Oxidation of unsaturated fatty acids by leaf tissue

C. HITCHCOCK and A. T. JAMES

Biosynthesis Division, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, England

SUMMARY Plant leaf systems have been incubated under various conditions with uniformly labeled oleic acid, and the breakdown products identified by gas–liquid chromatography. Young green leaves of the castor plant (*Ricinis gibsonii*) and of the field pea plant (*Pisum sativum*) catabolize oleic acid by an oxygen-requiring pathway to heptadec-8-enal which is then dehydrogenated by an NAD-coupled mechanism to heptadec-8-enoic acid. Further α-oxidation occurs with the production of hexadec-7-enoic acid and lower homologues. The α-oxidizing ability is associated with the fraction of homogenized leaves that sediments between 700 and 7000 $\times$ *g;* the partly purified acetone powders of leaves retain this enzymatic activity in soluble form. Preliminary studies with uniformly labeled linoleic acid suggest that α-oxidation of polyunsaturated acids also occurs in the leaf systems.

ALTHOUGH INFORMATION is now accumulating on the pathways of biosynthesis of unsaturated fatty acids in plant leaf systems (1, 2), little is known about their degradation. In this paper we report a study on the oxidation of oleic acid, and some preliminary results on the oxidation of linoleic and stearic acids, in cell-free systems obtained from leaves of *Ricinis gibsonii* (castor oil plant) and *Pisum sativum* (field pea plant).

EXPERIMENTAL METHODS

Materials

The castor oil plants were grown in an illuminated greenhouse (mercury vapor lamps) at 30° with a controlled day length of 11 hr. Leaves from other plants were obtained from field cultivated species. Seeds were germinated in vermiculite after swelling in water and dusting with commercial fungicide.

C^{14}-Labeled fatty acids were obtained from the Radiochemical Centre, Amersham, England; their specification is as follows:

(i) Generally labeled oleic acid. Specific activity: 88 mc/mmole (312 μc/mg). Radiochemical purity: 102% (by dilution analysis as 9,10-dihydroxystearic acid); 96% (by reversed phase paper chromatography).

(ii) Generally labeled linoleic acid. Specific activity: 39 mc/mmole (139 μc/mg). Radiochemical purity: 92% (by dilution and conversion to tetrabromostearic acid); 97% (by reversed phase paper chromatography).

(iii) Generally labeled stearic acid. Specific activity: 92 mc/mmole (324 μc/mg). Radiochemical purity: 98% (by dilution analysis).

(iv) Carboxyl-labeled oleic acid. Specific activity: 24.6 mc/mmole (87.2 μc/mg). Radiochemical purity: 100% (by dilution analysis as 9,10-dihydroxystearic acid); 97% (by reversed phase paper chromatography).

The radiochemical purity of each acid was found to be better than 95% by running a small sample as its methyl ester on the gas radiochromatograph (3). The acids were made up to a final concentration of 10 μc/ml by sonication in water containing a trace of sodium carbonate and Tween 80, after evaporation of the original benzene solvent. The solutions were stored frozen at −20°.

NAD, NADP, ADP, ATP, and CoA were obtained from L. Light & Co. Ltd., Colnbrook, Buckinghamshire, England, and stored at −20°.

Methods

Leaves (5–20 g) were homogenized at 0° in a blender for 45 sec in 150 ml of 0.5 M sucrose solution containing 1 g of sodium ascorbate, 2.5 ml of M NaCl, 2 ml of 0.2 M K_2HPO_4, and 0.5 ml of 0.2 M KH_2PO_4 (2). The resulting paste was filtered through four layers of cheesecloth, and then through eight layers by squeezing. The filtrate was centrifuged at 700 $\times$ *g* for 10 min at 5°; the supernatant fraction at 7000 $\times$ *g* for 30 min; and the second supernatant fraction at 100,000 $\times$ *g* for 30 min. The final supernatant solution contained soluble enzymes. The first sediment was washed by resuspension in the sucrose solution and recentrifugation: it was then dispersed in 0.2 M phosphate buffer and constitutes the "chloroplast" particulate fraction. The second and third sediments were washed once and dispersed in buffer as the "mito-

