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The Synthesis of Fatty Acids in Avocado Mesocarp and Cauliflower Bud Tissue

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1. Plastid and mitochondrial preparations were obtained by density-gradient centrifugation of homogenates made by gentle disintegration of avocado fruit mesocarp and cauliflower bud tissue. 2. The mitochondrial preparations had respiratory activity but did not incorporate [1-^{14}C]acetate into fatty acids. 3. The plastid preparations incorporated [1-^{14}C]acetate into the range of fatty acids found in the parent tissue. No fatty acid synthetase activity could be detected in the 12000*g* supernatant of these homogenates. 4. Homogenates produced by rupture of the tissue in an Ato-Mix blender and plastid preparations disintegrated by ultrasonic treatment both had fatty acid synthetase activity which did not sediment at 105000*g* and which formed mainly [^{14}C]stearate from [2-^{14}C]malonyl-CoA. 5. It is concluded that the plastids are the principal site of fatty acid biosynthesis in the tissues studied.

The present view of the site of fatty acid biosynthesis within the cells of higher plants is confused and the evidence in some respects is contradictory. The first observations of fatty acid biosynthesis in higher plants with cell-free preparations were made by Sisakyan & Smirnov (1956) who reported the incorporation of [1-^{14}C]acetate into fatty acids by chloroplast preparations from sunflower leaves. The earliest description of fatty acid biosynthesis by cell-free preparations from a non-photosynthetic tissue was made by Stumpf & Barber (1957) who reported the incorporation of [2-^{14}C]acetate predominantly into palmitate and oleate and, to a lesser extent, into stearate by a particulate preparation obtained by centrifuging at 10000*g* the brei obtained from the mesocarp of an avocado pear by use of a vegetable grater. The particulate preparation obtained was termed mitochondrial on account of its method of preparation.

The study of the biosynthesis of fatty acids in subcellular fractions derived from photosynthetic tissue was carried a stage further by Smirnov (1960) who showed light-stimulated incorporation of [1-^{14}C]acetate into fatty acids by a particulate preparation obtained from leaves of *Spinacea oleracea* by low-speed centrifugation. Stumpf & James (1963) demonstrated light-dependent incorporation of [^{14}C]acetate into fatty acids by chloroplast preparations obtained from lettuce leaves.

The biosynthesis of fatty acids by non-photosynthetic plant tissues was investigated further by Mudd & Stumpf (1961). A homogenate was obtained by grinding avocado mesocarp in a mortar; particles sedimenting from this homogenate at 2000–6000*g* displayed considerable fatty acid synthetase activity when [1-^{14}C]acetate was the substrate; particles sedimenting at 15000*g* had much less fatty acid synthetase activity, and the supernatant had no detectable synthetase activity with the same substrate. It was, however, noted that, whereas oleate formed about 70% of the total fatty acids of the avocado mesocarp, only about 30% of the total [1-^{14}C]acetate incorporated into fatty acids was incorporated into oleate by the active particles. Barron *et al.* (1961) prepared an extract of an acetone-dried powder of a particulate preparation, similar to that of Mudd & Stumpf (1961), which incorporated [1-^{14}C]acetate, [1-^{14}C]acetyl-CoA and [1,3-^{14}C]malonyl-CoA into fatty acids. The parent particulate fraction from which the extract was made was reported to sediment at 15000*g*, to have tricarboxylate-cycle activity, and to carry out oxidative phosphorylation.

Yang & Stumpf (1965) reported that a supernatant fraction of an avocado-pear homogenate prepared in a Waring blender incorporated [1,3-^{14}C]malonyl-CoA and [1,3-^{14}C]malonate but not [1-^{14}C]acetate or [1-^{14}C]acetyl-CoA into saturated fatty acids. On the other hand, the particles sedimenting at 15000*g* incorporated [1-^{14}C]acetate, [1-^{14}C]acetyl-CoA and [2-^{14}C]malonyl-CoA, but not [2-^{14}C]malonate into saturated and unsaturated fatty acids. From this it was concluded that the supernatant fraction lacked the acetyl-CoA carboxylase necessary to form malonyl CoA.

Overath & Stumpf (1964) showed that a fatty acid synthetase preparation obtained by $(NH_4)_2SO_4$ fractionation of the particle-free supernatant of the avocado-pear homogenate was dependent on a heat-stable factor from the supernatant. This factor could be replaced by a similar factor from the *Escherichia*

coli fatty acid synthetase system. Subsequently Simoni *et al.* (1967) obtained an acyl-carrier-protein preparation from the avocado pear that stimulated a spinach chloroplast fatty acid synthetase activity.

Harwood & Stumpf (1972) have reported that the particle-free supernatant obtained by centrifuging at 15000*g* a homogenate of the avocado mesocarp prepared in a Potter homogenizer was almost completely dependent on the addition of acyl-carrier protein for the demonstration of fatty acid synthetase activity.

From the above and from numerous other reports it might be concluded that fatty acid biosynthesis occurs in plants in at least three different subcellular locations. In photosynthetic tissues, isolated chloroplasts have been found to incorporate acetate into fatty acids, although whether preparations actively fixing CO_2 do this has been disputed (Everson & Gibbs, 1967; Boardman & Stumpf, 1970). Preparations described as mitochondrial have been found to form saturated, and to a much smaller extent unsaturated, fatty acids from acetate and acetyl-CoA, but the respiratory activities of these preparations have not been described in detail. Particle-free supernatants synthesize fatty acids from malonate and malonyl-CoA by using an enzyme system that appears to use acyl-carrier protein like the bacterial system. These preparations, however, appear to form saturated fatty acids only from malonyl-CoA or malonate and cannot utilize acetate or acetyl-CoA.

In view of this apparently complex situation the work reported in the present paper was undertaken to investigate the credibility of the attribution of synthesis of fatty acids *de novo* to the mitochondrial and cytoplasmic components of the cells of non-photosynthetic tissues of higher plants. Some of the observations described in this paper have been outlined in a preliminary communication (Weaire & Kekwick, 1970).

Materials and Methods

Materials

Plant material. Avocado fruit and cauliflowers were obtained from local retailers and were used on the day of purchase.

Special chemicals. Radiochemicals were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. The specific radioactivity of the [1-^{14}C]acetate was adjusted to 1.0Ci/mol and that of the [2-^{14}C]malonate, [U-^{14}C]glucose, [2,4-^{14}C]succinate and [1,5-^{14}C]citrate to 1.25Ci/mol.

[1-^{14}C]Acetyl-CoA (sp. radioactivity 0.67Ci/mol) was synthesized from [1-^{14}C]acetic anhydride by the procedure of Simon & Shemin (1953) as modified by Higgins & Kekwick (1973). [2-^{14}C]Malonyl-CoA (sp. radioactivity 0.2mCi/mol) was prepared from [2-^{14}C]malonic acid by the procedure of Trams & Brady (1960) and was purified by the procedure of Higgins & Kekwick (1973). Acyl-carrier protein was prepared from *E. coli* strain B (Whatman Biochemicals Ltd., Maidstone, Kent, U.K.) by the procedure of Majerus *et al.* (1969).

Coenzyme A, ATP, NADH, NADPH and bovine serum albumin were obtained from Sigma Ltd., London S.W.6, U.K.

Methods

Isolation of sub-cellular particles. All manipulations were carried out at 4°C and centrifuged preparations were made in MSE major and Superspeed 40 and 50 centrifuges.

