Biosynthesis of Cyclopropyl Long-Chain Fatty Acids from Cyclopropanecarboxylic Acid by Mammalian Tissues in vitro

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1. Radioactivity from cyclopropane[14C]carboxylic acid is incorporated into fatty acids in vitro by rat and guinea-pig adipose tissue, by rat liver slices and by the supernatant fraction of rat liver homogenate. 2. The labelled acids are different from endogenous straight-chain fatty acids, and evidence is produced that they consist of a cyclopropyl ring in the ω-position, the remainder of the chain being built up from C₃ units (not derived from cyclopropanecarboxylic acid) in the normal way via the malonate pathway. 3. It is suggested that these unnatural acids have some metabolic effect related to the hypoglycaemic action of cyclopropanecarboxylic acid.

Cyclopropanecarboxylate is an active oral hypoglycaemic agent in the guinea pig and the monkey. In some other species, including the rat and man, it is much less effective, but in all species studied it produces a marked synergistic effect with injected insulin (Stewart, 1962). The mechanism of its hypoglycaemic action is obscure, and some of its other biochemical effects, both in vivo and in vitro, are qualitatively different from those produced by a number of other hypoglycaemic agents (hypoglycin, tolbutamide, phenformin) (Stewart, 1962).

In view of the interactions between glucose and fatty acid metabolism in tissues, and their relationship to the control of glucose and fatty acid concentrations in blood and of insulin sensitivity (Randle, Garland, Hales & Newsholme, 1964), it seemed that information about the relation of cyclopropanecarboxylate to lipid metabolism might be of interest in connexion with its hypoglycaemic effect and synergism with insulin. We started by investigating the metabolism of cyclopropanecarboxylate via fatty acids in some mammalian tissues in vitro, the results of which are reported in this paper.

MATERIALS

Synthesis of 14C-labelled cyclopropanecarboxylic acid. Cyclopropyl bromide was prepared by the reaction of silver cyclopropanecarboxylate with bromine, with dichlorodifluoromethane as solvent (Roberts & Chambers, 1951). Preparation of the Grignard reagent followed by carbonation with 14CO₂ gave cyclopropanecarboxylic acid labelled in the carboxyl group. The methods used were similar to those described by Renk, Shafer, Graham, Mazur & Roberts (1961) for the preparation of the same acid labelled with 13C. In a typical preparation the radiochemical yield was 76·5%, starting with 3 mc of 14CO₂, and the acid had a specific radioactivity of 10·4 mc/mole. Purity was established by infrared spectroscopy, by thin-layer chromatography on silicic acid with the ethanol-water-ammonia system of Braun & Geenen (1962) followed by radioautography, and by gas-liquid chromatography of the ethyl ester, fractions being collected for radioactivity determinations as described below; the polyethylene column was for this purpose run at 140°C.

Other labelled materials were obtained from The Radiochemical Centre, Amersham, Bucks. Avidin was from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

METHODS

Fatty acid synthesis from labelled precursors in rat and guinea-pig adipose tissue and liver in vitro. General procedures for incubating epididymal fat pads and for isolating total fatty acids were as described by Duncombe (1968); similar methods were used for liver slices. About 20 ml. of Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) was used/g of tissue, with substrates and tracers as detailed in the Tables and Figures. When labelled cyclopropanecarboxylate, acetate or isobutyrate was used, fatty acids from incubated tissue were isolated by the method of Duncombe (1968). When labelled long-chain fatty acids were used, total lipids of adipose tissue were extracted by the procedure of Folch, Lees & Sloane-Stanley (1957), and the neutral lipids in the extract (predominantly triglycerides) were purified by chromatography on silicic acid treated with propan-2-ol–KOH, to remove contaminating labelled exogenous fatty acids (McCarthy & Duthie, 1962). Purity was checked by thin-layer chromatography on silica gel G in hexane–ether–acetic acid (30:20:1, by vol) and radioautography. Methyl esters for gas–liquid-chromatographic analysis were prepared from neutral lipid fractions or from total tissue fatty acids by acid-catalysed methanolysis (Bowyer, Leat, Howard & Gresham, 1963), by treatment
with boron trifluoride–methanol (deMan, 1967), or by reaction
with diazomethane (Gellerman & Schlenk, 1965). Completeness of esterification was checked by thin-layer
chromatography on silica gel G by the two-step develop-
ment of Skipski, Smolowe, Sullivan & Barclay (1965).
Radioactivity was determined in lipids by dissolving
weighed amounts in a toluene-based scintillator and count-
ing in a Packard Tri-Carb liquid-scintillation spectrometer,
followed by internal standardization and calculation of
absolute radioactivities.