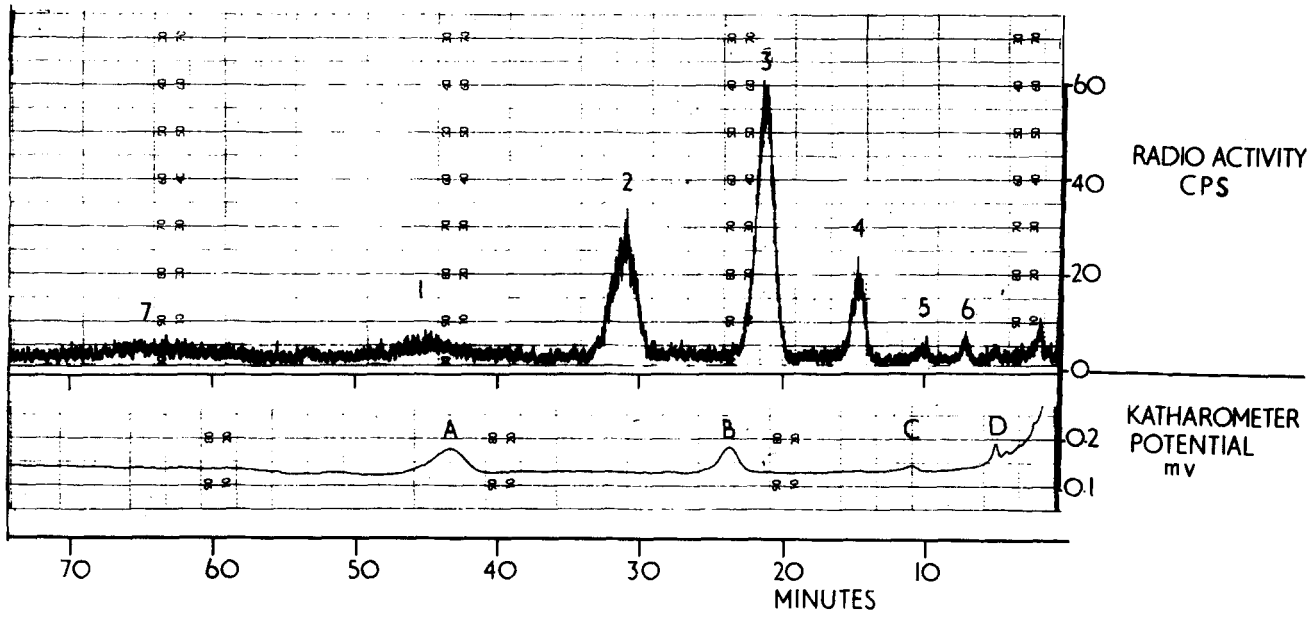

FIG. 1. α-Oxidation of oleic acid demonstrated as a radiochromatogram. Mitochondria from young pea leaves were incubated with oleic acid-U-C^{14} in 5 ml of 0.2 M phosphate buffer pH 7.0 containing 2 μmoles of NAD for 2 hr at 25°.

Peak identification: *1*, 18:1-C^{14} (added); *2*, 17:1-C^{14}; *3*, 16:1-C^{14}; *4*, 15:1-C^{14}; *5*, 14:1-C^{14}; *6*, 13:1-C^{14}; *7*, unknown. *A*, 18:0; *B*, 16:0; *C*, 14:0; *D*, 12:0.

Total radioactivity *1–7*, approx. 25 mμc. Total mass *A–D*, approx. 50 μg. Stationary phase: Apiezon L at 203°.

chondrial" and "microsomal" particulate fractions respectively. The association between a fraction and a particular subcellular particle was arbitrary, being based on centrifugal conditions only.

Freshly gathered pea leaves were homogenized in a blender for 5 min in about 20 times their weight of cold acetone; the wet powder was filtered off and similarly rehomogenized. The powder was washed with acetone on the filter, and finally with ether; after briefly drying on the filter, the powder was desiccated in vacuo over phosphorus pentoxide. It was stored in bulk at −20°.

GENERAL PROCEDURE

In a typical experiment, uniformly labeled oleic acid-C^{14} (1 μc) was added to 0.2 M phosphate buffer pH 7.0 (1.5 ml) containing either chopped plant tissue or a suspension or solution of plant material together with cofactors if appropriate. The mixture was incubated in air with reciprocal shaking in a water bath, usually at 25° for the appropriate time. For the time studies, incubations were carried out on ten times this scale, and aliquots of incubation mixture were withdrawn at intervals.

Extraction of Fatty Acids

The suspension was washed into excess (about 10 vol) chloroform–methanol mixture 2:1 (v/v) and left overnight; water was added and the lipid-containing lower layer removed and evaporated. The residue was refluxed with 5 ml of 10% methanolic sodium hydroxide for 2 hr; after making acid with excess 5 N sulfuric acid, the solution was extracted with ether. The ethereal layer was dried over sodium sulfate, and evaporated in a centrifuge tube whose tip touched the surface of a ventilated water bath at 80°. Immersion of the tube in ice-cold water then caused condensation of ether vapor on its sides; this washed the residue to the tip of the tube in about 0.1 ml of solvent. This solution was treated with an excess of freshly prepared diazomethane solution in ether (usually a few drops) and, after allowing 10 min at room temperature for the methylation, the volume of the solution was reduced to about 0.1 ml by evaporation as before. Some or all of this concentrated solution was carefully pipetted into an open stainless steel tube (internally threaded) and evaporated there in a gentle stream of oxygen-free nitrogen. If necessary, the centrifuge tube and pipette were washed by similarly evaporating about 0.5 ml of ether and adding the concentrated washings. The steel tube was now dropped into the radiochromatograph (3). The internal thread facilitated removal by means of a similarly tapped rod. Simultaneous records of mass and radioactivity of column effluent were obtained automatically.