Avocado preparations. Crude mitochondrial preparations were prepared from the mesocarp of the avocado pear (*Persea americana*) by the method of Hobson *et al.* (1966). The fruit was chilled at 4°C, peeled, and the seeds were removed. Chilled mesocarp tissue (120g) was passed through a plastic vegetable grater, having holes 6mm in diameter, into 240ml of a grinding medium, containing 0.3M-sucrose, 20mM-Tris–HCl buffer, pH7.2, 20mM-potassium phosphate buffer, pH7.2, 4mM-*N*-acetylcysteine, 10mM-KCl, 0.5mM-EDTA and 0.75mg bovine serum albumin/ml. The tissue was dispersed by gentle stirring and was filtered through three layers of surgical gauze. The filtrate was centrifuged at 1500*g* for 15min and the supernatant removed, and centrifuged at 12000*g* for 20min. The pellet thus obtained was resuspended in a suspending medium (5.0ml) of the same composition as the grinding medium except that *N*-acetylcysteine was omitted. This crude mitochondrial preparation was used for the initial studies of ADP-controlled respiration of tricarboxylate cycle intermediates and fatty acid biosynthesis.

Purified mitochondrial and plastid preparations were obtained from this preparation by centrifugation on a discontinuous sucrose density gradient by the procedure of Baker *et al.* (1968). The gradient consisted of the following sucrose solutions, all of which also contained 10mM-KCl, 2.0mM-Tris–HCl buffer, pH7.2, 20mM-potassium phosphate buffer, pH7.2, and 0.75mg of bovine serum albumin/ml, each being successively layered in a 20ml centrifuge tube: 2.5ml of 1.6M, 3.0ml of 1.4M, 3.0ml of 1.2M, 2.5ml of 1.0M, 2.5ml of 0.8M, 2.5ml of 0.6M. The tubes were centrifuged in a 3×20ml swing-out rotor at 15000rev./min for 1h. Fractions were removed with a syringe, diluted fivefold with the suspending medium (as above) and centrifuged at 12000*g* for 15min. The resultant pellets were resuspended in a volume of the suspending medium appropriate for the enzyme assay.

Plastid preparations were also obtained by direct centrifugation of avocado mesocarp homogenates without density-gradient centrifugation. The homogenate was prepared as described above and was filtered through six layers of surgical gauze. The filtrate was then centrifuged at 200*g* for 10min to remove cell debris and nuclei and the floating lipid layer was removed by suction. The remaining supernatant was centrifuged at 2000*g* for 10min; a plastid preparation was obtained by suspending the pellet thus obtained in 5–10ml of suspending medium. A cytoplasmic preparation was prepared by taking the supernatant obtained by centrifuging the 2000*g* supernatant at 12000*g*.

Cauliflower preparations. Sub-cellular preparations were obtained from cauliflower by a procedure similar to that outlined for avocado pears. Small cauliflowers were washed in distilled water, blotted dry and chilled to 4°C. The outer layer of buds (2–3mm thick) was removed and homogenized with a pestle, in a mortar containing 160ml of grinding medium. The homogenate was filtered through three layers of surgical gauze and the filtrate was centrifuged at 800*g* for 10min. The resulting supernatant was centrifuged at 12000*g* for 15min and the pellet thus obtained was resuspended in the standard suspending medium (see above) and constituted the crude cauliflower mitochondrial preparation.

Purified mitochondrial and proplastid preparations were obtained from this crude preparation by the discontinuous sucrose-density-gradient procedure of Baker *et al.* (1968) as described above.

Degradation of plastid preparations. Plastid preparations were ruptured in an MSE ultrasonic disintegrator at 360W. The optimum time of ultrasonic treatment for recovery of fatty acid synthetase activity from avocado plastids was 5s.

Extraction and analysis of fatty acids. Lipids were extracted from the incubation mixture by the procedure of Bligh & Dyer (1959) as modified by Galliard *et al.* (1965). The lipid extracts were dissolved in 10ml of 5% (v/v) methanol containing 5% (w/v) KOH and were saponified by refluxing for 1h. Incorporation of radioactive substrate into the non-saponifiable lipid fraction was negligible and the methanolic solution was therefore acidified with 2ml of 6M-HCl without prior extraction of non-saponifiable lipid. The lipid solution was then evaporated to dryness under decreased pressure; the residue was dissolved in distilled water and was extracted with three portions each of 10ml of chloroform.

The methyl esters of the fatty acids were prepared by reaction with diazomethane and were analysed in the Pye Argon gas chromatograph with a column [4ft (120cm)] of either 5% (w/v) polyethylene glycol adipate or 10% (w/v) Apiezon L grease supported on celite (80–120mesh). The mass peaks were identified by comparison with reference fatty acids. The radioactive fatty acids were trapped on glass wool in a Packard automatic gas fraction collector. The traps containing the separated fatty acid methyl esters were assayed for radioactivity by scintillation counting (see below). The average recovery of radioactivity was 85%.

To investigate the labelling pattern of fatty acids, the purified acids were degraded by the Schmidt (1923) reaction by the procedure of Madsen *et al.* (1964). A known amount of purified radioactive fatty acids was dissolved in light petroleum (b.p. 40–60°C) and the solvent was evaporated in the reaction vessel in a stream of N_2. The reaction vessel consisted of a 25ml round-bottomed flask fitted with a side arm to which was attached a small $CaCl_2$ tube containing glass wool. The glass wool was impregnated with a solution of 1.0M-Hyamine hydroxide in methanol mixed with methanol and toluene (1:2:3, by vol.). Sodium azide (50mg), followed by 0.5ml of a mixture of fuming and conc. H_2SO_4 (1:3, v/v), was introduced into the reaction vessel which was then immediately closed. The vessel was heated at 60°C for 1.5h and N_2 was flushed through the apparatus for 10min. The $CaCl_2$ tube was removed and the contents were washed into a scintillation vial with 10ml of the phosphor toluene solution (see below) and 5ml of light petroleum (b.p. 40–60°C). The recoveries of $^{14}CO_2$ from chemically synthesized [1-^{14}C]palmitic acid and [1-^{14}C]oleic acid were from 99.8 to 100% and 87 to 95% respectively.

Protein determination. The protein concentration of the subcellular preparation was determined by the biuret method of Gornall *et al.* (1949) after the removal of interfering pigments. A 1ml portion of the incubation mixture was treated with 0.5ml of 10% (w/v) trichloroacetic acid and the mixture was extracted with an equal volume of diethyl ether; the ether layer was separated by centrifugation and removed. The protein layer at the interface was dispersed in the aqueous layer, centrifuged down and the supernatant was removed. The protein precipitate was dissolved in 1ml of 2M-KOH and treated with 4.0ml of biuret reagent.

Measurement of oxygen uptake. The respiratory activity of mitochondrial preparations was measured polarographically by using a Clark oxygen electrode (Yellow Springs Instrument Co., Ohio, U.S.A.) adapted for following mitochondrial respiration by the procedure of Chappell (1964). The respiration medium was that of Hobson *et al.* (1966) and contained 0.25M-sucrose, 10mM-Tris–HCl buffer, pH7.2, 10mM-potassium phosphate buffer, pH7.2, 5mM-$MgCl_2$, 0.5mM-EDTA and 0.75mg of bovine serum albumin/ml. Mitochondria were added to the reaction media at 37°C followed by succinate

or other tricarboxylate-cycle intermediates to a final concentration of 20mM. The addition of ADP to give a concentration of 0.2mM resulted in the mitochondria being in steady state 3 as defined by Chance & Williams (1956). The rate of ADP-stimulated respiration of succinate was used as an index of mitochondrial respiratory activity.

