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Elongation of Fatty Acids by Microsomal Fractions from the Brain of the Developing Rat

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1. Elongation of fatty acids by microsomal fractions obtained from rat brain was measured by the incorporation of [2-^{14}C]malonyl-CoA into fatty acids in the presence of palmitoyl-CoA or stearoyl-CoA. 2. Soluble and microsomal fractions were prepared from 21-day-old rats; density-gradient centrifugation demonstrated that the stearoyl-CoA elongation system was localized in the microsomal fraction whereas fatty acid biosynthesis *de novo* from acetyl-CoA occurred in the soluble fraction. The residual activity *de novo* in the microsomal fraction was attributed to minor contamination by the soluble fraction. 3. The optimum concentration of [2-^{14}C]malonyl-CoA for elongation of fatty acids was 25μM for palmitoyl-CoA or stearoyl-CoA, and the corresponding optimum concentrations for the two primer acyl-CoA esters were 8.0 and 7.5μM respectively. 4. NADPH was the preferred cofactor for fatty acid formation from palmitoyl-CoA or stearoyl-CoA, although NADH could partially replace it. 5. The stearoyl-CoA elongation system required a potassium phosphate buffer concentration of 0.075M for maximum activity; CoA (1μM) inhibited this elongation system by approx. 30%. 6. The fatty acids formed from malonyl-CoA and palmitoyl-CoA had a predominant chain length of C_{18} whereas stearoyl-CoA elongation resulted in an even distribution of fatty acids with chain lengths of C_{20}, C_{22} and C_{24}. 7. The products of stearoyl-CoA elongation were identified as primarily unesterified fatty acids. 8. The developmental pattern of fatty acid biosynthesis by rat brain microsomal preparations was studied and both the palmitoyl-CoA and stearoyl-CoA elongation systems showed large increases in activity between days 10 and 18 after birth.

Myelination is a complex biological process by which oligodendroglial cells in the central, and Schwann cells in the peripheral nervous system produce a multilayered membrane around nerve fibres (Peters, 1964; Geren, 1954). We are interested in the biochemistry of myelogenesis and have concentrated our initial efforts on a process that results in one of the unique features of myelin, the occurrence of large concentrations of long-chain fatty acids ($C_{24:0}$, $C_{24:1}$) in the sphingolipids of myelin (O'Brien, 1965). Although all plasma membranes of animal cells appear to contain small concentrations of the sphingolipids, these compounds comprise approx. 25% (w/w) of the total lipids of human myelin (O'Brien, 1965). These sphingolipids and their long-chain acyl components may be responsible for the unique properties of myelin (Chapman, 1972). The crucial role of these lipids in myelin stability is emphasized by their decreased concentration in demyelinating diseases such as multiple sclerosis and globoid-cell leucodystrophy (Gerstl *et al.*, 1963; Svennerholm, 1963). Further evidence that these long-chain fatty acids are important in myelogenesis is the dramatic increase in their formation at the onset of myelination in the mouse (Goldberg *et al.*, 1973). In addition, the quaking mouse, which is a recessive autosomal mutant characterized by defective myelination, does not have normal concentrations of long-chain fatty acids in myelin (Baumann *et al.*, 1970) and Goldberg *et al.* (1973) have suggested that this may be caused by a defect in the elongation of C_{20}-CoA by the brain microsomal fraction.

Despite the importance of long-chain acyl residues in myelin, their synthesis in the rat has not been studied in detail. Aeberhard *et al.* (1969) demonstrated a transient rise in the rate of incorporation of malonyl-CoA into fatty acids during myelination. However, we wished to characterize more fully the properties of the microsomal system that elongates C_{16}-CoA and C_{18}-CoA and investigate its role in myelogenesis. The rat brain was selected because myelination begins approx. 10 days after birth (Davison, 1969).

