BBA 3875

# THE BIOSYNTHESIS OF LONG-CHAIN SATURATED AND UNSATURATED FATTY ACIDS IN ISOLATED PLANT LEAVES

A. T. JAMES*

*National Institute for Medical Research, Mill Hill, London (Great Britain)*

(Received June 15th, 1962)

SUMMARY

1. The incorporation of labelled acetic, octanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, octadecanoic and 9-octadecenoic acids into the saturated and unsaturated fatty acids of lipids from isolated leaves (mainly *Ricinus communis*) is described.

2. With the exception of hexadecanoic and octadecanoic acids all these labelled compounds act as precursors of oleic, linoleic and linolenic acids.

3. Hexadecanoic and octadecanoic acids are incorporated into leaf lipids.

4. In the absence of oxygen no oleic acid is synthesized.

5. Two separate pools of the longer-chain fatty acids are suggested. In one myristic acid is converted to palmitic and stearic acids which are then esterified to give galactolipid and phospholipid. In the second pool myristic acid is converted to palmitic acid, then to stearic acid and finally to oleic acid. Only the latter is freely available for esterification to combined lipids or for conversion to linoleic and linolenic acids.

## INTRODUCTION

The biosynthesis of unsaturated acids in animal tissues has been studied for some years, though but little information has been obtained until recently on the pathways concerned. Surprisingly little attention has been directed towards the higher plants even though both leaf and seed fats contain large amounts of unsaturated fatty acids.

SIMMONS AND QUACKENBUSH[1] studied the biosynthesis of the long-chain fatty acids by developing soyabean seed pods using uniformly labelled sucrose as the carbon source. After 6 h, oleic acid had the highest activity, after two days it accounted for 56 % of the total activity. The authors considered that this acid was the precursor of the other acids.

COPPENS[2] used slices of cotyledons of *Ricinus communis* as experimental material and found that the $C_{18}$ fatty acids contained most of the activity (except for ricinoleic acid which was almost unlabelled), when [1-$^{14}$C]acetate was used as precursor but when glucose was added to the medium, the $C_{14}$ and $C_{16}$ acids were found to be the most active.

* Present address: Unilever Research Laboratory, Colworth House, Sharnbrook, Bedfordshire (Great Britain).

STUMPF *et al.*[3,4] have studied the formation of the saturated and unsaturated $C_{18}$ acids by cell-free extracts from mesocarp of the avocado pear. Marked differences were found in the labelling of the saturated and unsaturated acids depending on the conditions and whether acetate or malonate was used as the precursor.

EBERHARDT AND KATES[5] described the synthesis of lipids by isolated runner bean leaves and showed that both [$^{14}$C]acetate and $^{14}CO_2$ were rapidly incorporated into long-chain fatty acids. No study was made of the nature of the acids synthesized.

This information prompted a study of the biosynthesis of fatty acids in isolated leaves. The plants used included castor oil (*Ricinus communis*), cabbage, lettuce, brussels sprouts and chicory, the results were essentially the same in all cases. Leaf tissue rapidly synthesises a variety of saturated and unsaturated fatty acids and is a particularly convenient material. Preliminary results have already been published by JAMES[6].

## MATERIALS AND METHODS

The radioactive substrates used were the following: sodium[2-$^{14}$C] acetate (2 mC per mmole), sodium [1-$^{14}$C]octanoate (3310 $\mu$C/mmole), sodium [1-$^{14}$C]decanoate (2150 $\mu$C/mmole), sodium [1-$^{14}$C]dodecanoate (2570 $\mu$C/mmole), sodium [1-$^{14}$C]-palmitate (2 mC/mmole), and [2-$^{14}$C]stearic acid (2 mC/mmole) (Radiochemical Centre, Amersham, Bucks.). [1-$^{14}$C]Oleic acid was supplied by Volks Radiochemical Co., Chicago, Illinois (U.S.A.).

The *Ricinus communis* (castor oil) plants used were grown both at the Royal Botanic Gardens, Kew, and in the greenhouses of the National Institute for Medical Research. A few plants of *Ricinus gibsonii* (Butchers Seeds Ltd., Croydon) were also used with similar results to *Ricinus communis*. Leaves were removed from the plants when they had grown to a width of 3–4 in. Cabbage, lettuce and chicory were obtained from commercial sources.