Incubations. Each incubation mixture contained 0.3ml of subcellular preparations, 2μmol of $MnCl_2$, 200nmol of NADH, 200nmol of NADPH, 30μmol of HCO_3^-, 50nmol of CoA and 10μmol of ATP in a total volume of 1.5ml of the standard suspending medium (see above). The concentration of radioactive substrate present is given after each experiment in the Results section. The mixtures were incubated at 30°C for 1h. The reaction was stopped by the addition of 0.1ml of 2M-HCl followed by 6.0ml of chloroform–methanol (1:2, v/v) and the lipids were extracted by the modified Bligh & Dyer (1959) procedure (see above).

Assay of radioactivity. The ^{14}C content of isolated lipids was assayed in a β scintillation spectrometer by the methods as previously described by Higgins & Kekwick (1973).

Electron microscopy. Samples of mitochondrial and plastid preparations were prepared for electron microscopy by a procedure similar to that described by Baker *et al.* (1968) for avocado mitochondria. Samples were taken from the centre of centrifuged pellets containing the organelle and fixed for 2h in 2.5% (w/v) glutaraldehyde dissolved in the standard suspending medium. The fixed pellets were then washed with suspending medium and post-fixed with 1% osmium tetroxide dissolved in the suspending medium. The samples were then washed, dehydrated with alcohol and embedded in 'Araldite' resin. Embedded samples were blocked out in gelatine capsules with Araldite resin, and were sectioned with an ultramicrotome. Sections were examined with an A.E.I. EM6 electron microscope.

Results

Fatty acid synthesis by mitochondrial preparations

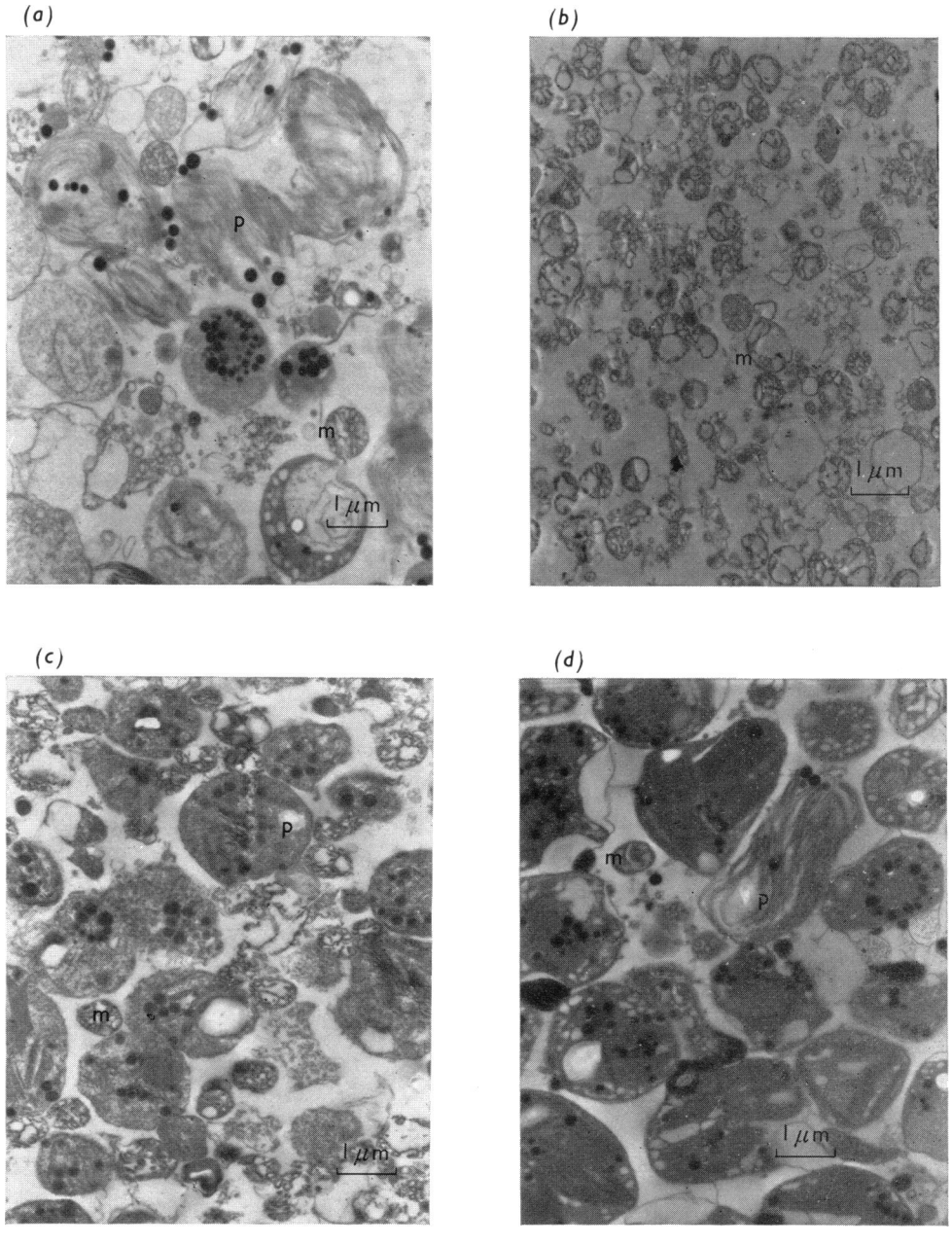
The first objective of the investigation was to study the incorporation of [1-^{14}C]acetate into fatty acids by mitochondrial preparations which showed good respiratory control of the phosphorylation of ADP, and had thereby satisfied one criterion by which their degree of intactness might be assessed. Mitochondrial preparations were therefore obtained both from avocado mesocarp and from cauliflower buds by the differential centrifugation procedure of Hobson *et al.* (1966). The respiratory characteristics of these preparations are shown in Table 1; the oxidation of succinate by both preparations was subject to respiratory control by ADP, but neither preparation showed this respiratory control of the oxidation of citrate or pyruvate.

Both preparations catalysed the incorporation of [1-^{14}C]acetate into fatty acids, and both preparations incorporated more of this substrate into oleate than into any other fatty acid (Table 2). The distribution of label amongst the fatty acids formed by both preparations was similar except that the cauliflower preparation incorporated a small amount of [1-^{14}C]-acetate into linolenate and the avocado mesocarp preparation incorporated some of the radioactive substrate into lower-molecular-weight fatty acids (C_{12}–C_{14}). The 12000*g* supernatant from neither preparation had fatty acid synthetase activity, but when [1-^{14}C]acetate was the substrate this fraction had a somewhat inhibitory effect on the incorporation of radioactivity into fatty acids by the crude mitochondria.

Table 1. *Respiratory characteristics of avocado and cauliflower mitochondria*

The mitochondrial suspension (0.1ml) was added to incubation mixtures containing 20mM substrate, 0.25M-sucrose, 10mM-potassium phosphate buffer, pH7.2, 10mM-Tris–HCl buffer, pH7.2, 5mM-$MgCl_2$, 0.5mM-EDTA, 0.75mg of bovine serum albumin/ml in a total volume of 1.2ml. State 3 of oxidation (Chance & Williams, 1956) was induced by the addition of ADP to a concentration of 200μM.