Materials and Methods

Materials

Chemicals. [2-^{14}C]Malonyl-CoA (specific radioactivity 20.6Ci/mol) was purchased from New England Nuclear, Boston, Mass., U.S.A. Acetyl-CoA was synthesized by the method of Simon & Shemin (1953) and the completion of the reaction was confirmed by the absence of free CoA thiol groups (Ellman, 1959). Malonyl-CoA, palmitoyl-CoA, stearoyl-CoA and CoA were obtained from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A. Precoated silica gel G t.l.c. plates were obtained from Machery-Nagel and Co., Düren, W. Germany. All cofactors as well as bovine serum albumin (fraction V) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Animals. Albino Wistar rats from the University of British Columbia Animal Unit were used. In each experiment males and females were of equal numbers.

Methods

Preparation of sub-cellular fractions. All operations were at 4°C.

Rats were killed by decapitation. The brains were quickly removed and washed in 50mM-Tris–HCl buffer, pH7.4, containing 0.25M-sucrose, 0.15M-NaCl and 2mM-dithiothreitol. Brain homogenates were prepared in this buffer (6ml/g wet wt. of tissue) with eight up-and-down strokes of a Dounce homogenizer and subsequently centrifuged at 17500$g_{av.}$ for 30min. The supernatant was re-centrifuged at 17500$g_{av.}$ for 30min; the resulting supernatant was centrifuged at 105000$g_{av.}$ for 60min to yield the crude microsomal and soluble fractions. The 6.3×10^6 g-min supernatant was re-centrifuged and the final supernatant was designated the soluble fraction. The crude microsomal pellet was suspended in 0.1M-potassium phosphate buffer, pH7.4, containing 0.25M-sucrose, 0.15M-NaCl and 2mM-dithiothreitol (1ml/g wet wt. of tissue) and was purified by one of two methods. In the first procedure (preparation I) the microsomal suspension was centrifuged at 105000$g_{av.}$ for 60min. Preparation II used a discontinuous sucrose gradient described by Brophy & Gower (1974). Sucrose solutions were in 0.1M-potassium phosphate buffer, pH7.4, containing 2mM-dithiothreitol. The tubes were centrifuged at 105000$g_{av.}$ for 60min and the resulting microsomal pellet was centrifuged again through a similar discontinuous sucrose gradient after resuspension as described above. The final microsomal pellet obtained by either method was resuspended in 0.1M-potassium phosphate buffer, pH7.4, containing 2mM-dithiothreitol (1ml/g wet wt. of tissue) and stored in 1ml portions at −70°C. Unless specified otherwise, all microsomal fractions were prepared by preparation II and the elongation activity was unaffected by storage at −70°C for 3 months. Samples that had been thawed were not refrozen.

The degree of separation of microsomal and soluble fractions was evaluated by continuous sucrose density gradients. These gradients were prepared with 50% (w/w) sucrose (5ml) in the bottom of cellulose nitrate tubes; a continuous gradient of 30ml of 10–25% (w/w) sucrose was subsequently introduced. Sucrose solutions used in the gradient were buffered with 50mM-Tris–HCl buffer, pH7.4, containing 1mM-NaCl and 1mM-EDTA. Samples (2ml) of either the soluble or the microsomal fractions were layered on the top of the gradient and the tubes centrifuged for 20h at 78000$g_{av.}$ in a Beckman SW27 rotor. The bottoms of the tubes were punctured with a needle and 1ml fractions collected after upward displacement of the contents of the tube with 65% (w/w) sucrose.

Enzyme assays. NADPH–cytochrome *c* oxidoreductase activity (EC 1.6.2.4) was assayed by the method of Sottocasa *et al.* (1967).