The esters were separated on a 4-ft coiled column (internal diameter 4 mm) containing 100–120 mesh Celite supporting 20% Apiezon L grease at 200° or 80–100 mesh Celite supporting 20% ethylene glycol adipate polymer at 170°.

Extraction of Aldehydes

The incubation mixture was dried in a rotary evaporator at 40° in vacuo and the residue refluxed with excess 5% methanolic hydrogen chloride for 2 hr. Esters were formed by interchange, but under these conditions aldehydes yield their dimethyl acetals. The latter conveniently chromatograph on Apiezon grease at 200° in between the peaks of the homologous series of corresponding normal esters (4, 5) (cf. Fig. 3). Otherwise acetals and esters may be separated before chromatography by saponification of the latter and extraction of the former from the aqueous alkaline solution.

The katharometers of the radiochromatographs are able to detect as little as 1 μg of fatty ester (3), but the mass of metabolites investigated was too small for significant peak areas to be observed.

The proportional counters of the radiochromatographs can estimate activities above 0.1 mμc; thus the biological fate of added fatty acids is easily observed provided they have a high specific radioactivity. However, it was impossible to measure the specific activity of the metabolites, because of their small mass.

The conversion of added substrate to metabolite is demonstrated by the appearance of peaks associated with a radioactive product (Fig. 1); such conversion is measured as the percentage of radioactivity in each peak relative to the total activity in all peaks ("Percentage Conversion"). This figure describes the distribution of radioactivity in the substrate and long-chain metabolites at the end of the incubation, though in general the total observed radioactivity falls as the catabolism proceeds.

RESULTS

Germinating seeds and cotyledons of castor, groundnut, sunflower, and maize plants were used in preliminary experiments, since the seed fat laid down by the parent plant would be metabolized to provide energy and nourishment for the seedling. No formation of radioactive metabolites could be demonstrated, possibly because of the speed with which the oleic acid was oxidized to materials of low molecular weight. Radiochromatograms showed peaks only of added oleate. Altering the incubation temperature, time, or pH had no apparent effect.

When young green leaves of the castor plant (*Ricinis gibsonii*) were tested, two peaks were observed, the second being evidently due to a monounsaturated fatty acid containing seventeen carbon atoms (17:1). When larger leaves were tested the yield of this metabolite decreased. Old leaves gave none at all, though the age of the whole plant was immaterial (Table 1).

Our initial experiments were therefore confined to young castor leaves. A homogenate was prepared and separated by differential centrifugation at 0° into various subcellular fractions which were tested. Table 2 shows that the ability to oxidize oleic acid to heptadecenoic acid (17:1) was associated with a particulate fraction containing the mitochondria or particles of similar size (e.g., aggregated microsomes). The chloroplasts, already shown to be active in fatty acid biosynthesis (2), the microsomal fraction, and the supernatant fraction had no such ability: the trace of activity associated with the cytoplasm was presumably due to leakage of the soluble enzymes, caused by damage to the active particulate fraction during homogenization.

The relative retention volume of the methyl ester of the major metabolite on two stationary phases suggested it was a heptadecenoate. After catalytic hydrogenation, it had the same relative retention volume as added unlabeled methyl heptadecanoate. Quantitative cleavage with buffered KIO_4–$KMnO_4$ reagent at pH 7.6 (6) and radiochromatography of the products as methyl esters showed (Fig. 2) only two peaks of equal radioactivity: these behaved identically on two stationary phases with methyl nonanoate and dimethyl octanedioate. There was therefore one double bond in the 8:9 position; hence the metabolite was *n*-heptadec-8-enoic acid. Moreover, when the particulate fraction was incubated with oleic

TABLE 1 PERCENTAGE CONVERSION OF 18:1 TO 17:1 IN CASTOR LEAVES OF VARIOUS AGES

Approx. Dimensions of Leaf	Approx. Age of Plant	
	4 months	2 years
cm		
5	5.4	17
10	2.6	—
25	0	0

Each flask contained approx. 2 g of chopped leaf covered with 2–3 ml of 0.2 M phosphate buffer, pH 7.4, containing 1 μc oleic acid-U-C^{14}. Incubation conditions: aerobic, 7 hr at 28°.