### *Uptake of radioactive substrates*

Single leaves were removed by cutting the stems under water and were placed in tap water until required. Labelled sodium acetate (2 $\mu$C) was dissolved in distilled water (0.2 ml) and the stem placed in the solution until absorbed, when a further 0.2 ml of distilled water was added. When this additional volume had been taken up the leaf was transferred to tap water and left under the lamps for the required time. Illumination was provided by four 2-feet fluorescent tubes (daylight emission) at a distance of 8 in. The lighting system used by EBERHARDT AND KATES[5] was frequently found to overheat the leaves even when a heat filter was used. Other radioactive substrates (1 $\mu$C of each) were dissolved in 0.2 ml of 0.1 M citrate–phosphate buffer (pH 6.0) with the addition of 1 mg of Tween 20 in the case of stearic and oleic acids to promote solubilisation.

At the end of the required time the leaf (or part of the leaf) was removed, the stem and ribs cut out and the fleshy part of the leaf cut into small pieces. It was then ground finely with powdered solid $CO_2$ in a mortar and washed into a flask with chloroform–methanol (2:1, v/v). At the end of 4–5 h at room temperature the residue was colourless and the solution of lipid, chlorophylls etc. was filtered into a separating funnel. One tenth of the volume of distilled water was added, shaken,

and the phases allowed to separate. The lower $CHCl_3$ layer was removed, dried over anhydrous sodium sulphate, evaporated to dryness *in vacuo* at 30–40° and saponified with 10% methanolic KOH. In some experiments the lipid material was separated into neutral lipids and phospholipid by evaporating the chloroform-lipid extract to dryness *in vacuo* at 30° and extracting with cold dry acetone. The two fractions were saponified in the usual way, the fatty acids were extracted with ether after acidification, dried over anhydrous sodium sulphate and methylated with freshly distilled diazomethane at 0°.

At the end of 30 min the diazomethane was removed by warming on the water bath and the ether reduced in volume by boiling. Samples were applied to the gas chromatogram by evaporating the ether solution on to a glass yarn plug, contained in a short length of glass tubing, in a stream of nitrogen. The outside diameter of the glass tube was such that it would just fit inside the column. The tube was dropped in, allowed to warm to column temperature (60 sec) and the gas stream turned on. In this way very small or very large samples can be quantitatively placed on the gas chromatogram.

*Chopped leaf preparations*

The leaf tissue was cut away from the ribs, folded and chopped into roughly 1-mm squares with a razor blade. The pieces were suspended in 0.2 M phosphate buffer (pH 7.4), as this buffer was found to give the highest incorporation of [2-$^{14}$C]-acetate into lipids. The labelled precursor was added, the flask placed in a shaker under fluorescent lamps and allowed free access of air for the time required. Anaerobic incubations were carried out in closed flasks through which a slow stream of either argon or argon–$CO_2$ (95:5) was passed. Labelled precursors were then added without access of air. At the end of the appropriate time the leaf tissues were filtered through fat-free cotton wool, washed several times with distilled water, and then extracted overnight with $CHCl_3$–MeOH. The procedure then followed that described above.

*Oxidative degradation of unsaturated acids and automatic counting*

Individual fatty acids were isolated by gas chromatography using 4-feet polyethyleneglycol adipate columns at 180°. Oxidation was carried out with $KMnO_4$ in glacial acetic acid by the method described by JAMES AND WEBB[6]. After extraction and methylation the mono- and dicarboxylic acids were separated on a 4-feet Apiezon L column at 200° using the automatic counter[7]. In later experiments a modified gas radiochromatogram was used[8] in which the column was connected directly to a combustion tube in which the eluted fatty acids were converted first to $CO_2$ and water, the water then being reduced with iron powder to hydrogen. These combustion products were then detected by a sensitive catharometer of the type described by STUVE[9] before being passed into the proportional counter.

## RESULTS

The major fatty acids of the castor leaf lipids are palmitic, linoleic and linolenic acids with oleic and stearic acids as minor constituents. No ricinoleic acid (the major component of the castor bean fat) could be detected (Table I). Leaves from lettuce, cabbage, chicory and brussels sprouts had a similar fatty acid composition.

In a previous publication[10] it was shown that 10 min after labelled acetate had been taken up by an isolated leaf, the oleic acid was extensively labelled, although palmitic acid and the other $C_{18}$ acids possessed a very low specific activity. Oleic acid reached its maximum specific activity after 20 min but had declined to roughly