Fatty acid biosynthesis was measured by the incorporation of [2-^{14}C]malonyl-CoA into fatty acids. Incubation mixtures contained 0.1M-potassium phosphate buffer, pH7.4, 25μM-[2-^{14}C]malonyl-CoA (0.13μCi, 10.3Ci/mol), 50μM-NADPH, 2mM-dithiothreitol and an acyl-CoA (15μM-acetyl-CoA, 8.0μM-palmitoyl-CoA or 7.5μM-stearoyl-CoA) in a total volume of 0.5ml. Assays were started with between 20 and 120μg of microsomal protein derived from pooled brains and after incubation at 37°C for 8min the assays were stopped by the addition of 20% (w/v) KOH (0.5ml). The incubation mixtures were saponified at 80°C for 30min and, after adjustment to pH1 with 5M-HCl, the incubations were extracted with 3×5ml of light petroleum (b.p. 30–60°C). The solvent was evaporated and the radioactivity of the residue determined. By these procedures the rate of incorporation of [2-^{14}C]malonyl-CoA into fatty acids was linear with respect to both time (up to 10min) and protein concentration (up to 130μg) in the presence of each of the three primer acyl-CoA esters. Control incubations without added primer acyl-CoA never resulted in the incorporation of more than 15% of the radioactivity present in the extracts of incubations containing primer, and this value was usually less than 10%. Other routine controls included incubations with no microsomal protein, with boiled microsomal protein and incubations that were stopped immediately after the addition of microsomal protein. Negligible incorporation of [2-^{14}C]malonyl-CoA into fatty acids was found in these studies (less than 55d.p.m.).

Radioactivity measurement. Radioactivity was measured with a Nuclear–Chicago liquid-scintillation spectrometer, model 300, with a counting efficiency of 84% for ^{14}C.

Identification of products. (*a*) Identification of fatty acids. Fatty acids were extracted from incubations as described above except that all steps were performed under N_2 wherever possible. The fatty acids were converted into their methyl esters (Vance & Sweeley, 1967) and analysed on a Hewlett–Packard gas chromatograph [model 7610A, operated at 170°C with a carrier-gas (N_2) flow rate of 50ml/min]. The column [180cm (6ft)] of 12% (w/v) polyethylene glycol succinate supported on Gaschrom P (80–100 mesh) was fitted with a stream splitter so that one-third of the effluent was collected. Fatty acids were collected in cooled glass tubes containing glass wool and were eluted with 15ml of scintillation fluid (Aeberhard *et al.*, 1969). The recovery of radioactivity was always within 10% of the theoretical quantity calculated from the split ratio.

(*b*) Phospholipid analysis. In these experiments incubations were stopped by the addition of 14vol. of chloroform–methanol (1:1, v/v) and the lipids extracted by the method of Folch *et al.* (1957). Each sample was then applied to a silica gel G t.l.c. plate and chromatographed in chloroform–methanol–water (65:25:4, by vol.). The lipids were located by brief exposure to I_2 vapour and identified by comparison with authentic standards. Once identified, the lipids were scraped off the plate, eluted from the silica gel with 3×1.5ml of chloroform–methanol (1:1, v/v) and the radioactivity of each fraction was determined.

(*c*) Unesterified fatty acid analysis. The possibility that the products of the elongation system were unesterified fatty acids was investigated by stopping duplicate incubations with 100µl of cold 1M-HCl. The tubes were immediately cooled to minimize the hydrolysis of esterified fatty acids and the unesterified fatty acids were extracted with 2×5ml of light petroleum (b.p. 30–60°C).

(*d*) Acyl-CoA analysis. The acyl-CoA fraction was separated by the procedure of Colli *et al.* (1969). For these analyses the incubations were stopped by the addition of cold 5% (w/v) $HClO_4$ (0.5ml).

Protein determination. Protein was measured by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin (fraction V) as the standard.