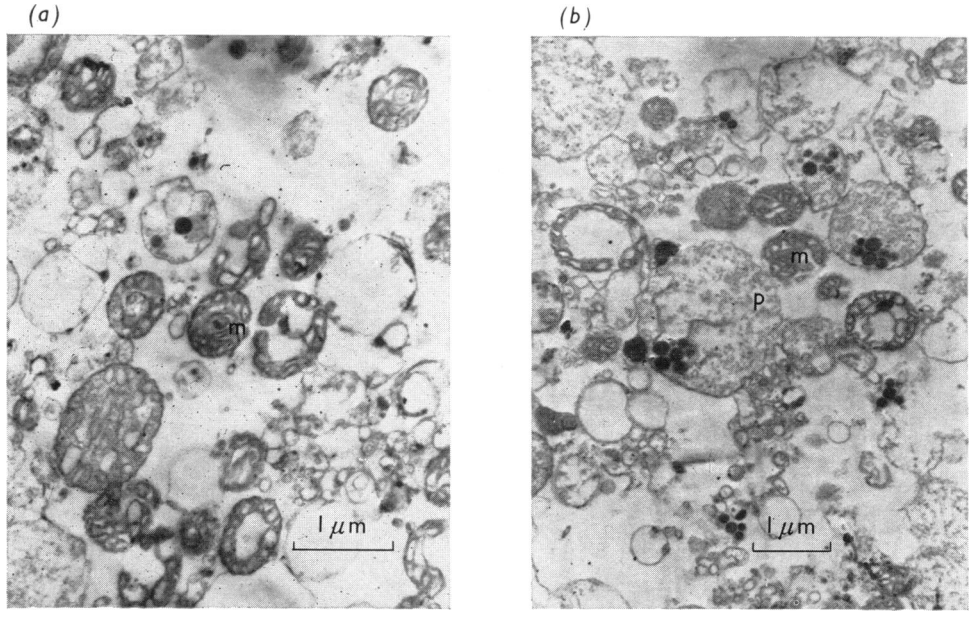
	Uptake of O_2 (nmol/min per mg of protein)					
	State 3		State 4		ADP/O	
	Avocado	Cauliflower	Avocado	Cauliflower	Avocado	Cauliflower
Succinate	104.0	87.0	44.7	13.4	1.5	1.2
Citrate	9.6	25.5	9.6	25.5	—	—
Malate	—	24.3	—	6.6	—	1.6
Citrate+1mM-malate	9.6	2.5	9.6	25.5	—	—
Pyruvate	0	0	0	0	—	—
Pyruvate+1mM-malate	9.3	12.7	9.3	12.7	—	—



EXPLANATION OF PLATE 1

Electron micrographs of subcellular preparations of avocado mesocarp

(*a*) Crude avocado mitochondrial preparation obtained by differential centrifugation of the mesocarp homogenate as applied to the sucrose density gradient; the preparation contains both plastids (p) and mitochondria (m). (*b*) Avocado mitochondrial preparation prepared by sucrose-density-gradient centrifugation of the crude mitochondrial preparation shown in (*a*); this corresponds to fractions 3 and 4 of Fig. 1(*a*). (*c*) Avocado plastid preparation prepared by sucrose-density-gradient centrifugation of the crude mitochondrial preparation shown in (*a*); this corresponds to fractions 1 and 2 of Fig. 1(*a*). (*d*) Avocado plastids prepared by centrifugation of the total mesocarp homogenate at 2000*g*.



EXPLANATION OF PLATE 2

Electron micrograhs of subcellular preparations of cauliflower buds

(*a*) Cauliflower mitochondrial preparation prepared by sucrose-density-gradient centrifugation of a crude preparation obtained by differential centrifugation of the homogenate of cauliflower buds; this material corresponds to fractions 3 and 4 of Fig. 1(*b*) and contains mainly mitochondria (m). (*b*) Cauliflower plastid preparation obtained by sucrose-density-gradient centrifugation as for Plate 2(*a*); this material corresponds to fractions 1 and 2 of Fig. 1(*b*), and contains mainly plastids (p) with a few mitochondria (m).

Table 2. *Incorporation of [1-^{14}C]acetate into fatty acids by mitochondrial and supernatant fractions from avocado mesocarp and cauliflower buds*

Incubations contained 0.3ml of subcellular preparation, 400nmol of [1-^{14}C]acetate, 10μmol of ATP, 30μmol of HCO_3^-, 2.0μmol of Mn^{2+}, 50nmol of CoA, 200nmol of NADH, 200nmol of NADPH, dissolved in the medium used for measuring oxygen uptake (see under 'Methods') in a total volume of 1.5ml. Values given are the means of duplicates that agreed to within 5%.

Subcellular fraction	Fatty acid	[1-^{14}C]Acetate incorporated (nmol/mg of protein) Avocado	Cauliflower
Crude mitochondria (avocado 1.0mg of protein, cauliflower 0.6mg of protein)	$C_{18:3}$	0	1.1
	$C_{18:2}$	2.7	1.5
	$C_{18:1}$	28.5	5.6
	$C_{18:0}$	3.2	1.1
	$C_{16:0}$	21.5	4.2
	C_{12-14}	1.0	0
	Total	56.9	13.5
Supernatant (12000*g*) (avocado 0.16mg of protein, cauliflower 1.0mg of protein)	Total	0	0
Crude mitochondria (avocado 0.6mg of protein, cauliflower 1.0mg of protein) +supernatant (12000*g*) (avocado 0.15mg of protein, cauliflower 0.15mg of protein)	Total	30.2*	9.73*

* Specific activity expressed in terms of mg of mitrochondrial protein present.

It seemed possible that these mitochondrial preparations could be contaminated with chloroplasts or plastid fragments, particularly as the preparation from avocado mesocarp was green. Electron microscopy showed that indeed this was the case; Plate 1(*a*) shows that the preparation from avocado mesocarp had a mixed population of plastids and mitochondria.

Fatty acid synthesis by purified mitochondrial and plastid preparations

Because of the heterogeneity of the mitochondrial preparations they were subjected to further fractionation on a discontinuous sucrose density gradient, by the method of Baker *et al.* (1968). Seven 2ml fractions were removed from the density-gradient separation of the crude avocado-pear mesocarp mitochondrial preparation and assayed for ADP-controlled respiration of succinate and for the incorporation of [1-^{14}C]acetate into fatty acids (Fig. 1*a*). Although the respiratory activity was predominantly in fractions 3 and 4, which contained the 1.2M-sucrose layer of the gradient, the highest incorporation of [1-^{14}C]acetate into fatty acids was obtained with fractions 1 and 2, which contained 1.4–1.6M-sucrose. Electron microscopy of material from these fractions revealed, as is seen in Plates 1(*b*) and 1(*c*), that whereas fractions 1 and 2 contained plastids somewhat contaminated with mitochondria, fractions 3 and 4 contained the mitochondria, virtually free of plastids.

An analogous fractionation of the crude cauliflower mitochondrial preparation yielded similar results. As is seen in Fig. 1(*b*) incorporation of [1-^{14}C]acetate was greatest with the material sedimenting in the 1.4–1.6M-sucrose region of the gradient. Electron microscopy (Plates 2*a* and 2*b*) again showed that the fraction oxidizing succinate contained almost pure mitochondria whereas those fractions incorporating [1-^{14}C]acetate into fatty acids contained mainly proplastids.