TABLE I

FATTY ACID COMPOSITION OF TOTAL LEAF LIPIDS

| *Acid* | *Percentage* |
|---|---|
| 8:0 → 12:0 | 0.2 |
| 14:0 | 0.2 |
| 16:0 | 21.0 |
| 18:0 | 1.6 |
| 16:1 | 1.1 |
| 18:1 | 3.2 |
| 18:2 | 13.0 |
| 18:3 | 60.0 |

half this level after 3 h. Linoleic acid increased steadily in specific activity up to 1 h whilst the values for stearic and palmitic acids remained roughly constant after 20 min. A typical gas radiochromatogram of acids synthesized in 2 h is shown in Fig. 1. At no time was any large fraction of the total radioactivity found in stearic acid and the expected precursor–product relationship between stearic and oleic acids was not observed. This has been demonstrated in a blue-green algae (*Anabaena variabilis*) by BLOCH[11]. Since a small pool of stearic acid could be turning over rapidly an attempt was made to follow the biosynthesis from [2-$^{14}$C]acetate and incorporation, of oleic and stearic acids, into both the neutral lipid (largely galactolipid) and phospholipid pools. The results are shown (in terms of count distribution at each time point) in Figs. 2 and 3. In both cases stearic acid contained very little radioactivity at all times from 10 min to 3 h after uptake of the labelled acetate. Fatty acid composition of the two fractions is given in Table II, the galactolipids contain most of the leaf linolenic acid. Isolation of the free fatty acids included in the neutral lipid fraction showed very little radioactivity.

In order to trace further any difference in the biosynthetic pathway of the saturated and unsaturated acids, experiments were carried out with labelled octanoic, decanoic, dodecanoic, hexadecanoic and octadecanoic acids using whole isolated

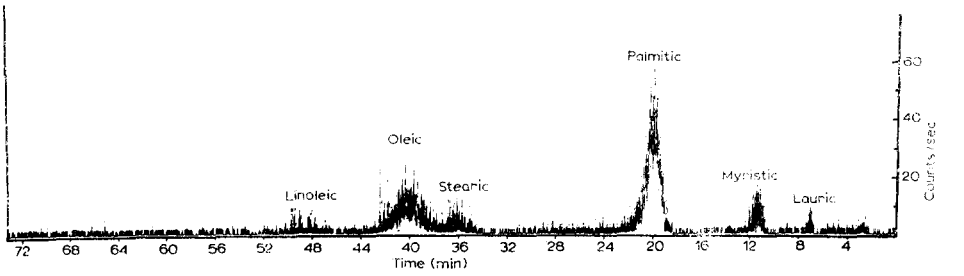



Fig. 1. Gas-radiochromatogram of the fatty acid synthesized in 2 h by a castor leaf from [2-$^{14}$C]-acetate. Stationary phase: poly-ethyleneglycol adipate at 180°. Gas flow rate, 71 ml/min. Counter response time, 0.5 sec.

leaves as before. The results are presented in Table III in terms of specific activity and in Table IV in terms of count distribution. The large dodecanoic acid count found when this acid was used as precursor is due to inclusion of the stem in the material extracted, much of the acid had not reached the leaf tissue. The main result stands out; the $C_8$, $C_{10}$ and $C_{12}$ acids act as precursors both of oleic and hence linoleic acids

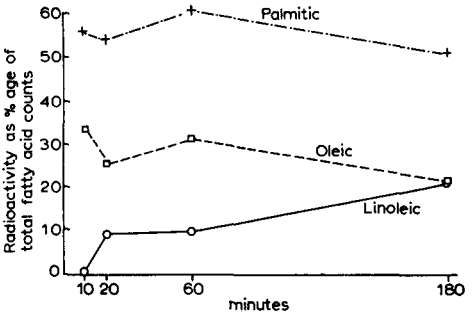



Fig. 2. Changes in distribution of radioactivity of long-chain fatty acids incorporated into castor leaf phospholipids, using [2-$^{14}$C]acetate as precursor, at various time intervals after uptake of precursor.

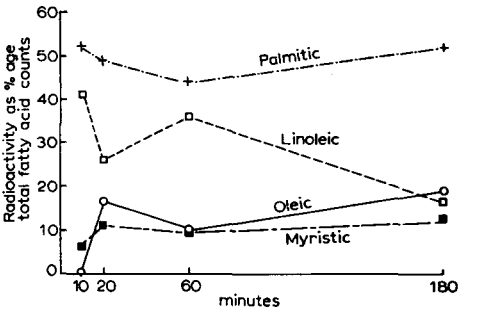



Fig. 3. Changes in distribution of radioactivity of long-chain fatty acids of the acetone-soluble lipids of the castor leaf using [2-$^{14}$C]acetate as precursor, at various intervals after uptake of precursor.