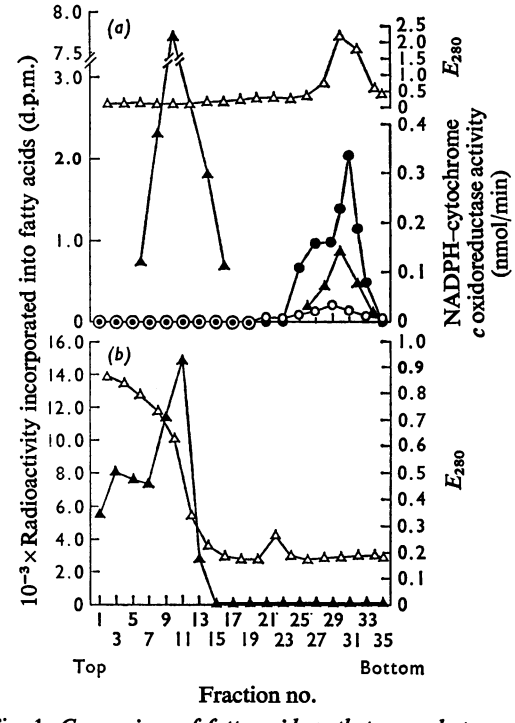

Fig. 1. *Comparison of fatty acid synthetase and stearoyl-CoA elongation activity of fractions obtained by density-gradient centrifugation of* (*a*) *the washed microsomal preparations and* (*b*) *the soluble fraction obtained from the brains of six 31-day-old rats*

A microsomal fraction (2.4mg of protein) obtained by preparation I (see the Materials and Methods section) and soluble fraction (3.0mg of protein) were subjected to sucrose-density-gradient centrifugation and portions (250µl) from fractions (1ml) were assayed for their ability to incorporate [2-^{14}C]malonyl-CoA (0.13µCi) into fatty acids in the presence of 25µM-NADPH, 2mM-dithiothreitol, 0.1M-potassium phosphate buffer, pH7.4, and either 25µM-acetyl-CoA (▲), or 3.0µM-stearoyl-CoA (○). Neither stearoyl-CoA elongation nor NADPH–cytochrome *c* oxidoreductase (●) activity was detected in the soluble fraction. △, E_{280}.

Results

Preparation of microsomal fractions

We designed these experiments to determine whether the microsomal fraction isolated from rat brain was free from contamination by the very active soluble fatty acid synthetase. Soluble and microsomal fractions were prepared by method I from the brains of six 31-day-old rats. Both fractions were analysed by continuous density-gradient centrifugation to determine the extent of contamination of the microsomal fraction by the soluble fraction.

Fig. 1(*a*) shows that the microsomal fractions from preparation I were contaminated with a soluble, acetyl-CoA-dependent fatty acid synthetase; however, most of the protein and the microsomal marker enzyme (NADPH–cytochrome *c* oxidoreductase) were associated with the microsomal fraction together with a low stearoyl-CoA elongation activity and fatty acid synthetase *de novo*. Although not shown in Fig. 1(*b*), none of the fractions from the density-gradient centrifugation of the soluble preparation contained either NADPH–cytochrome *c* oxidoreductase or stearoyl-CoA elongation activity.

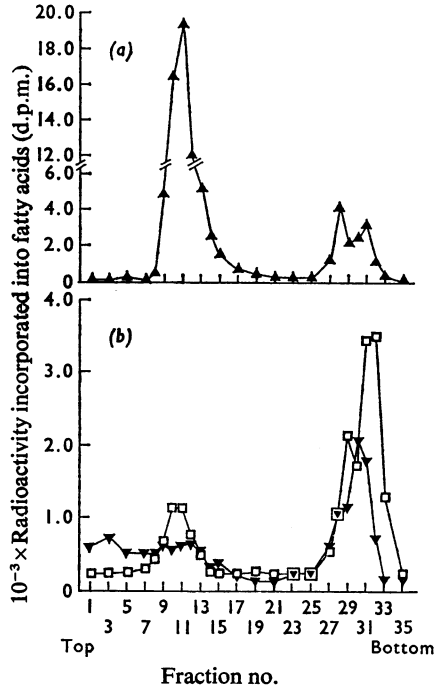

Fig. 2. *Fatty acid synthetase activity de novo in fractions obtained by density-gradient centrifugation of (a) washed microsomal fractions and (b) microsomal fractions after purification by discontinuous density-gradient centrifugation*

Crude microsomal preparations obtained by preparation I in the same manner as described in Fig. 1 were divided into three portions (7mg of protein each). The first sample (*a*; ▲) was subjected to density-gradient centrifugation as described in Fig. 1(*a*). The second sample was subjected to purification by discontinuous density-gradient centrifugation as described in the Materials and Methods section before analysis for contamination with the soluble fatty acid synthetase (*b*; □). The third sample was subjected to an additional passage through the discontinuous density gradient and subsequently analysed (*b*; ▼). The incorporation of [2-^{14}C]malonyl-CoA into fatty acids in the presence of 25μM-acetyl-CoA was measured as described in Fig. 1.