TABLE 2 PERCENTAGE CONVERSION OF 18:1 TO 17:1 AND 16:1 IN SUBCELLULAR FRACTIONS OF CASTOR LEAVES

Centrifugation Conditions	Major Component	Cofactors Absent		Cofactors Present*	
		% 17:1	% 16:1	% 17:1	% 16:1
Below 700 × *g*	Chloroplasts	—	—	0	0
700–7000 × *g*	Mitochondria	3.7	0	21.7	1.9
Above 7000 × *g*	Microsomes + cytoplasm	0	0	0.2	0
7000–100,000 × *g*	Microsomes	—	—	0	0

Each flask contained 2 ml of 0.2 M phosphate buffer pH 7.0, 2 ml of subcellular fraction, and approx. 1 μc of oleic acid-U-C^{14}. Incubation conditions: aerobic, 5 hr at 25°.

* The following cofactors were present: 5 μmoles of $MnSO_4$, 5 μmoles of $MgSO_4$, 1 μmole of $KHCO_3$, 2 μmoles of NADP, 2 μmoles of NAD, 1 μmole of ATP, 1 μmole of ADP, 0.5 μmole of CoA.

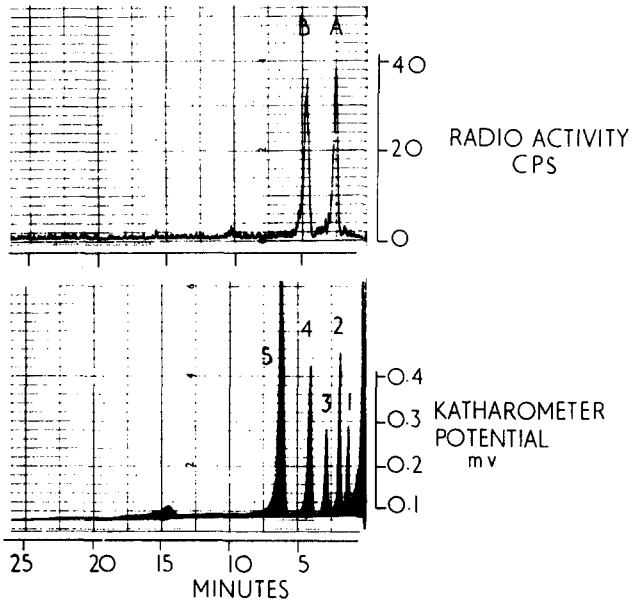

FIG. 2. Permanganate–periodate oxidation of radioactive 17:1 metabolite: radiochromatography of oxidation products as methyl esters. *A*, *B:* radioactive fragments. *1–5*, added markers: *1*, octanoate; *2*, nonanoate; *3*, decanoate; *4*, octandioate; *5*, nonandioate.

Stationary phase: Apiezon L at 198°.

acid-1-C^{14} no radioactivity was detected in any metabolite. Clearly the heptadecenoic acid was derived from oleic acid directly by an α-oxidation mechanism.

When the castor leaf subcellular fractions were supplemented with cofactors (Table 2) the conversion of oleic to heptadecenoic acid by the particulate "mitochondrial" fraction was greatly enhanced. The cofactors studied were: magnesium, manganese, carbonate, NADP, NAD, ADP, ATP, CoA. A partial requirement could be demonstrated for only NAD (Table 3); there was also an absolute requirement for molecular oxygen. The pH of the added buffer was evidently not critical (Table 4). Table 5 shows that the conversion is NAD-dependent and also does not require CoA.

In the absence of NAD, a second radioactive metabolite containing seventeen carbon atoms appeared at the expense of the heptadecenoate: this was detected only after the treatment (described above) of the incubation mixture to convert any aldehydes present to their dimethyl acetals (4, 5). Figure 3 shows the conversion of oleic acid-U-C^{14} to heptadecenoic acid-U-C^{14} by the

TABLE 3 COFACTOR REQUIREMENTS OF PARTICULATE FRACTION

Cofactor Omitted	Percentage Conversion to 17:1
None	12.7
Mn^{++}	15.3
Mg^{++}	14.2
HCO_3^-	14.8
NAD	7.0
NADP	12.9
ADP	13.1
ATP	14.3
CoA	11.5
All	1.0
Oxygen*	0.0

Each flask contained 1 ml of particulate fraction, 2 ml of 0.2 M phosphate buffer pH 7.0, 1 μc of oleic acid-U-C^{14}, and the following cofactors (except where omitted as noted in the table): 5 μmoles of Mn^{++}, 5 μmoles of Mg^{++}, 1 μmole of HCO_3^-, 2μmoles of NAD, 2 μmoles of NADP, 1 μmole of ATP, 1 μmole of ADP, 0.5 μmole of CoA. Incubation conditions: aerobic (unless otherwise stated), 4 hr at 25°.

* Incubation conditions: anaerobic (nitrogen), 4 hr at 21°.