TABLE II

FATTY ACID COMPOSITION OF PHOSPHOLIPID AND NEUTRAL FAT FRACTIONS OF LEAF LIPIDS

| *Acid* | *Phospholipid (%)* | *Neutral lipid (%)* |
|---|---|---|
| Saturated and unsaturated of chain length $C_8 \rightarrow C_{16}$ | 5.4 | 6.1 |
| 14:0 | 0.4 | 1.1 |
| 16:0 | 34.8 | 13.7 |
| 18:0 | 1.64 | 0.9 |
| 16:1 | 3.9 | 1.7 |
| 18:0 | 3.4 | 2.0 |
| 18:2 | 20.6 | 8.9 |
| 18:3 | 30.0 | 66.0 |

TABLE III

SPECIFIC ACTIVITY OF LEAF FATTY ACIDS USING DIFFERENT PRECURSORS (3 h INCUBATION) PRECURSORS INTRODUCED VIA THE STEM

Counts/cm$^2$ corrected peak area. Counting time, 10 sec. 1 cm$^2$ = 0.025 mg.

| *Precursor acid* / *Leaf fatty acid* | $C_8$ *Expt. 1* | $C_8$ *Expt. 2* | $C_{10}$ | $C_{12}$ | $C_{16}$ | $C_{18}$ |
|---|---|---|---|---|---|---|
| $C_8$ | 2750 | 360 | — | 71 | 0 | 0 |
| $C_{10}$ | — | — | 5400 | 260 | 0 | 0 |
| $C_{12}$ | — | — | — | 22 000 | 0 | 0 |
| $C_{14}$ | 330 | 148 | 68 | 1000 | 0 | 0 |
| $C_{16}$ | 42 | 71 | 53 | 35 | 493 | 0 |
| $C_{18}$ | 0 | 0 | 0 | 0 | 0 | 1200 |
| Oleic | 20 | 230 | 67 | 62 | 0 | 0 |
| Linoleic | 152 | 71 | 28 | 15 | 0 | 0 |

TABLE IV

PERCENTAGE DISTRIBUTION OF COUNTS IN LEAF ACIDS ISOLATED 3 h AFTER INTRODUCTION OF PRECURSOR VIA THE STEM

| *Precursor* | | $C_8$ | | $C_{10}$ | $C_{12}$ | | $C_{16}$ | $C_{18}$ |
|---|---|---|---|---|---|---|---|---|
| *Leaf fatty acid* | | *Expt. 1* (%) | *Expt. 2* (%) | (%) | *Incl.* $C_{12}$ (%) | *Excl.* $C_{12}$ (%) | (%) | (%) |
| | 8:0 | 13.9 | 6.9 | — | | 1.1 | | |
| 10:0 | $nC_{10}$ | — | | 15.3 | | 1.4 | 0 | 0 |
| 12:0 | $nC_{12}$ | | | | 92 | | 0 | 0 |
| 14:0 | $nC_{14}$ | 4.1 | 3.2 | 3.1 | 2.5 | 29 | 0 | 0 |
| 16:0 | $nC_{16}$ | 52 | 42 | 41 | 3.4 | 40 | 100 | 0 |
| 18:0 | $nC_{18}$ | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 18:1 | Oleic | 9.1 | 21 | 14.4 | 1.2 | 14.3 | 0 | 0 |
| 18:2 | Linoleic | 21.2 | 26 | 13.0 | 1.2 | 14.3 | 0 | 0 |

TABLE V

SPECIFIC ACTIVITY OF FATTY ACIDS FROM CHOPPED LEAF PREPARATIONS USING DIFFERENT PRECURSORS IN 0.2 M PHOSPHATE BUFFER (pH 7.4)

| *Precursors* | *3 h incubation acetate* | *5 h incubation* | | | | |
|---|---|---|---|---|---|---|
| | | $C_{10}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ |
| | | *Specific activity in counts/cm² peak area* | | | | |
| Leaf acid | | | | | | |
| 10:0 | 0 | 27 000 | 0 | 0 | 0 | 0 |
| 12:0 | 0 | 930 | 28 600 | 0 | 0 | 0 |
| 14:0 | 630 | 800 | 3900 | 21 800 | 0 | 0 |
| 16:0 | 96 | 155 | 216 | 455 | 840 | 0 |
| 18:0 | low | 83 | 218 | 354 | 860 | 955 |
| 18:1 | 212 | 205 | 660 | 753 | 0 | 0 |
| 18:2 | 11.8 | 34 | 25 | 14.5 | 0 | 0 |