We tried to remove the *de novo* synthetase from the microsomal fraction by centrifugation through discontinuous sucrose gradients. When the microsomal preparations were not sedimented through a sucrose gradient, they were severely contaminated by soluble fatty acid synthetase (Fig. 2*a*). Passage of the microsomal fractions through one (□) or two (▲) discontinuous sucrose gradients before analysis on the continuous sucrose gradients (Fig. 2*b*) revealed that the contamination by the soluble fraction was minimized to a considerable extent, but

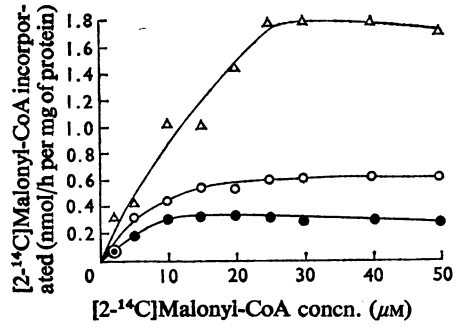

Fig. 3. *Effect of malonyl-CoA on the elongation of fatty acids by microsomal fractions from rat brain*

Microsomal preparations (53μg of protein), prepared from 21-day-old rats, were incubated with various concentrations of [2-^{14}C]malonyl-CoA (10.3Ci/mol) together with the constituents of the standard assay as described in the Materials and Methods section in the presence of 15μM-acetyl-CoA (●), 8.0μM-palmitoyl-CoA (△) or 7.5μM-stearoyl-CoA (○).

complete elimination of the new activity was not achieved, presumably because of occluded cytoplasm in the microsomal vesicles. In the light of these results, all subsequent microsomal fractions were purified by sedimentation through two successive discontinuous sucrose density gradients.

Determination of optimum assay conditions

Effect of malonyl-CoA. Each incubation contained microsomal fractions (53μg of protein) prepared from eight 21-day-old rats and the standard assay conditions were used with different concentrations of [2-^{14}C]malonyl-CoA. Fig. 3 shows that a [2-^{14}C]-malonyl-CoA concentration of 25μM is saturating for long-chain acyl-CoA elongation (8.0μM-palmitoyl-CoA or 7.5μM-stearoyl-CoA); 25μM-[2-^{14}C]-malonyl-CoA was therefore used in subsequent experiments. For comparative purposes the activity of the microsomal preparations with acetyl-CoA (15μM) was also measured as a function of [2-^{14}C]malonyl-CoA concentration.

Effect of primer acyl-CoA. Microsomal fractions (60μg of protein) from preparation II were included in the standard assays in the presence of different concentrations of primer acyl-CoA. Fig. 4 shows the optimum concentrations of palmitoyl-CoA and stearoyl-CoA to be 8.0 and 7.5μM respectively. Since these are above the critical micellar concentrations of these substrates, the elongation system appears to interact with both the monomers and the micelles. The low rate of incorporation of [2-^{14}C]-malonyl-CoA into fatty acids with acetyl-CoA depic-

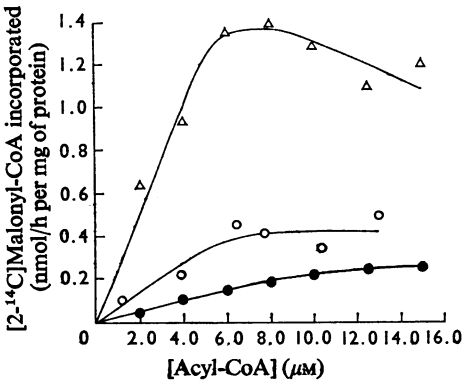

Fig. 4. *Influence of primer acyl-CoA on elongation of fatty acids by microsomal fractions from rat brain*