Oxidation of labelled substrates by tissues in vitro. Incuba-
tions were carried out by the same general methods as
described above, but with the apparatus and techniques of
Moss (1961), except that CO₂ was trapped in a solution con-
taining ethanolamine, and its radioactivity was determined
by liquid-scintillation counting (Jeffay & Alvarez, 1961).

Lipid synthesis from labelled precursors in rat liver
homogenate fractions. All operations before incubation were
carried out under ice-cold conditions. Liver was passed
through a small stainless-steel tissue mincer into homogeniz-
ing medium (Fletcher & Myant, 1961), about 3-5 ml of
medium/g of tissue being used. The suspension was homog-
ized in a Potter–Elvehjem homogenizer and centrifuged
at 800g for 10 min to remove tissue debris and nuclei, and
the supernatant was centrifuged at 10000g for 30 min to
sediment mitochondria. The resulting supernatant (includ-
ing the soluble fraction and microsomes) was designated
fraction S₁₀. The incubation medium used was the Fletcher
& Myant (1961) optimum medium for fatty acid synthesis.
Variations from this and additions of inhibitors and labelled
substrates were as detailed in the Results section. Incuba-
tions were carried out at 37° for 2 hr under air, each tube
containing 1 ml of incubation medium and 2-5 ml of fraction
S₁₀. Blanks consisted of incubation medium + radioactive
substrates only, and 2-5 ml of fraction S₁₀ was added to each
of these after incubation. Lipid biosynthesis was measured
in the incubated samples by the method of Goldfine (1966),
in which 0-1 ml samples were pipetted on to 25 mm. disks of
Whatman 3MM paper, dried and immersed successively in
ice-cold 10% (w/v) trichloroacetic acid (30 min.), ice-cold
5% (w/v) trichloroacetic acid (15 min.) and ice-cold water
(two washes each of 10 min). After being dried, the disks
were immersed in a tolulene-based scintillator solution in
counting vials and the radioactivity was determined. For
comparing different conditions within one experiment
observed counts/min. were used, since the nature of the
samples precluded internal standardization. With the com-
plete incubation system, the rate of lipid synthesis from
radioactive acetate was constant between 30 and 120 min.,
and at 180 min. was more than 90% of the value expected
at constant rate.

Gas–liquid-chromatographic separation of fatty acid methyl
ester. This was carried out with a Griffin Mk. III Kather-
ometer Chromatograph, with ½ in. × 6 ft. columns with flash
heaters. Two systems were used: (a) Celite (60–72 mesh)–
diethylene glycol succinate (4:1) + 0-5% polyethylene glycol
1500; carrier gas, H₂ at 5 lb./in.² or He at 10 lb./in.²;
column temperature 184°. (b) Celite (80–120 mesh)–Apiezon
L grease (4:1); H₂ at 15 lb./in.²; column temperature 197°.
Radioactive fractions were collected at 0-5 or 1 min. intervals
by means of a Packard gas chromatography fraction col-
lector with glass cartridges (approx. 45 mm. × 9 mm.).
Instead of the customary filling of silicone-coated
anthracene crystals, which permits the cartridge to be
counted directly in a liquid-scintillation counter, each
cartridge contained two cotton-wool cigarette filters, one at
each end. The one receiving the effluent from the gas chro-
matograph was damped with a few drops of toluene, and
after collection of the fraction this filter was removed from
the cartridge, the surrounding paper slit open and the com-
plete filter dropped into a vial of toluene-based scintillator
for counting. Lipids were readily extracted from the cotton
wool into the scintillator solution and internal standardiza-
tion for absolute measurements could be carried out if
required. This technique gives a greater counting efficiency
than the anthracene method and the efficiency of trapping
may be readily measured. As only about 1% of the radio-
activity passed through the first filter and was trapped on
the second, the second was not counted. It was always put
into each cartridge, however, so that the trapping efficiency
could be checked when necessary, and also because it was
thought that its omission might decrease the trapping
efficiency of the first filter by increasing the gas flow rate.