TABLE VI

COUNT DISTRIBUTION IN FATTY ACIDS ISOLATED FROM CHOPPED LEAF PREPARATIONS USING DECANOIC AND DODECANOIC ACIDS AS PRECURSORS

| *Leaf acid* | $C_{10}$ *Precursor* | | $C_{12}$ *Precursor* | |
|---|---|---|---|---|
| | *Percentage counts* | *Percentage counts* | *Percentage counts* | *Percentage counts* |
| | *incl.* $C_{10}$ | *excl.* $C_{10}$ | *incl.* $C_{12}$ | *excl.* $C_{12}$ |
| $C_{10}$ | 26.1 | 0 | 0 | |
| $C_{12}$ | 2.2 | 3.0 | 55 | 0 |
| $C_{14}$ | 5.2 | 6.9 | 7.4 | 16.6 |
| $C_{16}$ | 42.5 | 57.5 | 25.7 | 58.0 |
| $C_{18}$ | 3.2 | 4.3 | 1.2 | 2.7 |
| 18:1 | 13.2 | 17.8 | 8.0 | 18.1 |
| 18:2 | 7.7 | 10.4 | 2.0 | 4.5 |

and of the $C_{16}$ and $C_{18}$ acids, whereas the latter acids do not function as precursors of oleic acid. In the latter case it was difficult to get sufficient of the acids into the leaf via the stem, so the experiment was repeated using leaves cut into 1-mm squares and shaken at 22° with a solution of the appropriate acid in 0.2 M potassium phos-

phate buffer (pH 7.4). In this case the $C_{10}$, $C_{12}$, $C_{14}$, $C_{16}$ and $C_{18}$ labelled acids were used as substrates.

The results given in terms of specific activity in Table V, and in count distribution in Table VI, confirm the figures given in Tables III and IV. In this case palmitic acid gave rise to stearic acid of equal specific activity.

Isolation by gas chromatography of the oleic acid synthesized from octanoic, decanoic, dodecanoic and tetradecanoic acids was followed by oxidative degradation and analysis of the products on the gas radiochromatogram. The oleic acid derived from octanoate was labelled only in the monocarboxylic acid fraction whereas that derived from decanoate and dodecanoate was labelled only in the azelaic acid fragment. In Table VII is given the nature of the labelled products obtained by oxidation of oleic acid produced from a variety of precursors.

The known ability of the photosynthetic apparatus of the leaf to decompose water to hydrogen and molecular oxygen makes it difficult to create fully anaerobic conditions. For this reason yellow leaf tissue (chicory) that had been grown in the absence of light was incubated with labelled acetate in both air and argon. In air all the fatty acids were labelled but under argon oleic acid was inactive (Table VIII).

TABLE VII

THE SYNTHESIS OF OCTADECANOIC ACIDS IN PLANT LEAVES

| *Precursor* | *Labelled fragments produced by oxidation of isolated octadecenoic acid* | *Structure of corresponding octadecenoic acids* |
|---|---|---|
| [2-$^{14}$C]Acetic acid | Azelaic, nonanoic acids | $\Delta^9$-Octadecenoic acid |
| [1-$^{14}$C]Octanoic acid | Nonanoic acid | $\Delta^9$-Octadecenoic acid |
| [1-$^{14}$C]Decanoic acid | Azelaic acid | $\Delta^9$-Octadecenoic acid |
| [1-$^{14}$C]Dodecanoic acid | Azelaic acid | $\Delta^9$-Octadecenoic acid |
| [1-$^{14}$C]Tetradecanoic acid | Palmitic acid, myristic acid, azelaic acid | $\Delta^2$, $\Delta^4$ and $\Delta^9$-octadecenoic acids |

TABLE VIII

THE EFFECT OF OXYGEN ON THE BIOSYNTHESIS OF OLEIC ACID IN CHLOROPHYLL-FREE CHICORY LEAVES USING [2-$^{14}$C]ACETATE AS PRECURSOR

| *Fatty acid* | *Gas phase Specific activity in counts/min/mg* $\times 10^{-3}$ | |
|---|---|---|
| | *Air* | *Argon* |
| Lauric | 226 | 226 |
| Myristic | 141 | 178.1 |
| Palmitic | 33.8 | 16.08 |
| Stearic | 175.5 | 143 |
| Oleic | 63.5 | 0 |
| Linoleic | 0 | 0 |
| Linolenic | 0 | 0 |

In green leaf tissue containing active chloroplasts anaerobic conditions using argon gave a similar result (Table IX). With labelled acetate as precursor in the presence of light exclusion of oxygen resulted in a decreased ability to synthesize oleic acid (Fig. 4).