Brain microsomal preparations (60 μg of protein), from 21-day-old rats, were incubated with 25 μM-[2-^{14}C]-malonyl-CoA (0.13 μCi) together with the components of the standard assay as described in the Materials and Methods section in the presence of different concentrations of primer acyl-CoA; ●, acetyl-CoA; △, palmitoyl-CoA; ○, stearoyl-CoA.

ted in Fig. 4 indicates that the microsomal preparations were slightly contaminated by the soluble synthetase.

Cofactor requirements. The reduced nicotinamide nucleotide requirements for palmitoyl-CoA and stearoyl-CoA elongation were investigated. Each incubation contained 77 μg of microsomal protein with optimum concentrations of cofactors (50 μM each) in the standard assay. The results showed that NADPH is the preferred cofactor for fatty acid elongation, although NADH can replace it for palmitoyl-CoA and stearoyl-CoA elongation to a certain extent (47 and 49% of the maximum activity respectively). In the presence of acetyl-CoA, NADPH is the required cofactor and cannot be replaced by NADH; this would be expected if the *de novo* activity was caused by contamination with soluble fraction. In the light of these results, subsequent experiments used NADPH.

Properties of microsomal fatty acid synthetases

Effect of potassium phosphate buffer concentration on stearoyl-CoA elongation. Fatty acid synthetase complexes from various sources are unstable in phosphate buffers of low ionic strength (Yang *et al.*, 1965; Brindley *et al.*, 1969). We therefore investigated the effect of phosphate concentration on the elongation of stearoyl-CoA. Brain microsomal fractions (53 μg of protein) were incubated in the presence of different concentrations of potassium phosphate buffer, pH 7.4, with the components of the standard assay. The microsomal system is stimulated by increases in the phosphate concentration and requires a minimum of 0.075 M-potassium phosphate buffer for optimum activity.

Effect of CoA on stearoyl-CoA elongation. The effect of CoA on elongation of stearoyl-CoA was studied by incubation with 77 μg of microsomal protein together with the other components of the standard assay. CoA (1 μM) inhibited stearoyl-CoA elongation to about 68% of the maximum value obtained in its absence; moreover, this inhibition was not increased by concentrations of CoA up to 10 μM.

Products of stearoyl-CoA elongation. The object of these experiments was to determine if the products of stearoyl-CoA elongation were unesterified fatty acids, the CoA derivatives or fatty acids directly incorporated into phospholipids. The incubation mixtures were as described for the standard assay except that the [2-^{14}C]malonyl-CoA had a specific radioactivity of 20.6 Ci/mol. Incubations performed in duplicate (or triplicate for acyl-CoA analysis) agreed to within 10%. Relative to the incorporation of radioactivity into total fatty acids (920 d.p.m.), the products of stearoyl-CoA elongation were primarily present in incubations as their unesterified acids, (63%), although some radioactivity was associated with acyl-CoA esters (11%).

The incorporation of radioactively labelled fatty acids into phospholipids was studied. The pooled extracts from five incubations were applied to a t.l.c. plate and after development the following lipids were eluted and their radioactivity was determined: sphingomyelin, phosphatidylcholine, phosphatidylethanolamine and a fourth fraction that contained unesterified fatty acids and neutral lipids. Of the radioactivity applied to the t.l.c. plate (4500 d.p.m.), 71% was recovered in these four fractions, and the majority (60.5% of applied radioactivity) was recovered in the unesterified fatty acid–neutral lipid fraction. Only small quantities of radioactivity were recovered from the phosphatidylcholine, phosphatidylethanolamine and sphingomyelin fractions (4.6, 2.2 and 1.0% respectively). These data support the previous result which shows that the predominant products of stearoyl-CoA elongation are unesterified fatty acids.

