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Short-Chain Fatty Acid Synthases in Brain

SUBCELLULAR LOCALIZATION AND CHANGES DURING DEVELOPMENT

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Acetyl-CoA synthase (EC 6.2.1.1), propionyl-CoA synthase (EC 6.2.1.–) and butyryl-CoA synthase (EC 6.2.1.2) were measured in subcellular fractions prepared by primary and density-gradient fractionation from adult rat brain by a method resulting in recoveries close to 100%. Most of the activity of the three enzymes was recovered in the crude mitochondrial fraction. On subfractionation of this crude mitochondrial fraction with continuous sucrose density gradients, most of the activity of the three enzymes was found at a higher density than NAD^+–isocitrate dehydrogenase and at about the same density as glutamate dehydrogenase, confirming earlier reported data for acetyl-CoA synthase. The finding that propionyl-CoA synthase and butyryl-CoA synthase had about the same distribution in the gradients as acetyl-CoA synthase adds support to the hypothesis that mitochondria involved in the metabolism of these short-chain fatty acids (all three of which have been shown to result in a rapid and high labelling of glutamine *in vivo*) form a distinct subpopulation of the total mitochondrial population. The three synthase activities were found to differ from each other in their rate of change and their subcellular localization during rat brain development. This, in combination with the observation that in gradients of adult brain preparations the three activities did not completely overlap, suggests that the three synthase activities are not present in the same proportion to each other in the same subpopulation(s) of mitochondria in the brain.

Labelled acetate, propionate and butyrate are rapidly incorporated into glutamate, glutamine and aspartate of rat and mouse brains (O'Neal & Koeppe, 1966; O'Neal *et al.*, 1966; Van den Berg *et al.*, 1969; Cremer & Lucas, 1971). These three short-chain fatty acids are incorporated more extensively into glutamine than glutamate, in contrast with glucose, which is incorporated more extensively into glutamate. To account for this difference between acetate and glucose, a two citric acid-cycle model has been developed (see Van den Berg, 1973; Reijnierse *et al.*, 1975). Evidence obtained on the subcellular distribution of acetyl-CoA synthase, glutamate dehydrogenase, succinate dehydrogenase and glutaminase (Neidle *et al.*, 1969; Blokhuis & Veldstra, 1970), has led to the proposal that the two tricarboxylic acid cycles involved in the incorporation of glucose and acetate into glutamate and related amino acids are located in separable mitochondria (Neidle *et al.*, 1969). However, considerable difficulties were met in obtaining high and reproducible recoveries of acetyl-CoA synthase after density-gradient fractionation. Similar difficulties were encountered by others (Whittaker, 1965; Tucek, 1967; Tencati & Rosenberg, 1973).

We report further investigation on the subcellular distribution of acetyl-CoA synthase to discover whether in fact it differs from that of a number of other mitochondrial enzymes. For this we have developed a reliable synthase assay, resulting in satisfactory recoveries. In addition we have measured the activities of propionyl-CoA synthase and butyryl-CoA synthase to see if these two enzymes were also located in particles partially separable from the bulk of the mitochondria.

A considerable body of evidence is available indicating that during brain development there are quantitative and qualitative changes in the metabolic patterns shown by the use of precursors of glutamate and related amino acids (Berl, 1965; Patel & Balázs, 1970; Van den Berg, 1970). In addition to the three synthases we have assayed several other of the enzymes in the developing brain that may play a role in the metabolic patterns of amino acids.

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Materials and Methods

Chemicals

The following radioactive chemicals were from The Radiochemical Centre, Amersham, Bucks., U.K.: [2-^{14}C]acetic acid, sodium salt (40–60mCi/mmol); [1-^{14}C]propionic acid, sodium salt (40mCi/mmol); *n*-[1-^{14}C]butyric acid, sodium salt (10–30mCi/mmol); [1-^{14}C]acetyl-CoA (50mCi/mmol). Solutions containing ATP, CoA and CoA derivatives were freshly made; radioactive solutions were stored frozen. For further details see Reijnierse *et al.* (1975).

Measurement of radioactivity

Radioactivity was measured by liquid-scintillation counting. The scintillation fluid consisted of 5.1g of 2,5-diphenyloxazole and 100mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 400ml of Triton X-100 and 600ml of toluene. A Philips liquid-scintillation analyser was used. Quench correction was performed by external standardization.