Further evidence about the role of avocado plastids in fatty acid synthesis was obtained by assaying the synthesis of fatty acids by a plastid preparation obtained from avocado mesocarp by centrifuging the original homogenate at 2000*g*, as described under 'Methods'. Electron microscopy (Plate 1*d*) showed that this preparation contained similar organelles to those present in the bottom two fractions of the sucrose-density-gradient preparation. Table 3 shows that this preparation incorporated [1-^{14}C]acetate into the whole range of fatty acids found in the avocado mesocarp. From these results it appeared that, contrary to the conclusion of Yang & Stumpf (1965), fatty acid synthetase activity was located in the plastids of the avocado pear, and that the mitochondria, although possessing coupled respiratory activity, had no fatty acid synthetase activity. No evidence for the presence of a soluble fatty acid synthetase in the 12000*g* supernatant could be found. Both the preparations described above (Tables 2 and 3), containing intact plastids, incorporated [1-^{14}C]acetate into the whole range of fatty acids present in the avocado mesocarp. The principal difference between the experimental procedures used to produce the two sets of preparations having differing fatty acid synthetase activities was the method used to obtain the initial avocado mesocarp homogenate. Whereas Yang &

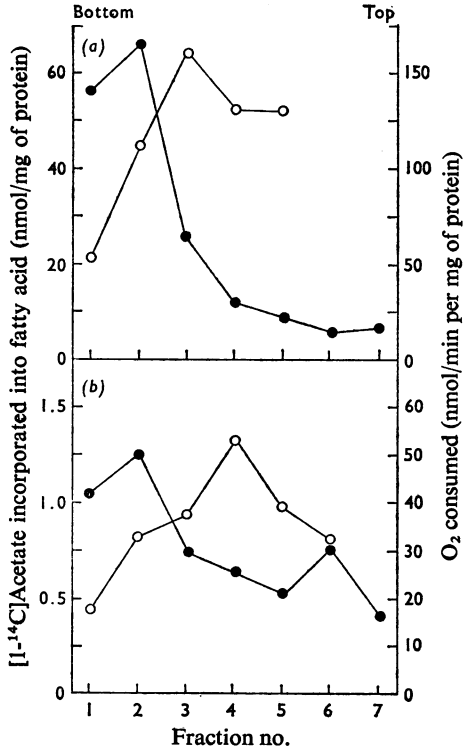


Fig. 1. *Comparison of respiratory and fatty acid synthetase activity of fractions obtained by density-gradient centrifugation of crude mitochondrial preparations from avocado mesocarp (a) and cauliflower buds (b)*

Oxygen uptake due to ADP-stimulated respiration of succinate was measured by the procedure given in Table 1. Incorporation of [1-^{14}C]acetate into fatty acids was measured under the conditions given in Table 2. ●, nmol of acetate incorporated; ○, nmol of oxygen consumed/min.

Stumpf (1965) used a Waring blender and obtained a mitochondrial preparation, the respiratory activity of which was not described, the present authors used a more gentle disintegration procedure with a plastic vegetable grater to obtain a preparation containing mitochondria the respiratory viability of which has been demonstrated.

To find out whether the discrepancy in the results could be ascribed to the disintegration procedure, the fatty acid synthetase activity of preparations obtained by each method from a single avocado pear was investigated. The mesocarp of one fruit was divided into two. One half was disintegrated with the plastic vegetable grater, and a plastid preparation was made by centrifugation at 2000*g* as described above. The remaining supernatant was then further centrifuged at 12000*g* to give a supernatant for assay. Material from the second half of the fruit was homogenized by means of an MSE Ato-Mix blender for 1 min by using the procedure of Yang & Stumpf (1965). The homogenate was centrifuged first at 2000*g* followed by a further centrifugation of the supernatant at 12000*g*, to give a second supernatant. The 2000*g* precipitate and the 12000*g* supernatant were assayed for fatty acid synthetase activity. The results of the experiments are given in Table 3. The active fractions obtained from the brei made with the vegetable grater had a much greater specific fatty acid synthetase activity than those obtained from the Ato-Mix homogenates. The activity of the vegetable grater brei was concentrated almost entirely in the 2000*g* precipitate which converted [1-^{14}C]acetate into the whole range of fatty acids found in the avocado mesocarp, the most predominant of which was oleate. The activity of the Ato-Mix homogenate on the other hand was concentrated in the 12000*g* supernatant which incorporated [2-^{14}C]-malonate and to a markedly lesser extent [1-^{14}C]-acetate into a mixture of fatty acids, the most predominant of which was stearate.

From this it appeared that the fatty acid synthetase activity of the 12000*g* supernatant from the Ato-Mix homogenate could have arisen by rupture of the plastids followed by release of the fatty acid synthetase. Accordingly a preparation of avocado mesocarp plastids obtained by centrifuging the vegetable-grater homogenate at 2000*g* was disintegrated ultrasonically. Electron microscopy showed that the plastids were almost entirely ruptured. This preparation was centrifuged at 105000*g* for 20 min and the capacity of the resultant supernatant to incorporate [1-^{14}C]acetate and [2-^{14}C]malonyl-CoA into fatty acids was compared with that of the intact plastids. When [1-^{14}C]-acetate was the substrate the specific synthetic activity of the supernatant was considerably less than that of the intact plastids but when [2-^{14}C]malonyl-CoA was the substrate the synthetic activity of the supernatant was much greater than that of these intact plastids (Table 4*a*). Whereas the plastid preparation incorporated [1-^{14}C]acetate into the whole range of fatty acids present in the avocado mesocarp, the 105000*g* supernatant formed mainly stearate from [1-^{14}C]acetate or [2-^{14}C]malonyl-CoA (Table 4*b*).

Somewhat similar results were obtained when a proplastid preparation from cauliflower buds was subjected to ultrasonic disintegration and the disrupted material was fractionated by centrifugation at 105000*g*. The parent plastid preparation incorporated [1-^{14}C]acetate and, to a slight extent, [2-^{14}C]malonyl-CoA into fatty acids. The 12000*g*

Table 3. *Comparison of fatty acid synthetase activity in subcellular fractions of the avocado mesocarp obtained after homogenization with a vegetable grater with those obtained after homogenization with a MSE Ato-Mix homogenizer*

The method of preparation of the subcellular fractions from a single fruit is given in the text. Samples of these fractions (0.3ml) were incubated at 30°C for 1h in a medium which contained 400nmol of [1-^{14}C]acetate or 400nmol of [2-^{14}C]malonate, together with 10μmol of ATP, 30μmol of HCO_3^- (omitted when [2-^{14}C]malonate was the substrate), 2μmol of Mn^{2+}, 50nmol of CoA, 200nmol of NADH, 200nmol of NADPH made up to a total volume of 1.5ml with the suspending medium (see the text). Values given are the means of duplicates which agreed to within 5%.

(*a*) Total substrate incorporated into fatty acids (nmol/mg of protein)

Method of homogenization ...		Vegetable grater		Ato-Mix	
Fraction	Substrate ...	[1-^{14}C]Acetate	[2-^{14}C]Malonate	[1-^{14}C]Acetate	[2-^{14}C]Malonate
Precipitate (2000*g*)		19.5	0.6	1.0	0.4
Supernatant (12000*g*)		0	0	0.6	2.2

(*b*) Percentage distribution of radioactivity between fatty acids

Fraction	Substrate	$C_{18:3}$	$C_{18:2}$	$C_{18:1}$	$C_{18:0}$	$C_{16:1}$	$C_{16:0}$
Vegetable grater (2000*g* precipitate)	[1-^{14}C]Acetate	3.0	5.0	47	13	10	22
Ato-Mix (12000*g* supernatant)	[2-^{14}C]Malonate	0	0	32	60	0	8

cytoplasmic fraction obtained from the supernatant of this 2000*g* plastid preparation had very little fatty acid synthetase activity with [1-^{14}C]acetate but somewhat more activity when [2-^{14}C]malonyl-CoA was the substrate (Table 4). Ultrasonic rupture of these plastids followed by centrifugation at 105000*g* gave a supernatant that had a high fatty acid synthetase activity when [2-^{14}C]malonyl-CoA was the substrate and a much lower specific enzyme activity when [1-^{14}C]acetate was the substrate (Table 4*a*).

From these results it appeared that the fatty acid synthetase activity of the avocado mesocarp and of the cauliflower buds was present almost exclusively in the plastids. The fatty acid synthetase activity found in the 12000*g* supernatant of avocado preparations made in the Ato-Mix homogenizer could be ascribed to rupture of the plastids followed by the liberation of the enzyme system.

