when liver homogenates are incubated together with citrate (c). This difference can be shown to be statistically significant at the level $p < 0.001$ when it is tested against a pooled standard deviation with $6 \times (5 + 3 + 1) = 54$ degrees of freedom. A comparison between the results from the medium (b) and the results from incubations in a medium (d) containing another NADPH generating system than the one based on citrate, shows no enhancement of myristic acid synthesis. In the latter case the relative percentage of ^{14}C activity found in myristic acid rather resembles the small values seen after synthesis by the liver slices. When the shown percentages do not add up to 100 %, the rest consisted mainly of a saturated C_{15} acid together with small amounts of a double-unsaturated C_{18} acid.

The observed citrate effect should be viewed in relation to the general problem of what makes the *de novo* synthesis of saturated fatty acids normally stop at the palmitic stage. A closer examination of the mechanism of citrate stimulation may help to clarify this matter. In view of the findings by Lorch, Abraham and Chaikoff [5] it could seem at present as if malonyl-CoA generated by citrate, in contrast to artificially synthesized malonyl-^{14}C-CoA, had difficulties in elongating myristic acid to palmitic acid. Our results show that the relative myristic enhancement is not due to stimulation by NADPH.

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A New Sennoside from *Cassia* Species

PENTTI K. HIETALA and ANERI PENTTILÄ

The Research Laboratories, Medica Ltd, Helsinki, Finland

The extensive studies by Stoll *et al.*[1] on anthrone glycosides of *Senna* species resulted in the isolation and characterization of two isomeric sennosides, the optically active (+),(+)-bis-rheinanthrone-8-glycoside or sennoside A and the intramolecularly compensated mesoform (+),(−)-bis-rheinanthrone-8-glycoside or sennoside B. We have now isolated a new sennoside from *Cassia* species. The name sennoside C has already been given to another compound.[2,3] Therefore, the name sennoside III is proposed for the new substance.

For the isolation of sennoside III finely ground leaves of *Cassia acutifolia* or *Cassia angustifolia* are defatted by extraction with chloroform. The fat-free leaves can then be extracted with any of the conventional solvents previously used for isolation of sennosides A and B. However, the best results have been obtained by using glacial acetic acid as extractant; the sennosides,