Preparation of homogenates, primary fractionation and density gradient centrifugation

Rats were killed by decapitation, and the whole brain was removed and homogenized in 0.32M-sucrose (pH7.0–7.4) (20vol./g wet wt.). Male adult Wistar rats were used but for the developmental studies rats of either sex were used. In the latter case litter sizes were decreased to eight, and there was free access to food and water.

The homogenates were prepared and fractionated as described by Reijnierse *et al.* (1975).

Assay of the dehydrogenase enzymes

Lactate dehydrogenase, NAD^+–isocitrate dehydrogenase and glutamate dehydrogenase were assayed as described in the preceding paper (Reijnierse *et al.*, 1975).

$NADP^+$–isocitrate dehydrogenase was measured fluorimetrically (Blokhuis, 1970). The 1.5ml incubation mixture consisted of 100mM-potassium phosphate buffer, pH7.6, 0.3mM-$NADP^+$, 0.5mM-$MnCl_2$, 2mM-trisodium DL-isocitrate and a suitably diluted enzyme preparation, pretreated with 0.5% (v/v) Triton X-100 for 30min. Standards containing 2 or 20μM-NADPH replacing isocitrate were always incubated simultaneously. The reaction was allowed to proceed for 30min at 37°C. The rest of the procedure was as described for glutamate dehydrogenase (Reijnierse *et al.*, 1975).

Assay of the synthase activities

As one of the major objects of this study was to obtain recoveries of acetyl-CoA synthase close enough to 100% to allow a quantitative interpretation of the distribution curves obtained with sucrose density gradients to be made, we have taken great care to develop a method giving a satisfactory recovery; details of this method will be described elsewhere.

The principle of the method is the separation of the CoA derivatives of the short-chain fatty acids from the fatty acids by the use of Dowex 1 (X4; 200–400 mesh: Cl^- form), the radioactive CoA derivatives then being counted by liquid-scintillation spectrometry.

It appeared that the amount of sucrose in the incubation medium did affect the activity measured. When enzyme preparations were assayed with increasing amounts of sucrose in the incubation mixture we observed a linear relation between activity and sucrose concentration. For acetyl-CoA synthase this relation can be described by the equation $y=-121x+100$, for propionyl-CoA synthase by $y=-77x+99$ and for butyryl-CoA synthase by $y=32x+101$, where y signifies percentage of activity at 0.01M-sucrose and x the sucrose molarity in the incubation medium. This equation was found to hold for acetyl-CoA synthase and propionyl-CoA synthase in the range 0.0–0.5M-sucrose and for butyryl-CoA synthase in the range 0.0–0.18M-sucrose. Sucrose (0.5M) did not influence the blanks.

Activities measured in the primary fractions can be compared directly as they were all taken up in 0.32M-sucrose and diluted with 0.32M-sucrose. All activities in these preparations and those measured in fractions from density gradients have been corrected for variations in sucrose concentration; the equations given above have been used to correct for incubation at 0.01M-sucrose. The sucrose concentration in the incubation medium was calculated from that measured in the fractions obtained and from the dilution factor.

It should be noted that the stability of acetyl-CoA synthase with time was found to depend on the sucrose concentration in the medium in which it was stored; this, however, does not appear to be a problem because of the short centrifugation time used by us, and because we did the assays as soon as possible after the fractions were obtained.

Assay of acetyl-CoA synthase

A 0.3ml portion of enzyme preparation was added to 0.2ml of a solution containing 22.5mM-$MgCl_2$, 1.25% (v/v) Triton X-100 and 4.5mg/ml bovine serum albumin. Enzyme preparation (heat-denatured at 100°C for 5min) was added to the controls. After 30min in an ice–water bath 0.3ml of 1.8mM-CoA–9mM-ATP–150mM-potassium phosphate buffer, pH8.0, and 0.1ml of 30mM-sodium [2-^{14}C]acetate (2.2×10^6d.p.m./ml) were added. Tubes were incubated for 30min at 37°C and the reactions stopped by heating for 5min at 100°C. After cooling, the tubes were centrifuged for 15min at 1400*g*. Each supernatant was transferred to a column (0.8cm×2cm) of Dowex 1 (X4; 200–400 mesh: Cl^- form). Free acid

was eluted with 50ml of 2M-formic acid. The CoA derivative was then eluted with 4.5ml of 2M-HCl. (It was shown that with this procedure the separate recoveries of the free acids and their CoA derivatives were more than 99%, with negligible cross-contamination.) The HCl fractions were assayed for radioactivity.

Assay of propionyl-CoA synthase

Sodium [1-^{14}C]propionate (30mM, 2.2×10^6 d.p.m./ml) replaced acetate. In other respects the procedure was as for acetyl-CoA synthase.