TABLE 4 pH REQUIREMENTS OF PARTICULATE FRACTION

pH of Added Buffer	Percentage Conversion to 17:1	Percentage Conversion to 16:1
4.7	0	0
6.0	34	1
6.5	53	6
7.0	59	11
7.5	59	9
8.0	56	6
8.8	50	1

Each flask contained 1 ml of particulate fraction, 2 ml of 0.2 M phosphate buffer at the pH given, all cofactors given under Table 3 and 1 μc of oleic acid-U-C^{14}. Incubation conditions: aerobic, 5 hr at 25°.

TABLE 5 COFACTOR REQUIREMENTS OF PARTICULATE FRACTION

Cofactor Added	Percentage Conversion to 17:1
NAD + CoA	26
NAD	28
CoA	3
None	2

Each flask contained 0.7 ml of particulate fraction, 1 ml of 0.2 M phosphate buffer pH 7.0, 1 μc of oleic acid-U-C^{14}, and (where shown) 2 μmoles of NAD, 0.5 of μmole CoA. Incubation conditions: aerobic, 2 hr at 25°.

TABLE 6 α-OXIDIXING ABILITY OF VARIOUS PLANT LEAVES

Family	Plant	Percentage Conversion to 17:1
Rosaceae	Strawberry	0
Compositae	Lettuce	0.3
Leguminosae	Pea	64
Labiatae	Deadnettle	0.3
Umbelliferae	Cowparsnip	0
Cruciferae	Wallflower	2.3
Ranunculaceae	Buttercup	2.8
Gramineae	Rye grass	0

Each flask contained youngest leaves of the plant shown, picked on 5th June and chopped; these were covered with 5 ml of 0.2 M phosphate buffer pH 7.0 containing 1 μc of oleic acid-U-C^{14}. Incubation conditions: aerobic, 2 hr at 25°.

