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

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The stimulating effect of citrate on lipo-
genesis was first shown by Brady and Gurin.1 Later Brady, Mamoun and Stad-
tman2 showed that this effect was due partly to citrate generating reduced nico-
tinamide-adenine-dinucleotide phosphate (NADPH) and Martin and Vagelos3 and
Abraham, Lorch and Chakoff4 among others showed that citrate specifically
stimulated the acetyl-coenzyme A car-
boxylase reaction. Many workers have
since added citrate to their incubation
media. Recently Lorch, Abraham and
Chakoff5 have compared the synthetic
patterns of long chain fatty acids formed
from 14C-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 homo-
genates 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 Tietz6 with
citrate stimulated pigeon liver homog-
genates and acetate as the precursor
resulted in 23 % myristic acid and 64 %
palmitic acid. Bhaduri and Srere7 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 14C-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 McGarrah,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
investigation10. The total amounts of radio-
activity and the relative distributions of
these activities among the individual fatty
acids were determined by paper chromato-
graphy as previously described10 except
that the assay of the fatty acids from the
homogenates in addition also included
a more detailed analysis based on extrac-
tion 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).

<table>
<thead>
<tr>
<th>Reaction system</th>
<th>Number of animals</th>
<th>Specific activity after incorp. (µC/g f.a.)</th>
<th>Percentage of incorporated $^{14}$C in product fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Myristic</td>
</tr>
<tr>
<td>Slices $^a$</td>
<td>10</td>
<td>$17.6 \pm 6.0$</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Homogenates acc. to Bucher and McGarrah $^b$</td>
<td>6</td>
<td>$2.4 \pm 1.0$</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Homogenates acc. to Catravas and Anker $^c$</td>
<td>4</td>
<td>$14.0 \pm 5.3$</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Homogenates as above with citrate substituted by glucosephosphate $^d$</td>
<td>2</td>
<td>$0.8 \pm 0.5$</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

Incubation media: $^a$ Krebs-Ringer phosphate buffer with $2 \times 10^{-7}$ 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 $7 \times 10^{-8}$ 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 $7 \times 10^{-8}$ 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 $7 \times 10^{-8}$ 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 $\lambda = 338 \text{ nm}$.

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 $s \times (5 + 3 + 1) = 54$ 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$_{16}$ 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 Lorich, Abraham and Chaikoff 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
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The extensive studies by Stoll et al. 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. 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,