TABLE IX

THE EFFECT OF ANAEROBIC CONDITIONS ON OLEIC ACID BIOSYNTHESIS FROM [2-$^{14}$C]ACETATE BY GREEN LEAVES IN THE LIGHT

| *Leaf fatty acid* | *Gas composition* | | | | | |
|---|---|---|---|---|---|---|
| | *Air* | | *Argon* | | *Argon–$CO_2$ (95:5)* | |
| | *Per cent total counts in each acid* | *Specific activity (counts/min/mg $\times 10^{-3}$)* | *Per cent total counts in each acid* | *Specific activity (counts/min/mg $\times 10^{-3}$)* | *Per cent total counts in each acid* | *Specific activity (counts/min/mg $\times 10^{-3}$)* |
| Myristic | 1.05 | | 1.3 | 204 | 1.6 | |
| Palmitic | 44.0 | 650 | 44.6 | 418 | 46.2 | 109.5 |
| Stearic | 27.2 | 2260 | 42.3 | 2222 | 43.0 | 715 |
| Oleic | 23.6 | 1640 | 9.3 | 402 | 9.4 | 156 |
| Linoleic | 1.6 | 61.5 | 0 | 0 | 0 | 0 |

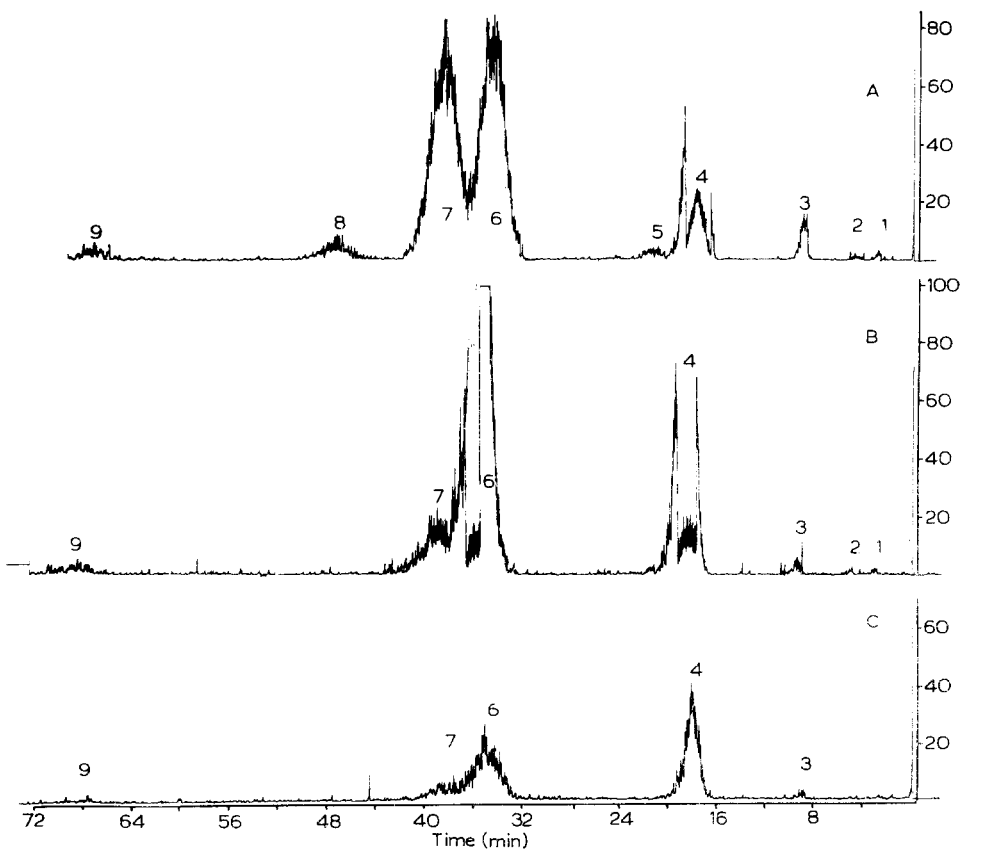



Fig. 4. The effect of lack of oxygen on the biosynthesis of oleic acid by chopped green leaves (*Ricinus communis*) for 3 h at 20°. Gas phase: Curve A, air; curve B, argon; curve C, argon–$CO_2$ (95:5). Peaks in order of appearance: 1, capric; 2, lauric; 3, myristic; 4, palmitic; 5, unknown, possible unsaturated $C_{16}$; 6, stearic; 7, oleic; 8, linoleic; 9, unknown, probably arachidic. Column: ethyleneglycol adipate at 178°. Counting time, 8.5 sec. Response time, 2 sec. Ordinate, radioactivity in counts/sec.

*Biosynthesis of more highly unsaturated acids*

The major fatty acid of all the leaves studied is linolenic acid but in periods up to 5 h very little label appears in this acid. In a single experiment a leaf was allowed to take up 1 $\mu$C of [2-$^{14}$C]acetate and was then kept standing in tap water under illumination for 24 h. In Fig. 5 are shown the changes of specific activity with time, of palmitic, oleic, linoleic and linolenic acids. Stearic acid had a low activity at all times. The expected cross-over of specific activity of oleic and linoleic acids occurs and linolenic acid shows appreciable activity after 24 h. Since linolenic acid is the major component, even with a low specific activity it possesses 15 % of all the fatty acid counts (Fig. 6).