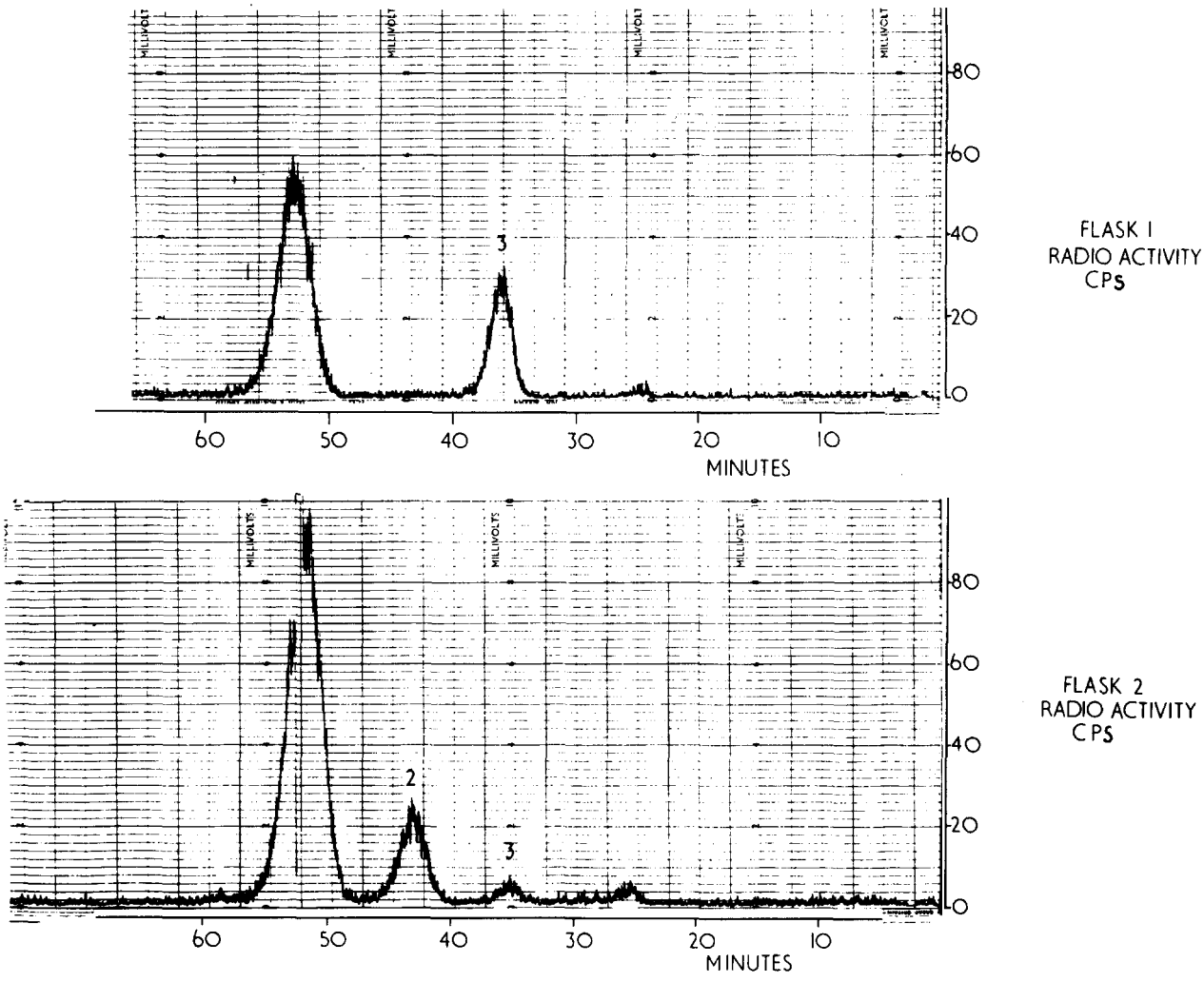

FIG. 3. Radiochromatography of oleic acid-U-C^{14} and its metabolites.

Flasks contained 2 ml of 0.2 M phosphate buffer pH 7.0, 1.5 ml of castor leaf "mitochondrial" fraction, 1 μc of oleic acid-U-C^{14}. Flask 1 contained in addition 2 μmoles of NAD. After aerobic incubation at 25° for 2 hr, the acids and aldehydes were radiochromatographed as their methyl esters and dimethyl acetals respectively. The corresponding katharometer records are not shown. *1*, methyl oleate-C^{14}; *2*, dimethyl acetal of heptadec-8-enal-C^{14}; *3*, methyl heptadec-8-enoate-C^{14}.

Stationary phase: Apiezon L at 200°.

particulate fraction: in the presence of NAD (Flask 1) heptadecenoic acid is formed in good yield, but in the absence of NAD (Flask 2) the radioactivity accumulates instead in a new peak corresponding to the dimethyl acetal of heptadecenal, as yet identified only by its chromatographic properties.

Chopped young leaves obtained from plants belonging to several families were tested. Table 6 shows that no α-oxidizing activity was observed in the leaves of strawberry and of cowparsnip and in rye grass; slight activity in lettuce and deadnettle; appreciable activity in wallflower and buttercup leaves; and considerable activity in pea leaves. Pea leaves were more efficient than castor leaves, and the efficiency was even more marked in their subcellular fractions (Table 7). The particulate fraction

TABLE 7 PERCENTAGE CONVERSION TO RADIOACTIVE METABOLITES IN SUBCELLULAR FRACTIONS OF PEA LEAVES

Fatty Acid	Particulate Fraction (700–7000 × g)	Supernatant Fraction (over 7000 × g)
	%	%
Unknown metabolite*	6	6
18:1 (unchanged)	5	30
17:1	17	54
16:1	63	8
15:1	7	2
14:1	1	0
13:1	1	0

Each flask contained 5 ml of subcellular fraction shown, 1 μc of oleic acid-U-C^{14}, and 2 μmoles of NAD. Incubation conditions: aerobic, 2 hr at 25°.

* Retention volume relative to 18:1 on Apiezon L at 203°: 1.45.

(700–7000 × *g*) yielded a homologous series of radioactive metabolic products apparently involving five successive α-oxidations (Fig. 1). The supernatant fraction (about 7000 × *g*) retained much activity: this suggested that (*a*) more leakage of soluble enzymes occurred during homogenization of pea leaves than that of castor leaves; or (*b*) two oxidizing systems (associated with different sizes of particle) were present; or (*c*) a single microsomal system (present in the supernatant solution) caused α-oxidation, though most of these particles were aggregated and so sedimented in the particulate fraction.

Because of the high activity and abundant availability of young pea leaves, these were used in experiments designed to obtain a soluble α-oxidizing system. Crude acetone powders of the leaves supplemented with NAD proved to give high yields of α-oxidation products of oleic acid-U-C^{14}. These yields were not affected by using veronal instead of phosphate buffer at pH 7.0.

The protein obtained from the acetone powder by 50% saturation with ammonium sulfate at pH 7.0 exhibited efficient α-oxidizing ability; the fact that it was subject to the same cofactor requirements and yielded the same products as the castor leaf particulate fraction indicated the similarity of the systems. Table 8 shows the partial requirement for NAD, and also that no exogenous hydrogen peroxide source is needed. When the isolated metabolite heptadec-8-enoic acid-U-C^{14} was incubated with the active castor leaf particulate fraction none of the expected formation of hexadecenoate was observed. Apparently the particulate system accepts octadecenoate, but not heptadecenoate under identical conditions. When the pea leaf acetone powder system was used, however, a 45% conversion to hexadecenoate occurred: the identity of this metabolite was confirmed by permanganate–periodate cleavage as already described. It proved to be hexadec-7-enoic acid, the expected isomer of the usual palmitoleic acid. The variation with time of the radioactivity of oleic acid-U-C^{14} and its metabolites during oxidation by pea leaf acetone powder (in the presence of NAD) is illustrated by Fig. 4.