Assay of butyryl-CoA synthase

The potassium phosphate buffer was at pH7.1; the ATP addition was at 18mM; sodium [1-^{14}C]-butyrate (36mM, 1.1×10^6 d.p.m./ml) replaced acetate and the enzyme addition was 0.1ml. In other respects the procedure was as for acetyl-CoA synthase.

The incubation conditions chosen were not optimal for all three enzymes; this was done for reasons of uniformity and because many assays had to be run together, long delays resulting in enzyme inactivation (see above). We have ascertained, however, that the conditions chosen were such that the activities obtained were linearly related to the amount of enzyme present.

We have also shown by paper chromatography that the products formed are indeed the CoA derivatives of acetate, propionate and butyrate.

Protein

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Activities of acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase in adult rat brain and their distribution in primary fractions

The activity of acetyl-CoA synthase in unfractionated homogenized adult rat brain was found to be 17.4 μmol/h per g wet wt. This value is higher than that previously reported (Schuberth, 1965; Neidle *et al.*, 1969; Aas, 1971; Tencati & Rosenberg, 1973). In sheep and guinea-pig brains activities of the order of 2–3 μmol/h per g wet wt. were observed (Tucek, 1967). The activity of propionyl-CoA synthase was found to be 38.3 μmol/h per g wet wt. and that of butyryl-CoA synthase 278 μmol/h per g wet wt. These activities are higher than those reported earlier, 20 and 30 μmol/h per g wet wt. respectively for these two enzymes (Aas, 1971).

By differential centrifugation of rat brain sucrose homogenates the three synthase enzymes were found to be primarily present in the crude mitochondrial fraction (Table 1). Small amounts of activity were recovered in the postmitochondrial fractions. More of the three synthase enzymes, especially acetyl-CoA synthase, were found in the supernatant than NAD$^+$–isocitrate dehydrogenase and glutamate dehydrogenase. The distribution of lactate dehydrogenase, present in the cytosol in the nerve endings and elsewhere, differed substantially from the three synthase enzymes.

The distribution of NADP$^+$–isocitrate dehydrogenase was intermediate between that of the mitochondrial enzymes and that of lactate dehydrogenase, indicating that it is partially a mitochondrial and partially a cytosolic enzyme (Salganicoff & Koeppe, 1968; Blokhuis & Veldstra, 1970).

The percentage of the mitochondrial enzymes glutamate dehydrogenase and NAD$^+$–isocitrate dehydrogenase recovered in the crude nuclear fraction was quite high, some 20–30% of the total. This was because the crude nuclear fraction was not washed (Van Kempen *et al.*, 1965).

Small amounts of propionyl-CoA-synthase and butyryl-CoA synthase were recovered in the combined postmitochondrial fractions (Table 1). This may be due in part to mitochondrial damage, as some glutamate dehydrogenase and NAD$^+$–isocitrate dehydrogenase were also found in the postmitochondrial fraction, or it may be that these enzymes are to some extent located extramitochondrially, though from the data given in Table 1, this could amount at most to 2–4%. The acetyl-CoA synthase present in the postmitochondrial fractions (about 9%) was higher than that observed for any of the other mitochondrial enzymes. Very likely there is some cytosolic acetyl-CoA synthase in brain. Similar data have been published (Schuberth, 1965; Tucek, 1967; Neidle *et al.*, 1969; Tencati & Rosenberg, 1973).

Changes in the activity and subcellular localization of the three synthase enzymes and four dehydrogenase enzymes during rat brain development

The activities of lactate dehydrogenase, NAD$^+$–isocitrate dehydrogenase, glutamate dehydrogenase, acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase were all found to increase during the development of the rat brain in the postnatal period (Fig. 1, Table 1). The rate of increase of these enzymes was greatest in the second and third postnatal week. The activity of NADP$^+$–isocitrate dehydrogenase decreased during this period.

Over the whole period the activities of lactate dehydrogenase, NAD$^+$–isocitrate dehydrogenase and glutamate dehydrogenase increased about fourfold. Acetyl-CoA synthase and butyryl-CoA synthase activity also increased about fourfold, whereas that of propionyl-CoA synthase increased less than twofold. Glutamate dehydrogenase differed from NAD$^+$–isocitrate dehydrogenase in that it reached its adult activity somewhat earlier in development. Protein

Table 1. *Distribution of enzymes in primary subcellular fractions prepared from rat brain at various stages of its development*

The activity of each fraction, mean±S.E.M., is given as a percentage of the total recovered activity. Pooled homogenates from three brains were used for the 4-day-old animals. The results on the distribution are for three experiments, the activities in the unfractionated homogenate for the numbers of experiments indicated in parentheses.