Identification of fatty acids. The chain lengths were determined for the fatty acids synthesized by the microsomal fractions in assay mixtures that contained no added primer acyl-CoA, acetyl-CoA (15.0 μM), palmitoyl-CoA (8.0 μM) or stearoyl-CoA (7.5 μM). The fatty acids extracted from replicate incubations (at least five) were pooled and the distribution of radioactivity among the various chain lengths of their methyl esters was determined by preparative g.l.c. Five fractions were collected for each sample. The first fraction was composed of the

Table 1. *Chain lengths of fatty acids synthesized by rat brain microsomal fractions with or without various primer acyl-CoA esters*

Each incubation contained brain microsomal fractions (100 μg) from 23-day-old rats, 25 μM-[2-^{14}C]malonyl-CoA (0.26 μCi), 50 μM-NADPH and the other components of the assay system as described in the Materials and Methods section. Fatty acid methyl esters, derived from replicate incubations, were collected from the effluent of a g.l.c. column as described in the Materials and Methods section. The splitting ratio was 2:1 in favour of the flame; this ratio was checked by applying a portion of each sample to the g.l.c. column and measuring the total radioactivity collected during a period long enough to ensure complete elution of $C_{24:0}$ and $C_{24:1}$ fatty acids. The results are expressed as percentages of the total radioactivity collected for each sample. The radioactivity in each sample applied to the column from incubations containing no primer acyl-CoA, acetyl-CoA, palmitoyl-CoA or stearoyl-CoA was 1.9×10^3, 17.9×10^3, 18.0×10^3 and 19.0×10^3 d.p.m. respectively.

	Primer acyl-CoA			
Chain length	None	Acetyl-CoA	Palmitoyl-CoA	Stearoyl-CoA
$\leq C_{16}$	45.0	79.0	5.7	5.5
C_{18}	16.0	6.1	68.0	10.2
C_{20}	4.5	2.1	8.8	26.3
C_{22}	7.3	1.7	4.6	35.0
C_{24}	25.0	11.0	14.2	23.0

methyl esters of fatty acids with a chain length of C_{16} or less and included $C_{16:0}$ and $C_{16:1}$; the other four fractions were: $C_{18:0}+C_{18:1}$, $C_{20:0}+C_{20:1}$, $C_{22:0}+C_{22:1}$ and $C_{24:0}+C_{24:1}$. The results (Table 1) show that, as might be expected, the predominant fatty acids formed in the presence of acetyl-CoA or palmitoyl-CoA are C_{16} or less (79.0%) and C_{18} (68.0%) respectively. In the presence of stearoyl-CoA, the majority of the radioactivity (84.3%) was distributed evenly among the C_{20}, C_{22} and C_{24} fractions. The balance of the radioactivity in the C_{16} and C_{18} fractions was presumably due either to fatty acid synthesis *de novo* after decarboxylation of [2-^{14}C]malonyl-CoA or to the presence of endogenous primer acyl-CoA esters in the microsomal fraction. The chain lengths of fatty acids synthesized in the absence of added primer acyl-CoA were predominantly C_{16} and C_{18} (61%), although a substantial portion of the radioactivity was also present in the C_{24} fraction (25%). Total incorporation of [2-^{14}C]malonyl-CoA into fatty acids in the absence of primer acyl-CoA never exceeded 15% of that found in the presence of added primer. This suggested that the long-chain products obtained in the presence of palmitoyl-CoA and stearoyl-CoA were elongation products of the added acyl-CoA.

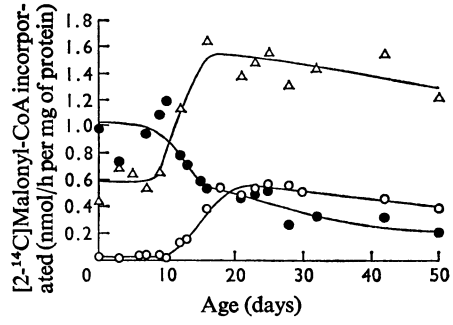

Fig. 5. *Microsomal elongation of acyl-CoA esters by rat brain preparations as a function of age*

Each incubation contained brain microsomal preparations from a minimum of four rats (six, when younger than 12 days) and the components of the standard assay as described in the Materials and Methods section. All values are the means of duplicates that agreed to within 10%: ●, 15.0 μM-acetyl-CoA; △, 8.0 μM-palmitoyl-CoA; ○, 7.5 μM-stearoyl-CoA.