TABLE 8 COFACTOR REQUIREMENTS OF SOLUBLE PROTEIN FROM PEA LEAF ACETONE POWDER

Cofactors Added	Percentage Conversion to 17:1	Percentage Conversion to 16:1
None	25	0
NAD (2 μmoles)	47	12
NAD (2 μmoles) + glucose–glucose oxidase	48	7

Each flask contained the soluble protein from 100 mg of crude pea leaf acetone powder, 1 ml of 0.2 M phosphate buffer pH 7.0, approx 0.5 μc of oleic acid-U-C^{14}, and the cofactors given. Incubation conditions: aerobic, 1 hr at 25°.

Stearic acid-U-C^{14} is α-oxidized by the acetone powder to heptadecanoate-C^{14} and palmitate-C^{14}: these metabf olites were identified by their relative retention volumes- Radioactive metabolites obtained during the oxidation o. linoleic acid-U-C^{14} by the acetone powder include two whose relative retention volumes suggest a heptadecadienoate (17:2) and a hexadecadienoate (16:2). The identity of these metabolites, the α-oxidation of other polyunsaturated fatty acids, and the isolation of the enzyme system responsible are now being investigated.

DISCUSSION

These results clearly indicate an α-oxidation (Fig. 5) of stearic, oleic, and linoleic acids in the green leaf systems. No long-chain fatty acid intermediates expected from a β-oxidation system were observed, though simultaneous β-oxidation could be occurring in the particulate systems if its rate were such that no buildup of intermediates occurred. The α-oxidation of saturated long-chain fatty acids has been previously observed in plant systems by Martin and Stumpf (7). They reported that a specific peroxidase (8, 9), requiring an exogenous hydrogen per-

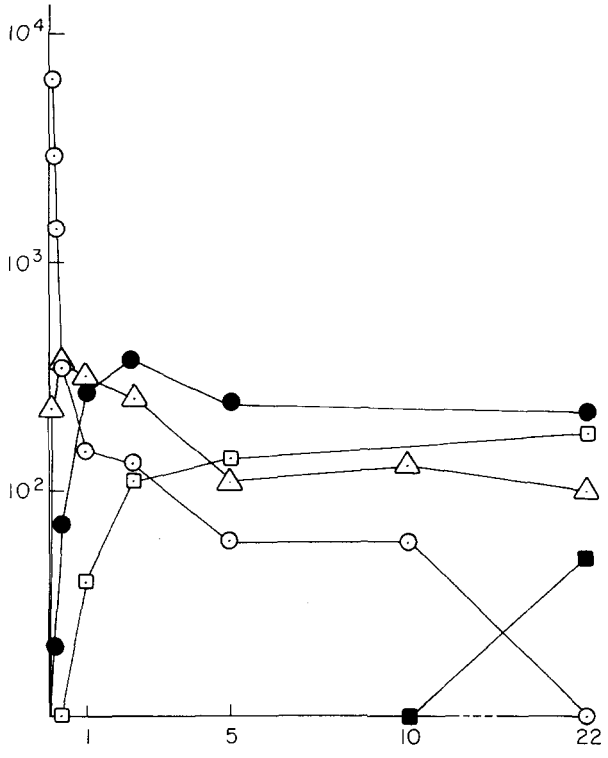

FIG. 4. Variation with time of the distribution of radioactivity of metabolites during incubation at 25° of 1 g of pea leaf acetone powder dissolved in 20 ml of 0.2 M phosphate buffer pH 7.0 containing 20 μmoles of NAD with 10 μc of oleic acid-U-C^{14}. Vertical axis: total radioactivity recovered in each fatty acid, counts. Horizontal axis: time of incubation, hr.
⊙ 18:1-C^{14} (added); △ 17:1-C^{14}; ● 16:1-C^{14}; ⊡ 15:1-C^{14}; ■ 14:1-C^{14}.

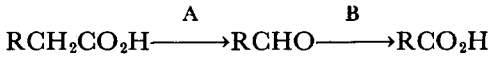

FIG. 5

oxide source, caused decarboxylation to the lower aldehyde (Fig. 5, route A); this was then oxidized to the corresponding acid by an NAD-specific dehydrogenase (Fig. 5, route B). They found the enzymes in fresh homogenates and in acetone powders of germinating cotyledons; in the peanut, the dehydrogenase was associated with the microsomes while the peroxidase remained in the supernatant solution. Palmitic acid-C^{14} was mainly used as substrate, though all normal saturated acids from lauric to stearic inclusive were found to be acceptable. Preliminary experiments with oleic acid-1-C^{14} indicated that unsaturated fatty acids were also subject to α-oxidation, though more slowly. The oxidation was followed (7) by using substrate labeled at particular positions along the carbon chain, and (*a*) determining the amount and radioactivity of carbon dioxide evolved, (*b*) isolating the metabolites by paper chromatography, and (*c*) estimating the concentration of metabolic aldehydes by the enzyme luciferase.