Enzyme	Age	Enzyme activity (μmol/h per g wet wt.)	Activity in fractions (%)				Recovery (%)
			Nuclear	Mitochondrial	Microsomal	Supernatant	
Acetyl-CoA synthase	4 day	4.3±0.4 (5)	27.9±3.5	49.3±5.5	4.2±2.3	18.7±2.3	92±6
	14 day	11.9±1.2 (3)	28.6±1.2	49.6±1.1	6.6±1.7	15.2±1.9	89±5
	Adult	17.4±1.1 (5)	28.6±0.8	62.3±1.8	1.1±0.8	8.0±1.1	92±2
Propionyl-CoA synthase	4 day	20.6±1.4 (6)	62.3±5.3	30.3±4.2	2.8±0.9	4.6±0.5	106±17
	14 day	23.0±2.5 (3)	48.7±1.5	44.7±1.1	2.5±0.2	4.2±1.1	101±5
	Adult	38.3±3.1 (7)	37.8±1.9	56.3±2.0	1.8±0.2	4.2±0.3	90±3
Butyryl-CoA synthase	4 day	65±9 (4)	36.8±1.9	56.8±1.0	4.3±0.6	2.1±0.3	99±6
	14 day	216±9 (3)	30.8±0.9	66.6±1.0	1.6±0.3	1.1±0.2	76±8
	Adult	278±7 (5)	39.9±5.0	55.7±4.5	1.3±0.5	3.1±0.4	73±7
Lactate dehydrogenase	4 day	2176±83 (12)	12.6±2.3	26.7±1.3	17.5±1.2	43.1±3.1	100±7
	14 day	5700±315 (12)	8.9±0.9	34.3±1.4	22.1±1.3	34.7±1.9	91±3
	Adult	8052±345 (12)	8.2±0.8	38.5±2.3	14.7±1.2	38.7±2.9	92±7
NAD^+–isocitrate dehydrogenase	4 day	246±16 (7)	20.7±1.3	69.8±2.6	6.8±1.0	2.7±0.2	100±5
	14 day	526±29 (7)	20.0±2.3	75.4±1.8	3.3±0.4	1.2±0.1	88±3
	Adult	1070±66 (9)	21.1±0.1	75.8±0.9	2.3±1.0	0.7±0.1	95±2
Glutamate dehydrogenase	4 day	147±11 (6)	27.9±3.1	66.4±5.1	5.1±1.7	0.6±0.3	101±0
	14 day	469±57 (6)	27.9±2.9	69.2±2.5	1.9±0.2	1.1±0.2	84±10
	Adult	590±32 (9)	30.0±2.7	67.5±2.1	1.3±0.5	1.2±0.1	99±14
$NADP^+$–isocitrate dehydrogenase	4 day	580±33 (4)	17.3±0.6	40.0±2.2	17.3±1.6	25.4±1.0	87±8
	14 day	463±27 (4)	17.6±1.2	42.7±2.0	16.2±0.9	23.5±1.4	92±4
	Adult	314±23 (4)	25.2±1.2	46.4±1.8	6.8±0.7	21.7±1.2	88±3