Harwood & Stumpf (1972) have reported that the addition of acyl-carrier protein is essential for the demonstration of fatty acid synthetase activity in their 12000*g* supernatant preparation from the avocado mesocarp. The effect of addition of *E. coli* acyl-carrier protein on the fatty acid synthetase activity of the 12000*g* supernatant of the avocado mesocarp homogenate and of the 105000*g* supernatant of the disintegrated plastid preparation was therefore investigated. Whereas the 12000*g* supernatant showed no fatty acid synthetase activity when [2-^{14}C]malonyl-CoA was the substrate despite the addition of *E. coli* acyl-carrier protein, the soluble supernatant of the disrupted plastids was demonstrably dependent on this cofactor (Table 5). Further investigation of the effect of acyl-carrier protein on the fatty acid synthetase activity released from disrupted plastids showed that maximal activity was obtained when 0.3mg of acyl-carrier protein was added to each incubation mixture.

Synthesis of fatty acids de novo

The remaining point to be investigated was whether the observed incorporation of [1-^{14}C]acetate into fatty acids by the avocado and cauliflower plastids could be ascribed to synthesis of the individual fatty acids *de novo* or whether it arose merely from chain elongation of pre-existing lower-molecular-weight fatty acids. Radioactive oleic acid and stearic acid were isolated from incubations of both avocado and cauliflower plastids with [1-^{14}C]acetate. The percentage of the radioactivity present in the whole of the molecule that was present in the carboxyl group was assayed by comparing the radioactivity released as $^{14}CO_2$ by the Schmidt (1923) degradation with that of the parent fatty acids. The results in Table 6 show that the percentages obtained were near to those that would be predicted for synthesis *de novo*.

Substrates utilized by plastids for fatty acid biosynthesis

Although it was clear from the results in Table 4 that acetate was more efficiently incorporated into fatty acids than was acetyl-CoA, we decided to investigate the incorporation of other substrates which might give rise to acetyl-CoA and which might pass through the plastid membrane more

Table 4. *Effect of ultrasonic rupture on the biosynthesis of fatty acids by avocado and cauliflower plastids*

Plastid preparations were obtained by centrifuging the homogenates obtained with the plastic vegetable grater at 2000*g*. The supernatant was centrifuged at 12000*g* and the clear supernatant taken. The plastids were subjected to ultrasonic rupture for two periods each of 5s. The disintegrated plastids were centrifuged at 105000*g* and the supernatant fraction was assayed for fatty acid synthetase activity by incubating either 500nmol of [1-^{14}C]acetate (0.5μCi), 168nmol of acetyl-CoA (0.25μCi) or 250nmol of malonyl-CoA (0.22μCi) with 0.3ml of preparation for assay together with the same cofactors and buffer as in Table 3, for 1h at 30°C. Values given are the means of duplicates which agreed to within 5%.

(*a*)

	Total fatty acid formed (nmol of substrate incorporated/h per mg of protein)				
Source ...	Avocado mesocarp			Cauliflower buds	
Substrate ...	[1-^{14}C]-Acetate	[1-^{14}C]-Acetyl-CoA	[2-^{14}C]-Malonyl-CoA	[1-^{14}C]-Acetate	[2-^{14}C]-Malonyl-CoA
Preparation					
Homogenate					
Precipitate (2000*g*) (avocado 0.89mg of protein, cauliflower 0.7mg of protein)	65.4	1.65	12.6	0.535	0.183
Supernatant (12000*g*) (avocado 0.62mg of protein, cauliflower 0.42mg of protein)	0.670	—	10.1	0.049	1.27
Sonicate					
Supernatant (105000*g*) (avocado 0.55mg of protein, cauliflower 0.18mg of protein)	18.2	24.0	133	0.449	15.9

(*b*)

		Percentage distribution of radioactivity between fatty acids					
Preparation	Fatty acid Substrate ...	$C_{18:3}$	$C_{18:2}$	$C_{18:1}$	$C_{18:0}$	$C_{16:1}$	$C_{16:0}$
Avocado plastids	[1-^{14}C]Acetate	9.0		43.0	13.0	10.0	25.0
Supernatant of disrupted avocado plastids (105000*g*)	[1-^{14}C]Acetate	0.7		1.4	90.0	0.6	7.3
	[2-^{14}C]Malonyl-CoA	1.1		1.8	86.3	4.4	6.4
Cauliflower plastids	[1-^{14}C]Acetate	6.5	11.3	59.3	5.4	<1	17.5
Supernatant of disrupted cauliflower plastids	[2-^{14}C]Malonyl-CoA	17.3	11.7	35.6	11.2	<1	24.3

(*c*)

	Percentage composition of fatty acid					
Fatty acid ...	$C_{18:3}$	$C_{18:2}$	$C_{18:1}$	$C_{18:0}$	$C_{16:1}$	$C_{16:0}$
Avocado plastids	3.5	13	53	5.0	5.5	20
Cauliflower plastids	40	12	9.0	4.0	5.0	30

Table 5. *Effect of E. coli acyl-carrier protein on fatty acid biosynthesis by the cytoplasm and the supernatant of the disrupted plastids of the avocado mesocarp*

The 12000*g* supernatant of the avocado homogenate and the plastid fragments were obtained by the procedure given in Table 4. Mixtures containing 0.3ml (0.513mg of protein) of the 12000*g* supernatant or 0.3ml (0.476mg of protein) of the 105000*g* supernatant of the disrupted plastids together with 250nmol of malonyl CoA (0.05μCi), the amounts of acyl-carrier protein shown and the cofactors given in Table 3, in a total volume of 1.5ml were incubated for 1h at 30°C. Values given are the means of duplicates, which agreed to within 5%.

	Malonyl-CoA incorporated (nmol/h per mg of protein)					
Acyl-carrier protein (mg) ...	0	0.05	0.1	0.2	0.3	0.4
Fraction						
Supernatant of mesocarp homogenate (12000*g*)	0	—	—	0	—	—
Supernatant of disrupted plastids (105000*g*)	86.5	122.5	134	142	163	156

readily than acetyl-CoA. The results in Table 7 show that [U-^{14}C]glucose was also an efficient precursor of fatty acids in the avocado plastid system. At the concentrations used very little of the tricarboxylate-cycle intermediates [2,4-^{14}C]succinate or [1,5-^{14}C]citrate was incorporated into fatty acids even when the incubation mixture was fortified with the cytoplasmic and mitochondrial fractions. The range of [^{14}C]fatty acids formed from [U-^{14}C]-glucose was similar to that formed from [1-^{14}C]acetate.

Cofactor requirement for fatty acid synthesis by plastids

The results presented in Table 8 show the effect of omission of one or other of the cofactors used on the incorporation of [1-^{14}C]acetate into the various fatty acids by the avocado and cauliflower plastid preparations. The most pronounced features were the requirements for ATP, HCO_3^- and Mn^{2+}, absence of any of which caused a marked depression in the formation of all the radioactive fatty acids. The observation that the omission of NADPH causes a smaller decrease in fatty acid labelling than omission of NADH is of some interest. The omission of individual cofactors appeared to affect the biosynthesis of most of the fatty acids equally.

Variation of incorporation of [1-^{14}C]acetate into individual fatty acids

The relative increase in the ^{14}C content of the individual fatty acids during the course of incubations was found to be similar with both avocado mesocarp and cauliflower-bud plastid preparations. Early in the incubation most of the radioactivity incorporated into fatty acids was in [^{14}C]palmitate but later [^{14}C]oleate was the principal radioactive fatty acid (Fig. 2).