Hydrogenation of fatty acid methyl esters. Three methods
were used: (1) with Pd–CaCO₃ in ethanol (Nunn, 1952),
which reduces the double bond in cyclopropane rings without
affecting olefinic double bonds; (2) with PtO₂ in methanol,
which reduces both cyclopropene and olefinic double bonds
(Gellerman & Schlenk, 1966); (3) with PtO₂ in acetic acid,
which in addition opens cyclopropane rings, giving pre-
dominantly a mixture of branched-chain isomers (Gellerman
& Schlenk, 1966; McCloskey & Law, 1967).

Decarboxylation of ¹⁴C-labelled fatty acids. This was
carried out by the method of Brady, Bradley & Trams
(1960).

RESULTS

Fatty acid synthesis from cyclopropanecarboxylate
in rat and guinea-pig tissues in vitro. The results shown
in Table 1 demonstrate the incorporation of carbon from cyclopropane[¹⁴C]carboxylate into
long-chain fatty acids of rat adipose tissue in vitro.
The incorporation increased considerably when glucose
was also present in the medium, and in this
respect was similar to the incorporation of acetate into
rat adipose-tissue fatty acids (Masoro, 1962).
Moreover, the two compounds were incorporated to a
similar degree (Table 1).

To investigate the nature of the labelled fatty
acids formed from cyclopropanecarboxylate, the
acids were methylated and separated by gas–liquid
chromatography, and successive fractions of the ef-
fluent were examined for radioactivity. Fig. 1 shows
a typical gas chromatogram (b) and the correspond-
ing radioactive scan (a) for material from rat adipose
tissue incubated with cyclopropane[¹⁴C]carboxylate
plus non-radioactive glucose. A notable feature was
the appearance in the radioactivity scan of five un-
usual labelled fatty acids (peaks A–E). The typical
pattern shown was found quite consistently in about
ten different experiments, and virtually identical
patterns appeared also with samples from rat liver
and guinea-pig adipose tissue. Close examination of
all the results showed that none of the radioactive
Table 1. Incorporation of cyclopropane[14C]carboxylate and [carboxy-14C]acetate into fatty acids of rat adipose tissue in vitro

Experimental details were as given in the Methods section. Some flasks contained 16.7 mM-glucose in addition to the radioactive substrate.

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Radioactive substrate and concen. (mM)</th>
<th>Carboxyl C incorporated (μg. atoms/mg. of fatty acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyclopropane carboxylate (0.06)</td>
<td>Without glucose: 44, 49, 87</td>
</tr>
<tr>
<td>2</td>
<td>Cyclopropane carboxylate (0.1)</td>
<td>With glucose: 223, 274, 337</td>
</tr>
<tr>
<td>3</td>
<td>Cyclopropane carboxylate (24)</td>
<td>16.7 mM-glucose: 101, 79, 74</td>
</tr>
<tr>
<td>4</td>
<td>Cyclopropane carboxylate (24)</td>
<td>16.7 mM-glucose: 354, 300, 370</td>
</tr>
<tr>
<td>5-11</td>
<td>Acetate (0.1)</td>
<td>16.7 mM-glucose: —</td>
</tr>
</tbody>
</table>

* Mean ± s.d.

Fig. 1. Gas-chromatographic separation of fatty acids (as methyl esters) from rat adipose tissue incubated with cyclopropane[14C]carboxylic acid. General conditions of incubation and sample preparation were as described in the Methods section. Additions were: sodium cyclopropane-[14C]carboxylate (24 mM; 7 μC in 20 ml); glucose (16.7 mM).

(a) Radioactivity; (b) mass-detector record.

peaks coincided with the major endogenous tissue fatty acids (14:0, 16:0, 16:1, 18:0, 18:1, 18:2) or with the positions of added straight-chain odd-numbered fatty acids. The pattern was unchanged when the fatty acid methyl esters were made by any of the three methods described, or when helium was used instead of hydrogen as the flow gas in the gas-liquid-chromatographic separations. It therefore seems unlikely that the typical pattern was an analytical artifact.

The gas-liquid-chromatographic trace (Fig. 1b) showed no peak corresponding to even the most radioactive fraction; the weight of the latter synthesized during the incubation was thus very small compared with the weight of endogenous fatty acids.