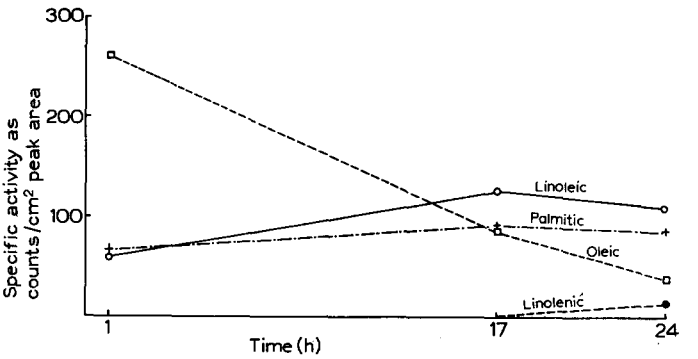



Fig. 5. Changes in specific activity of the fatty acids of a castor leaf with time, using [2-$^{14}$C]acetate as precursor.

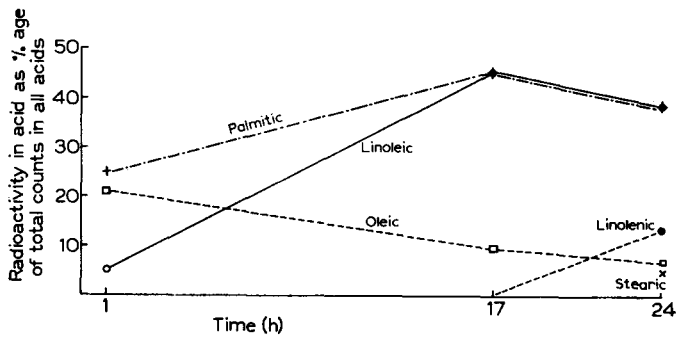



Fig. 6. Changes in the distribution of radioactivity among the fatty acids with time.

## DISCUSSION

The major fatty acids of the castor leaf lipids are palmitic (21 %), linoleic (13 %) and linolenic acid (60 %). The greater part of the linolenic acid is found in neutral lipid (Table II) (largely galactolipid), whereas the greater part of the linoleic acid is found in the phospholipids. It is notable that this fatty acid pattern is common

to the leaves of many species. The occurrence of unusual fatty acids containing epoxy or hydroxy groups seems to be limited to seeds of particular species.

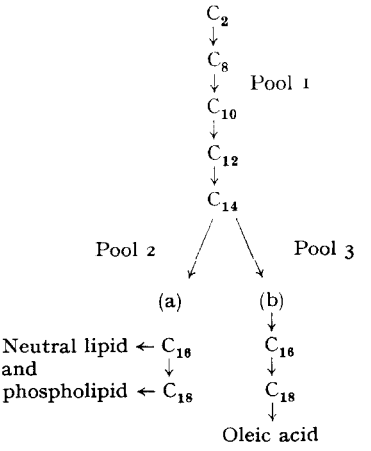
The acids synthesized most readily from labelled acetate are the $C_{12}$, $C_{14}$, $C_{16}$ and $C_{18}$ saturated acids, and oleic acid. This acid whilst only a minor component of the unsaturated acids contains most of the label (Fig. 1) and usually has a higher specific activity than stearic acid.

Two pathways of biosynthesis of oleic acid are known. In aerobic organisms such as yeast[11] and a blue-green alga (*Anabaena variabilis*), stearic acid is the precursor of oleic acid and the system requires both oxygen and TPNH. The same system occurs in rat liver[13].

In anaerobic microorganisms a different mechanism operates. The precursor of 9-octadecenoic acid is a $\Delta^3$ unsaturated dodecanoic acid which is chain lengthened by addition of three $C_2$ units[11]. In an earlier publication[10] a time study of the formation of both saturated and unsaturated acids from labelled acetate by an isolated castor leaf showed extensive labelling of oleic acid without any indication of prior labelling of stearic acid.

In these experiments no attempt was made to separate the free fatty acids, neutral lipid and phospholipid pools. When this was done similar results were obtained to those reported before (Figs. 2 and 3). At all times from 10 min to 3 h after uptake of acetate, stearic acid contained very little of the label. The small amounts of free acids present also showed this distribution of radioactivity.