The effect of ultrasonic rupture of the plastid preparation on the distribution of radioactivity among the fatty acids obtained from [1-^{14}C]-acetate is shown in Fig. 3. Ultrasonic treatment for 5s resulted in a decrease in the [^{14}C]oleate and [^{14}C]palmitate and an increase in the [^{14}C]-stearate compared with the radioactive fatty acids produced by the intact plastid preparation. The stearate-synthesizing activity was liberated from the plastid by the ultrasonic treatment; after 15–20s of such treatment even this stearate-synthesizing activity diminished.

Table 6. *Percentage of total radioactivity present in the carboxyl carbon of fatty acids formed from [1-^{14}C]acetate by avocado and cauliflower plastids*

Plastids were obtained by the procedure given in Table 4 and were incubated with 500nmol (0.5μCi) of [1-^{14}C]-acetate for 2h under the conditions given in Table 4.

	Percentage of total radioactivity present in carboxyl carbon			
Fatty acid ...	$C_{18:2}$	$C_{18:1}$	$C_{18:0}$	$C_{16:0}$
Expected percentage	11.1	11.1	11.1	12.5
Source of plastids				
Avocado mesocarp	11.5	10.0	9.6	13.0
Cauliflower buds	10.7	14.7	16.0	13.4

Table 7. *Incorporation of possible radioactive precursors of [^{14}C]acetyl-CoA into fatty acids by subcellular fractions of the avocado pear mesocarp*

Plastid preparations were obtained by the method given in Table 4, and the mitochondrial preparation was made by the method of Baker *et al.* (1968) (see under 'Methods'). Radioactive substrates (400nmol, 0.5μCi) were incubated with the buffer and cofactor mixture given in Table 3. Values of the incorporations are the means of duplicates which agreed to within 5%.

(*a*) Total substrate incorporated into fatty acids

	^{14}C incorporated (ng-atoms/h per mg of protein)			
Substrate ... / Fraction	[1-^{14}C]Acetate	[U-^{14}C]Glucose	[2,4-^{14}C]Succinate	[1,5-^{14}C]Citrate
Precipitate (2000*g*)	57.4	40.0	0.708	0.515
Precipitate (2000*g*)+supernatant (12000*g*)	26.6	17.0	0.490	0.167
Precipitate (2000*g*)+supernatant (12000*g*)+ mitochondrial fraction	27.2	17.3	0.432	0.166

(*b*) Distribution of radioactivity among fatty acids derived from [U-^{14}C]glucose

	Percentage of total ^{14}C incorporated				
Fatty acid ... / Fraction	$C_{18:3}+C_{18:2}$	$C_{18:1}$	$C_{18:0}$	$C_{16:1}$	$C_{16:0}$
Precipitate (2000*g*)	10	32	18	10	30

Table 8. *Effect of cofactors on the incorporation of* [1-^{14}C]*acetate into fatty acids by plastid fractions from avocado mesocarp and cauliflower buds*

The plastid fractions were obtained by the procedure given in Table 4 and were incubated for 1 h at 30°C with 400 nmol (0.5 μCi) of [1-^{14}C]acetate, the complete system being as given in Table 3, omissions being made as shown.

	[1-^{14}C]Acetate incorporated (nmol/h per mg of protein)				
Fatty acid ... Factor omitted	$C_{18:3}$	$C_{18:2}$	$C_{18:1}$	$C_{18:0}$	$C_{16:1}+C_{16:0}$
(*a*) Avocado mesocarp					
None	1.43	7.90	50.4	1.95	42.4
CoA	2.18	11.5	25.6	1.45	20.0
ATP	0.835	1.46	3.13	0.955	4.20
NADH	0.882	4.65	20.1	0.441	14.0
NADPH	2.37	11.8	47.5	1.275	28.2
HCO_3^-	1.63	5.83	15.0	0.687	13.1
Mn^{2+}	0.492	1.47	7.34	0.454	9.15
(*b*) Cauliflower buds					
None	0.700	0.622	1.60	0.770	1.14
CoA	0.425	0.412	0.541	0.422	0.905
ATP	0.342	0.337	0.321	0.232	0.332
NADH	—	0.259	0.492	0.348	0.340
NADPH	0.427	0.474	1.44	0.288	1.25

Discussion

The results presented above show that subcellular fractionation of disintegrated avocado mesocarp or cauliflower-bud tissue on a sucrose density gradient resulted in the separation of a dense particulate fraction which had fatty acid synthetase activity. This fraction was clearly resolved from the mitochondrial fraction which, although it showed controlled respiration, had little fatty acid synthetase activity. No fatty acid synthetase could be detected in the cytoplasmic fraction remaining in the supernatant after centrifugation of the homogenate at 12000*g*, even when [2-^{14}C]malonyl-CoA was used as the substrate and incubations were fortified by the addition of *E. coli* acyl-carrier protein.

The large particulate fractions from both the avocado mesocarp and the cauliflower buds, although somewhat different from each other, were found by electron microscopy to have the morphological features of plastids or proplastids. The biochemical characteristics of these two preparations were similar, but different in some significant features from those of other fatty acid synthetase preparations obtained from the same two tissues (e.g. Barron *et al.*, 1961; Mazliak *et al.*, 1972). The two salient biochemical features of fatty acid synthetase preparations described in the present paper concern the substrates they used and the fatty acids they produced. Acetate was the preferred substrate for both plastid preparations studied in the present paper, [1-^{14}C]acetyl-CoA was utilized to some extent but [2-^{14}C]malonyl-CoA was hardly utilized at all for fatty acid synthesis by the intact plastids. The principal products obtained from [1-^{14}C]acetate were [^{14}C]oleate and [^{14}C]palmitate; both the avocado and the cauliflower preparations incorporated some of this substrate into linoleate and in addition the cauliflower preparation formed some radioactive linolenate. Neither preparation formed appreciable amounts of radioactive stearate but in any case this fatty acid is only a minor component of the lipids of these tissues. Whereas the relative incorporation of [1-^{14}C]acetate into the fatty acids of the avocado plastids corresponds closely to the fatty acid composition of the organelle the correspondence between ^{14}C fatty acids formed and the fatty acid composition of the cauliflower proplastids is not so close. The cauliflower plastids contain a much higher proportion of C_{18} polyunsaturated acids but most of the radioactivity from [1-^{14}C]acetate was found in octadecenoic acid (Table 4). It seems that the preparation studied has a relatively poor capacity to form these polyunsaturated fatty acids for, as is seen in Fig. 2(*b*), there is little increase in the radioactivity present in these fatty acids after incubation times of up to 2 h.

The morphology of the crude mitochondrial fraction obtained by differential centrifugation and the plastid and mitochondrial fractions obtained by sucrose-density-gradient centrifugation was shown by electron microscopy to be very similar to that of fractions obtained from avocado mesocarp by Baker *et al.* (1968) with the same procedure. These observations, together with the comparative biochemical investigations of the brei made by using

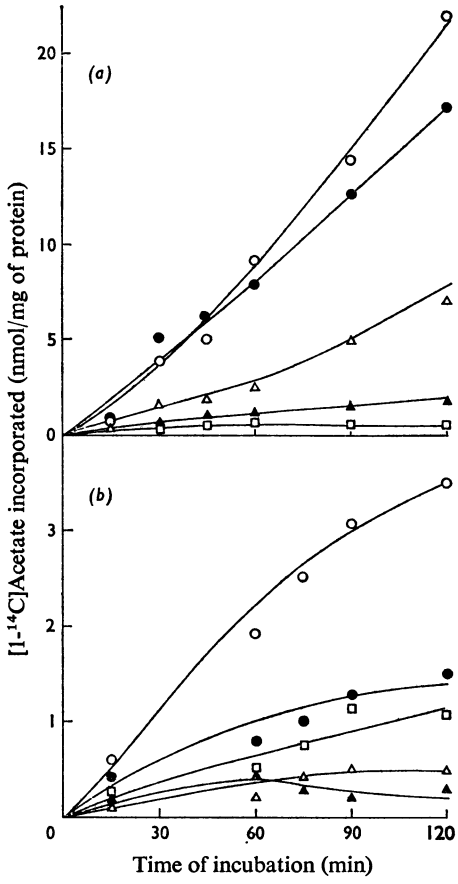


Fig. 2. *Time-course of incorporation of* [1-^{14}C]*acetate into fatty acids by intact plastids from avocado mesocarp* (*a*) *and cauliflower buds* (*b*)

Incubation conditions were as given in Table 2. The figure shows [1-^{14}C]acetate incorporation into the different fatty acid types. ▲, $C_{18:3}$; △, $C_{18:2}$; ○, $C_{18:1}$; □, $C_{18:0}$; ●, $C_{16:0}$.