The question whether these new fatty acids were synthesized exclusively from cyclopropane-carboxylate or whether other small molecules could be involved was investigated by incubating rat adipose tissue with [1-14C]acetate together with non-radioactive cyclopropane-carboxylate. The results (Fig. 2) showed that radioactivity appeared not only in the fatty acids expected to be synthesized from acetate (e.g. 14:0, 16:0, 16:1, 18:0, 18:1) but also in two positions (peaks D and E) corresponding exactly to peaks D and E of the radioactive cyclopropane-carboxylate experiment (Fig. 1). Peaks corresponding to peaks A, B and C of that experiment would have been obscured by the radioactive fatty acids normally synthesized from acetate. When radioactive acetate was incubated in the absence of cyclopropane-carboxylate no radioactivity was seen at positions D and E. It is therefore evident that, for the biosynthesis of the fatty acids D and E (and possibly also acids A, B and C), both cyclopropane-carboxylate and endogenous acetate are required.

Similar experiments with other labelled and unlabelled compounds were carried out in an attempt to show whether the cyclopropane-carboxylate was metabolically degraded before utilization for fatty acid synthesis. The radioactive acids D and E were not produced when adipose tissue was incubated with labelled acetate plus unlabelled propionate or isobutyrate, or with labelled isobutyrate (in all cases in the presence of glucose). Incubation with labelled
laurate or palmitate plus unlabelled cyclopropane-carboxylate also failed to produce them. These results suggest that cyclopropane-carboxylate is not converted into propionate or isobutyrate before incorporation into fatty acids, and also that the fatty acids that are formed do not arise by elongation of existing long-chain acids. The possibility that the cyclopropane ring is opened after incorporation into long-chain fatty acids is not supported by the changes in the radioactive acids caused by hydrogenation (see below).

On chemical decarboxylation of the fatty acids derived from adipose tissue incubated with cyclopropane\(^{14}\)C\)carboxylate, less than 5% of the radioactivity appeared in the carbon dioxide evolved.

Further evidence relating to the structure of the new acids was sought by comparing the gas–liquid-chromatographic radioactivity patterns of the fatty acid methyl esters from adipose tissue incubations before and after selective hydrogenation procedures. Owing to the very small amounts of material available, it was not possible to measure hydrogen uptake quantitatively, so that only qualitative conclusions could be drawn. The following observations were made. (1) Hydrogenation for cyclopropene double bonds produced no difference in the pattern obtained on the polyester column; (2) reduction of olefinic double bonds (as confirmed by disappearance of the mass peaks for the endogenous unsaturated fatty acids) caused a diminution of the radioactive peak D (Fig. 1) and the appearance of a faster-running radioactive peak (‘peak X’) having an elution time slightly longer than that of palmitic acid; (3) hydrogenation capable also of breaking cyclopropane rings again produced the peak X, together with an increase in peak C (Fig. 1). With an increase in the time of hydrogenation peak X disappeared and there was substantial conversion of acid D into acid C (or into a compound having a similar elution time). Changes in the other radioactive peaks of Fig. 1 were difficult to follow, owing to their smaller size. With non-polar columns similar qualitative changes were seen.

The simplest explanation for the observed behaviour can be found by assuming that acid D is a long-chain fatty acid containing one or more olefinic double bonds and one or more cyclopropane rings, but no cyclopropene ring. Reduction of the unsaturation would give acid X, containing a saturated chain and a cyclopropane ring or rings, and further reduction, also breaking the rings, would give rise predominantly to a mixture of isomeric long-chain acids, bearing methyl-group side chains, appearing

<table>
<thead>
<tr>
<th>System</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>3534</td>
<td>10930</td>
</tr>
<tr>
<td></td>
<td>3212</td>
<td>9590</td>
</tr>
<tr>
<td></td>
<td>3617</td>
<td>9700</td>
</tr>
<tr>
<td>Avidin (150 μg.) added</td>
<td>897</td>
<td>919</td>
</tr>
<tr>
<td></td>
<td>843</td>
<td></td>
</tr>
<tr>
<td></td>
<td>919</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>384</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>504</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>253</td>
<td>180</td>
</tr>
<tr>
<td>Malonate omitted</td>
<td>4990</td>
<td>4630</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3070</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3950</td>
<td></td>
</tr>
<tr>
<td>Malonate and HCO(_2^-) omitted</td>
<td>1820</td>
<td>1590</td>
</tr>
<tr>
<td></td>
<td>1510</td>
<td></td>
</tr>
</tbody>
</table>
at position C. The present evidence is insufficient to show whether this radioactive hydrogenation product appearing at peak C is identical with the compound found at peak C before hydrogenation.