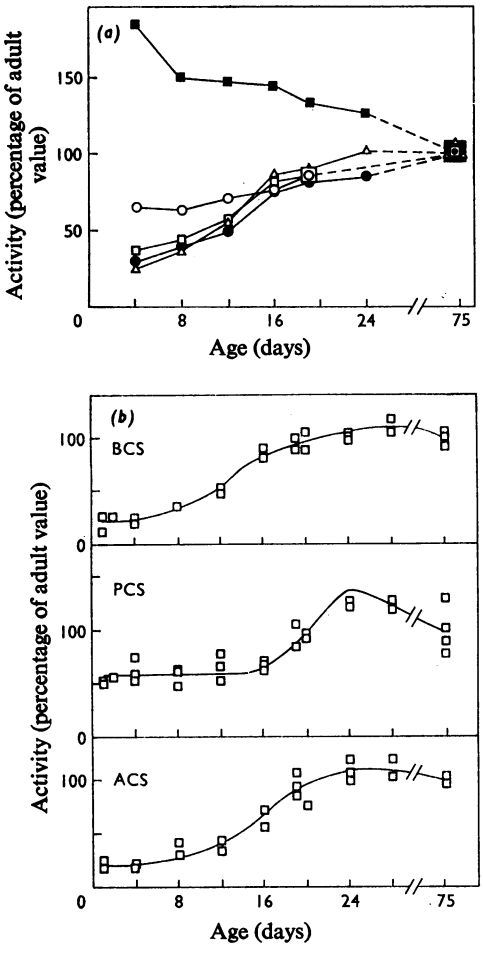

Fig. 1. *Enzyme activities in brain during postnatal growth of the rat*

(*a*) Activities of lactate dehydrogenase (□), NAD^+–isocitrate dehydrogenase (●), glutamate dehydrogenase (△), $NADP^+$–isocitrate dehydrogenase (■) and protein. (○) [Protein content in the adult rat brain was 130.4±3.9mg/g wet wt. (5)]. The values are averages for three to five experiments per age point. (*b*) Activities of acetyl-CoA synthase (ACS), propionyl-CoA synthase (PCS) and butyryl-CoA synthase (BCS). Each symbol indicates a value for one animal. All enzyme activities are given in percentages of the adult values, as given in Table 1.

increased by about 50% over the same period of development. Obviously, there are many different patterns of developmental changes of enzymes or groups of enzymes.

The subcellular distribution of the enzymes measured, except the synthase enzymes, did not change very much during development; the percentage distribution over the primary fractions being constant (Table 1). The percentage activity of acetyl-CoA synthase in the postmitochondrial fractions decreased by more than half, but as the total activity of the enzyme increased there was no decrease in the measured enzyme activity in the postmitochondrial fraction (cytosol). Buckley & Williamson (1973) measured acetyl-CoA synthase in 35000*g* (45min) supernatants of homogenates prepared in 4vol. of 10mM-Tris–0.25M-sucrose and obtained results very similar to ours, although an exact comparison is not possible owing to the differences in the preparations assayed. The possibility that the relatively high activity of cytosolic acetyl-CoA synthase is the result of more extensive mitochondrial damage during homogenate preparation and fractionation of brain in the early stages of the development seems unlikely from the results obtained for the other mitochondrial enzymes. At the most, some 3% of these enzymes was present in the postmitochondrial supernatant and somewhat more in the crude microsomal fraction at any of the ages investigated.

The most marked change in the distribution of any of the enzymes measured was for propionyl-CoA synthase (Table 1). More than 60% of this enzyme was present in the crude nuclear fraction from the brain of 4-day-old animals, whereas this percentage was less than 40% for adult rat brain. The percentage of the total adult activity for propionyl-CoA synthase in the immature brain was relatively high when compared with acetyl-CoA synthase and butyryl-CoA synthase; when a comparison is made of the total activity in the crude mitochondrial fraction between these three enzymes (during development), there is a much smaller difference.

At each of the developmental ages investigated, glutamate dehydrogenase, acetyl-CoA synthase and butyryl-CoA synthase were present at about 30% of the total activity in the crude nuclear fraction and NAD^+–isocitrate dehydrogenase at about 20%. Differences in distribution of this kind between the crude mitochondrial and the crude nuclear fractions could be taken as evidence of mitochondrial heterogeneity and the present results suggest that such heterogeneity occurs at all developmental stages.

Distribution of acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase in fractions obtained by sucrose density fractionation of crude mitochondrial fractions from adult rat brain

The three synthase enzymes and lactate dehydrogenase, NAD^+–isocitrate dehydrogenase and glutamate dehydrogenase were determined in each of the 19 fractions obtained after centrifugation in a sucrose density gradient for 1h at 53000$g_{av.}$. The results are presented in Fig. 2. The three synthase enzymes and glutamate dehydrogenase sedimented at higher sucrose densities than did lactate dehydrogenase.

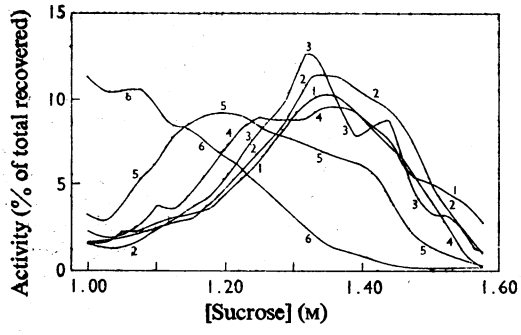

Fig. 2. *Distribution of acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase and some reference enzymes in sucrose density gradients from mitochondrial preparations from adult rat brain*