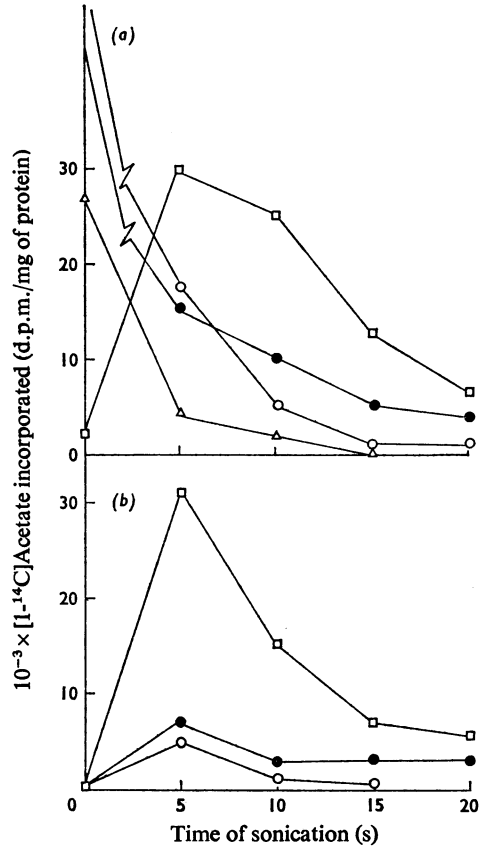


Fig. 3. *Synthesis of fatty acids by ultrasonically ruptured avocado plastids* (*a*) *and* 105000*g supernatant from ultrasonically ruptured avocado plastids* (*b*)

Incorporation of [1-^{14}C]acetate was assayed under the conditions given in Table 2. The figure shows [1-^{14}C]-acetate incorporated into △, $C_{18:2}$; ○, $C_{18:1}$; □, $C_{18:0}$; ●, $C_{16:0}$.

a vegetable grater, with the Ato-Mix homogenate and the ultrasonically ruptured plastids, revealed the reasons for the differences in the properties of the fatty acid synthetase preparations described above compared with those reported by other workers studying the same tissues. In their pioneer studies on the fatty acid synthetase of the avocado mesocarp, Stumpf & Barber (1957) did in fact use a vegetable grater to obtain the homogenate and attributed a considerable portion of the radioactive fatty acids obtained to [^{14}C]oleate, but because of the inadequate chromatographic techniques then available were not able to determine the proportion of [^{14}C]oleate with precision. In subsequent reports Stumpf and co-workers used what appear to have been somewhat more vigorous disruptive procedures. Mudd & Stumpf (1961) used a mortar to produce a homogenate of the avocado mesocarp from which fairly dense particles sedimenting between 2000*g* and 6000*g* incorporated [^{14}C]acetate into a mixture of fatty acids, 30% of which was [^{14}C]oleate. In contrast Yang & Stumpf (1965) found fatty acid synthetase activity in the supernatant of the homogenate obtained with a Waring blender. The product of this preparation was principally stearate and the preferred substrate was malonyl-CoA. The results of more recent experiments of Harwood & Stumpf (1972) in which a soluble fatty acid synthetase was obtained from a homogenate prepared by use of a Potter homogenizer may also be attributed to the rigour of the disintegration procedure.

Mazliak *et al.* (1972) have reported the results of a study of the fatty acid synthetase activity of a homogenate obtained from cauliflower buds with a 'Moulinex' grinder, fractionated by differential centrifugation. A mitochondrial preparation apparently similar to that used for the experiments described in Table 2 of the present paper was obtained, which showed coupled respiration and which was said to contain mainly mitochondria. Malonyl-CoA was the preferred substrate and the major product was stearate. It is significant that there was no incorporation of radioactivity into linolenate although the preparation contained an appreciable amount of this acid. Octadecenoate was formed, but it is of considerable interest that the specific radioactivity of this acid varied inversely with the proportion of mitochondrial protein present in the incubation. It is suggested that these results of Mazliak *et al.* (1972) may be attributed to the whole and disrupted plastid content of the mitochondrial preparation which would form octadecenoate and stearate respectively from malonyl-CoA.

The general concept that plastids are the principal site of fatty acid biosynthesis in higher plants is in accord with the observations on the fatty acid biosynthesis by chloroplasts reported by, for example Smirnov (1960), Stumpf & James (1963) and Boardman & Stumpf (1970). It is, however, of particular interest that Zilkey & Canvin (1969) were able to obtain a homogenate of the castor-bean (*Ricinus communis*) endosperm that on sucrose-density-gradient centrifugation, showed fatty acid synthetase activity to be associated with large particles clearly separated from the mitochondria, which were characterized by high succinoxidase activity. Again the principal radioactive fatty acid formed was oleate; the substrate used was [1-^{14}C]acetyl-CoA and it is perhaps unfortunate for this argument that no comparable results were reported for the incorporation of [^{14}C]acetate.

It is therefore concluded from the results reported here that intact plastids in the two tissues studied incorporate [^{14}C]acetate into palmitate, oleate, linoleate and, in the case of cauliflower plastids, linolenate, and that rupture of the plastids liberates a synthetase system which forms stearate from malonyl-CoA.

The properties of the isolated plastid system are of interest with respect to the substrates utilized and the cofactors required. It is noteworthy that appreciable amounts of radioactive fatty acids were formed when [U-^{14}C]glucose was the substrate but the poor incorporation of the tricarboxylate-cycle intermediates [2,3-^{14}C]succinate and [1,5-^{14}C]citrate in the presence of added mitochondria is probably not surprising, for any [^{14}C]acetyl-CoA that may have been formed from these substrates probably could not have reached the fatty acid synthetase. The pronounced requirement of CoA for fatty acid biosynthesis by the plastids is noteworthy because, if the relatively low rate of incorporation of [^{14}C]acetyl-CoA into fatty acids is due to low permeability of the plastid membrane it is surprising that the same membrane should be permeable to the unacetylated thiol. The apparent permeability of the plastids to NADH and NADPH is less surprising, for avocado and cauliflower mitochondria were also found to be permeable to these two cofactors.

In conclusion it seems pertinent to recall the remarks of de Duve (1967): 'Homogenization is the necessary prerequisite of all mass-fractionation experiments. It results in considerable loss of morphological information but such is the price we pay for being able to apply henceforth the innumerable techniques of biochemistry. Our problem is to conduct the operation in such a manner as to lose no more information than is needed, while making what is left retrievable with as little distortion as possible'. It is the contention of the present authors that when appropriate methods are developed for the homogenization of other tissues of higher plants, which can be shown not to rupture an appreciable portion of any plastids that may be present in such tissues, it is likely that fatty acid synthetase activity will be found to be present in these organelles.

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