**Lipo genesis from cyclopropanecarboxylate in cell-free preparations: mechanism of biosynthesis.** Since we usually observe much greater lipogenic activity in liver homogenates than in adipose-tissue homogenates, on a basis of fresh tissue weight, we used rat liver preparations to investigate the mechanism of biosynthesis from cyclopropanecarboxylate.

Table 2 shows the results of experiments under conditions known to influence fatty acid synthesis from acetate in this preparation (Vagelos, 1964). The requirement for HCO₃⁻ and malonate and the inhibitory effect of avidin suggest that a mechanism similar to the malonate pathway is involved in the incorporation of cyclopropanecarboxylate into fatty acids.

**Oxidation of cyclopropanecarboxylate by rat adipose tissue** in vitro. The conversion of the carboxyl carbon of cyclopropanecarboxylate into carbon dioxide was found to be about 1% of its incorporation into fatty acids.

**DISCUSSION**

The incorporation of the radioactivity of cyclopropane[¹⁴C]carboxylate into tissue fatty acids raises three main questions. (1) In what chemical form does the incorporation of the labelled carbon atom occur? (2) What is the mechanism involved? (3) Are these findings relevant to the hypoglycaemic action of cyclopropanecarboxylate?

Chemical identification of the labelled biosynthetic acids was difficult because of the very small amounts produced compared with the endogenous acids. The radioactive material isolated by gas-liquid chromatography was undoubtedly contaminated by comparatively large quantities of non-radioactive material arising from tailing of the major fatty acid peaks and from ‘bleeding’ of the stationary phase, making spectroscopic examination of little value. Evidence about chemical structure was therefore obtained indirectly by observing changes in the chromatographic behaviour of radioactive samples subjected to various hydrogenation procedures.

The results of such experiments show that the cyclopropane-containing acid D also contains units derived from acetate, and at least part of the incorporation of cyclopropanecarboxylate is due to a mechanism stimulated by HCO₃⁻ and malonate and sensitive to avidin. This is presumably analogous to, or identical with, the malonate pathway for fatty acid synthesis from acetate. The enzymic formation from cyclopropanecarboxylate of a dicarboxylic acid, similar to the formation of malonate from acetate, seems rather unlikely. Further, if the fatty acid chain were made up by the addition of acetate and cyclopropanecarboxylate units randomly, a considerable variety of species would be expected, their character depending on the number and position of cyclopropyl groups included and the total chain length. In fact, only five acids were observed, and these appeared consistently in a number of different experiments with different tissues and animal species. The most plausible mechanism for the formation of these acids is that cyclopropanecarboxylate forms the start of the chain, analogous to the methyl end of a long-chain fatty acid synthesized from acetate, and that the rest of the chain is made up in the usual way from acetate units by the malonate pathway. Support for this as the major pathway is given by the finding of less than 5% of the total radioactivity of the acids in the carboxyl group. By analogy with the naturally occurring acids synthesized from acetate units, it seems likely that all five acids contain the ω-cyclopropyl group, differing only in unsaturation or the number of C₃ units in the chain (or both).

If we assume that these acids contain one cyclopropane group, some deductions can be made about their chain length. In a gas–liquid-chromatographic system similar to the one used here (polyester column), the elution time of a normal straight-chain fatty acid is shorter than that of a monocyclopropyl acid of the same carbon number but longer than that of the branched-chain acid resulting from hydrogenation of the cyclopropyl ring (Kaneshiro & Marr, 1961). Consideration of the present results suggests that the major peak D might correspond to a C₁₆ atom fatty acid containing an ω-cyclopropyl group and probably also some unsaturation, for which evidence from hydrogenation experiments has been presented. The other radioactive peaks would correspond to saturated or unsaturated (or both) even-numbered homologues.