Changes in the microsomal elongation of fatty acids during development. Standard assays were performed in the presence of either acetyl-CoA (15.0 μM), palmitoyl-CoA (8.0 μM) or stearoyl-CoA (7.5 μM). All incubations, which included controls with no added primer acyl-CoA, were in duplicate and agreed to within 10%.

Fig. 5 depicts the changes in the activities of fatty acid biosynthesis from acetyl-CoA, palmitoyl-CoA and stearoyl-CoA during development. The synthesis *de novo* of fatty acids from acetyl-CoA by microsomal fractions exhibits a steady decline in activity from birth to 50 days. A similar developmental pattern was observed for the soluble fatty acid synthetase of rat brain (Volpe & Kishimoto, 1972) and provides further evidence that the activity *de novo* in the microsomal fraction can be attributed to contamination by the soluble fatty acid synthetase. Almost no stearoyl-CoA elongation activity is present before 10 days, but there is a subsequent rapid rise in activity to a maximum at 18 days. During the same period there is also an increase in the microsomal elongation of palmitoyl-CoA. In contrast with the results of Goldberg *et al.* (1973) with mouse brain microsomal preparations, the palmitoyl-CoA elongation system is present in the microsomal fractions before its increase in activity at 10 days, although at a lower activity.

Discussion

We have studied the elongation of palmitoyl-CoA and stearoyl-CoA by microsomal fractions from the brains of rats at different stages of development. A major difficulty that we encountered was contamination of microsomal preparations by the soluble

de novo synthetase. We were, however, able to minimize this contamination by passage of the microsomal fractions through sucrose gradients. Although our experiments have not completely eliminated the possibility that the *de novo* activity might reside in the microsomal fractions, the properties of the activity *de novo* are virtually identical with those of the soluble fatty acid synthetase described by Volpe & Kishimoto (1972). The residual activity *de novo* probably results from occluded cytoplasm in the microsomal vesicles.

Carey & Cantrill (1975) have also observed that microsomal preparations from rabbit brain (Carey & Parkin, 1975) were significantly contaminated by a soluble synthetase. Similarly, the activity *de novo* reported by Bourré *et al.* (1973) from microsomal fractions from mouse brain may be largely caused by contamination by the soluble synthetase.

CoA inhibited the elongation of stearoyl-CoA, and this is in marked contrast with the elongation system of the high-molecular-weight fatty acid synthetase from *Mycobacterium smegmatis*, which is stimulated by CoA (Vance *et al.*, 1973). This difference may be explained by our observation that brain microsomal fractions synthesize elongated unesterified fatty acids. By contrast the products of elongation by the mycobacterial system are acyl-CoA esters.

The chain lengths of the fatty acids formed by elongation show a distribution similar to that found for mouse brain microsomal fractions (Pollet *et al.*, 1971) except that in the rat, a higher proportion of C_{22} and C_{24} fatty acids is synthesized when stearoyl-CoA is the primer.

The increase in activity of the microsomal elongations system during the active period of myelogenesis is similar to that observed in microsomal preparations from mouse brain (Goldberg *et al.*, 1973). The elongation of palmitoyl-CoA by microsomal preparations from rat brain does, however, increase to a distinctly higher activity than that of the stearoyl-CoA elongation system and is present (at a lower activity) from birth. This appears to differ from the mouse brain, in which no palmitoyl-CoA-elongation activity was observed before myelination (Goldberg *et al.*, 1973). The ability to elongate palmitoyl-CoA before myelogenesis may be explained by the requirement for C_{18} fatty acids in the formation of phospholipids which are used in cellular membranes.

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