The finding reported here that only the $C_8$, $C_{10}$, $C_{12}$ and $C_{14}$ saturated acids gave rise to labelled oleic acid in both isolated leaves and chopped leaves is not easily explicable on the basis of either of the mechanisms put forward by BLOCH *et al.*[11]. The failure to demonstrate conversion of either palmitic or stearic acid to oleic acid cannot be explained by lack of activation to the CoA esters since both acids were converted to phospholipid and galactolipids, processes proceeding via the thiol esters. The shorter-chain acids were not broken down into smaller molecules and then rebuilt into oleic acid, since isolation and degradation of the latter in each case demonstrated preservation of label position and hence an *in toto* incorporation into solely 9-octadecenoic acid. The octadecenoic acid obtained when [1-$^{14}$C]tetradecanoic acid was




A necessary assumption is that Pool 3 is impermeable to free palmitic and stearic acids.

used as precursor was found to be a mixture (Table VII), no explanation for this can be found at the moment.

The contradiction between these results and those reported by BLOCH *et al.*[11] for the blue-green algae was resolved only when both yellow (chlorophyll deficient) and normal green leaves were allowed to metabolize labelled acetate under anaerobic as well as aerobic conditions. In the absence of oxygen the synthesis of oleic and linoleic acids is inhibited whilst that of stearic acid is unaffected. The lower specific activity obtained in the presence of $CO_2$ is presumably due to dilution of labelled acetate by fixation of the unlabelled $CO_2$ (see Tables VIII and IX and Fig. 4).

Thus the synthesis of oleic acid does require oxygen and the $C_8$, $C_{10}$, $C_{12}$ and $C_{14}$ saturated acids can act as direct precursors whereas the $C_{16}$ and $C_{18}$ acids cannot. Although labelled palmitic acid gave rise to labelled stearic acid no conversion to oleic acid occurred. One explanation of the results would be the existence of three separate pools of free acids, one for acids from $C_2$ to $C_{14}$ capable of supplying precursors to either a system synthesizing palmitic and stearic acids or to one synthesizing palmitic, stearic and oleic acids. This scheme is shown below.

*Linoleic and linolenic acids*

In the earlier publication[6], it was shown that leaf tissue readily converted [1-$^{14}$C]-oleic acid to labelled linoleic acid. The absence of other labelled fatty acids except for those impurities already present (*i.e.* palmitic acid) demonstrated that no breakdown and resynthesis from smaller fragments had occurred. This was confirmed by oxidative degradation of isolated labelled linoleic acid which gave the expected pattern of mono and dicarboxylic acids with label present only in azelaic acid (*cf.* the results reported by CHING YUAN AND BLOCH[12]).

Although linolenic acid is the major fatty acid of leaf tissue, its rate of synthesis is very slow. Only after 24 h was this acid appreciably labelled when [2-$^{14}$C]acetate was used as precursor (see Fig. 6). The time curve suggests a slow conversion of linoleic acid to linolenic acid in agreement with the results of CHING YUAN AND BLOCH[12] who put forward the sequence oleic acid → linoleic acid → linolenic acid.

ACKNOWLEDGEMENTS

I should like to thank Mr. RUSSELL and the Director of the Royal Botanic Gardens, Kew, for growing the castor oil plants. Thanks are also due to Mr. A. WINFIELD of the National Institute for Medical Research for caring for the plants.

REFERENCES

1 R. O. SIMMONS AND F. W. QUACKENBUSH, *J. Am. Oil Chemists' Soc.*, 31 (1954) 441.
2 N. COPPENS, *Nature*, 177 (1956) 279.
3 J. B. MUDD AND P. K. STUMPF, *J. Biol. Chem.*, 236 (1961) 2602.
4 F. J. BARRON, C. SQUIRES AND P. K. STUMPF, *J. Biol. Chem.*, 236 (1961) 2610.
5 F. M. EBERHARDT AND M. KATES, *Can. J. Botany*, 35 (1957) 907.
6 A. T. JAMES AND J. P. W. WEBB, *Biochem. J.*, 66 (1957) 515.
7 A. T. JAMES AND E. A. PIPER, *J. Chromatog.*, 5 (1961) 265.
8 A. T. JAMES AND E. A. PIPER, to be published.
9 N. STUVE, *Gas Chromatography*, Butterworths, London, 1958.
10 A. T. JAMES, *Biochim. Biophys. Acta*, 57 (1962) 167.
11 K. BLOCH, P. BARONOWSKY, H. GOLDFINE, W. J. LENNARZ, R. LIGHT, A. T. NORRIS AND G. SCHEUERBRANDT, *Federation Proc.*, 20 (1962) 921.
12 CHING YUAN AND K. BLOCH, *J. Biol. Chem.*, 236 (1961) 1277.
13 J. B. MARSH AND A. T. JAMES, *Biochim. Biophys. Acta*, 60 (1962) 320.