Other possible modes of synthesis of these acids seem to be much less likely. No elongation of palmitate or laurate by cyclopropanecarboxylate was demonstrated. No mechanism involving degradation of the cyclopropyl ring before fatty acid synthesis seems to occur, since labelled propionate or isobutyrate did not give rise to the novel acids; further, ring fission to give n-butyrate or two acetate units does not occur since this would result only in normal even-chain-length acids, which were not detected. Decarboxylation before incorporation is conceivable, since the radioactive label would be lost and the cyclopropyl group would give rise to unlabelled acids; the very small conversion of the labelled carboxyl group into carbon dioxide (1%) compared with its incorporation into fatty acids makes this unlikely as a major pathway.

We are unaware of any reports of long-chain fatty

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acids containing an \( \omega \)-cyclopropyl group, either natural or synthetic. Naturally occurring cyclopropyl and cyclopropenyl long-chain fatty acids are well known in plants and micro-organisms, but the ring is never terminal. In micro-organisms typical acids are cis-11,12-methyleneoctadecanoate (lactobaccilate) and cis-9,10-methylenehexadecanoate, either or both of which have been found in lactobacilli, Escherichia coli, Salmonella typhimurium and other organisms (Chalk & Kodicek, 1961; Kaneshiro & Marr, 1961; Macfarlane, 1962; O'Leary, 1962). In plant families (Sterculaceae and Malvaceae) unsaturated fatty acids such as 9,10-methyleneoctadec-9-enoate (sterculate) and 8,9-methyleneheptadecai-8-enoate (malvalate) acid are commonly found (Shenstone & Vickery, 1961). In acids of both micro-organisms and plants the ring is believed to be formed by addition of a \( \Delta_1 \) fragment (e.g. formate or methionine methyl) across an unsaturated bond (Liu & Hofmann, 1962; Chalk & Kodicek, 1961; Smith & Bu'Lock, 1964).

Our finding that the cyclopropene ring is not degraded during the incorporation of cyclopropenecarboxylate into animal-tissue fatty acids is consistent with the findings of other workers. Chung (1966) found no conversion into carbon dioxide of the cyclopropene methane carbon in labelled cis-9,10-methylenehexadecanoate or cis-9,10-methyleneoctadecanoate (biosynthesized by Clostridium butyricum) when these acids were incubated with rat liver mitochondria or whole-liver homogenates, though the carbon chain was shortened. Wood & Reiser (1965) reported the accumulation of cis- or trans-3,4-methyleneoctadecanoate in the adipose tissue of rats fed with synthetic cis- or trans-9,10-methyleneoctadecanoate respectively. Another hypoglycaemic agent, 2-amino-3-methylenecyclopropylpropionate (hypoglycin), is rapidly metabolized by rats in vivo, ultimately giving rise to methyleneacyclopentylacetate (Holt, 1968). Cyclopropene itself and its methyl, ethyl and vinyl ethers are biochemically inert and are eliminated unchanged (Williams, 1959). All these results suggest that the cyclopropene ring is not readily broken in mammalian tissues.

The present demonstration that cyclopropenecarboxylate can lead to the synthesis of unnatural fatty acids in the body does not throw any direct light on the mechanism of its hypoglycaemic action. Such action of the cyclopropenyl amino acid, hypoglycin, has been attributed to the inhibition of fatty acid oxidation by its metabolite, methyleneacyclopropylacetate (Holt, Holt & Böhm, 1966). Also, it is known that cyclopropenecarboxylate can cause inhibition of fatty acid oxidation (Williamson & Wilson, 1965; Senior & Sherratt, 1967). One possibility would therefore seem to be that cyclopropenecarboxylate exerts its hypoglycaemic effect through interference with the metabolism of endogenous fatty acids by the unnatural acids formed from it. Certainly the onset of hypoglycaemia in sensitive species is slow (Stewart, 1962), which is consistent with the active agent's being a metabolite not rapidly synthesized. Our finding that synthesis of the unnatural acids is similar in tissues both of rat and of guinea pig, whereas the hypoglycaemic response is very different in the two species, indicates that if this possibility is correct then considerable differences must exist between these two species as far as the magnitude of the metabolic interference is concerned. As yet there is no definite evidence for the suggested mechanism or that the synthesis of the cyclopropenyl long-chain acids has any physiological significance.

We are indebted to Mr A. R. Elphick for the preparation of cyclopropyl bromide.

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