The castor leaf particulate and pea leaf acetone powder systems described in this paper appear to be similar to the cotyledon systems of Stumpf et al. Both cause α-oxidation to the lower aldehyde (Fig. 5) which is dehydrogenated by an NAD-specific mechanism to the corresponding acid. However, we have not yet been able to demonstrate any requirement for a hydrogen peroxide source, and our systems are particle-bound. Moreover, the catabolism of seed fat is expected in a germinating seedling which must rise above ground before photosynthesis is possible; but the leaf systems described in this paper originate from mature plants. It is not at all clear why such systems exist. If α-oxidation is a significant metabolic pathway in the growing plant, it must be that free fatty acids as such exist at the site of oxidation, and since they are evidently not synthesized there, they must be transported (*a*) from storage fat, (*b*) from the chloroplasts of the same leaf, or (*c*) from the chloroplasts of another leaf. Factor (*a*) is obviously important in seedlings utilizing seed fat, but no obvious fat reserves exist in mature plants. Factors (*b*) and (*c*) raise the possibility of translocation of fatty acids to and within young leaves. Since the leaf loses its ability to α-oxidize as it matures (Table 1) it would appear that young photosynthesizing leaves whose growth is not complete require exogenous nutrient, and receive part of it as fatty acid from neighboring leaves. It is generally thought that the mature leaves of the growing plant export to the young leaves, while no exchange of products occurs between mature leaves (e.g., Kursanov, 10). No direct evidence for the translocation of fatty acid in plants has yet been found. The main nutrient transported in the phloem is presumed to be carbohydrate, but small concentrations of fatty acids may be of importance if their turnover is rapid.

α-Oxidation occurs in the absence of phosphate and CoA; apparently no preliminary activation of the substrate is required. In this respect α-oxidation differs sharply from β-oxidation. The precise site of oxidation in the leaf is uncertain. The enzymes concerned are evidently bound to subcellular particles which are smaller than chloroplasts: they may be mitochondria (which are associated with other oxidizing systems) or microsomes (as in the systems of Stumpf et al.).

The mechanism of α-oxidation is not clear: the lower aldehyde is apparently an intermediate, though our experiments do not preclude the possibility of another route not involving the aldehyde. The unique nature of the peroxidase system has been pointed out by Martin and Stumpf, who also suggest a possible mechanism of its action (7).

The appearance of radioactive metabolites having a greater retention volume (on Apiezon L) than oleate (e.g., Table 7) suggests the formation of hydroxy- or oxo-intermediates, which are being studied. Fulco and Mead (11) have presented evidence that, in rat brain, cerebronic acid is formed by direct hydroxylation of lignoceric acid (24:0); Mead and Levis (12,13), using the same system, have proposed an α-oxidation pathway for the production of tricosanoic (23:0) from cerebronic acid.

A preliminary report of these results has already been made (14).

The valuable assistance of K. Harris is gratefully acknowledged. We would also like to thank Mr. W. Kovachich for putting the facilities of the Basic Horticulture laboratory at our disposal and Mr. B. Parker for growing many of the plants used.

Manuscript received January 20, 1964; accepted April 23, 1964.

REFERENCES

1. James, A. T. *Biochim. Biophys. Acta* **70:** 9, 1963.
2. Stumpf, P. K., and A. T. James. *Biochim. Biophys. Acta* **70:** 20, 1963.
3. James, A. T., and E. A. Piper. *Anal. Chem.* **35:** 515, 1963.
4. Farquhar, J. W. *J. Lipid Res.* **3:** 21, 1962.
5. Gray, G. M. *J. Chromatog.* **4:** 52, 1960.
6. Lemieux, R. U., and E. von Rudloff. *Can. J. Chem.* **33:** 1701, 1955.
7. Martin, R. O., and P. K. Stumpf. *J. Biol. Chem.* **234:** 2548, 1959.
8. Castelfranco, P., P. K. Stumpf, and R. Contopoulou. *J. Biol. Chem.* **214:** 567, 1955.
9. Stumpf, P. K. *J. Biol. Chem.* **223:** 643, 1956.
10. Kursanov, A. L. *Endeavour* **20:** 19, 1961.
11. Fulco, A. J., and J. F. Mead. *J. Biol. Chem.* **236:** 2416, 1961.
12. Mead, J. F., and G. M. Levis. *J. Biol. Chem.* **238:** 1634, 1963.
13. Levis, G. M., and J. F. Mead. *J. Biol. Chem.* **239:** 77, 1964.
14. Hitchcock, C., and A. T. James. *Biochem. J.* **89:** 22P, 1963.