Centrifugation was for 1h at 53000$g_{av.}$. Data were obtained from two experiments and averaged as described by Reijnierse *et al.* (1975) to obtain the lines shown. All six enzymes were assayed in each fraction. The average total recoveries were: acetyl-CoA synthase 98%, propionyl-CoA synthase 94%, butyryl-CoA synthase 96%, glutamate dehydrogenase 98%, NAD^+-isocitrate dehydrogenase 85% and lactate dehydrogenase 102%. Symbols: 1, acetyl-CoA synthase; 2, propionyl-CoA synthase; 3, butyryl-CoA synthase; 4, glutamate dehydrogenase; 5, NAD^+-isocitrate dehydrogenase; 6, lactate dehydrogenase.

Neidle *et al.* (1969) obtained similar results for acetyl-CoA synthase and glutamate dehydrogenase; however, in comparison with the present work the acetyl-CoA synthase activity was low and variable. Our recovery data are close enough to 100% to allow a more definite interpretation of the difference in distribution found for the enzymes to be made. It should be noted that the distribution curves of glutamate dehydrogenase, acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase were not identical.

Discussion

Distribution of acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase in primary fractions from brain

Less than 10% of acetyl-CoA synthase and less than 5–6% of propionyl-CoA synthase and butyryl-CoA synthase were found in the postmitochondrial fraction from rat brain. Some 3% of glutamate dehydrogenase and NAD^+-isocitrate dehydrogenase was also recovered in this fraction, indicating some mitochondrial damage, and we infer that at the most 7% of acetyl-CoA synthase and 2–3% of propionyl-CoA synthase and butyryl-CoA synthase are located in the cytosol in brain. The absence of a peak or shoulder for these three enzymes from the sucrose density gradients in the region where lactate dehydrogenase is present supports this conclusion. In the cytosol fraction from rat liver, kidney, heart and adipose tissue substantial amounts of acetyl-CoA synthase have been found (Barth *et al.*, 1971). In the guinea-pig heart about 18% of the acetyl-CoA synthase was found in the cytosol (Scholte *et al.*, 1971), which, according to these authors, was most likely owing to mitochondrial damage. Evidently, there are large organ and/or species differences in the intracellular localization of acetyl-CoA synthase.

During development, the greatest change was observed for propionyl-CoA synthase, which had a relatively high activity in the crude nuclear fraction of the immature brain; for acetyl-CoA synthase a relatively greater localization in the cytosol was observed in the immature brain. The distribution of at least some enzymes seems therefore also to be a function of the developmental stage.

There were differences in enzyme recoveries in the crude nuclear fraction from adult brain: about 30% for acetyl-CoA synthase and glutamate dehydrogenase, close to 40% for propionyl-CoA synthase and butyryl-CoA synthase, but only 21% for NAD^+-isocitrate dehydrogenase. Assuming that all these enzymes are largely or completely present within mitochondria, the differences in the proportional distribution of these enzymes between the crude nuclear and crude mitochondrial fraction could indicate that the mitochondria in these two fractions do not have the same enzymic composition. It may be that the mitochondria present in the crude nuclear fraction are heavier (larger) than those present in the crude mitochondrial fraction. It is also evident that there are three different patterns of distribution of the mitochondrial fraction in the adult tissue, or even more if the results obtained in developmental studies are also considered. No doubt there are changes in the qualitative and quantitative composition of mitochondria during brain development (Van den Berg, 1974.

Distribution of acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase in mitochondria separated by density-gradient fractionation

In previous investigations on the localization of acetyl-CoA synthase the recoveries obtained for this enzyme were low and variable, and a reliable interpretation of the data obtained was impossible (Tucek, 1967; Neidle *et al.*, 1969; Tencati & Rosenberg, 1973). Total enzyme recoveries from density gradients in the present work were close enough to 100% to allow a definite conclusion; a large proportion, at least, of acetyl-CoA synthase, propionyl-CoA synthase and butyryl-CoA synthase, is present in mitochondria which can be separated from those that contain NAD^+-isocitrate dehydrogenase. It does

seem that the enzymes that play a role in the metabolism of those compounds that result in rapid and extensive labelling of glutamine compared with glutamate are localized in a distinct and separable subpopulation of mitochondria from brain. This subpopulation may itself be heterogeneous. This follows from small differences seen in the distribution curves in the density gradient (Fig. 2), from the observations on the different patterns of distribution over primary fractions from the immature and adult brain, and from the finding that especially after centrifugation for a short time, glutamate dehydrogenase and γ-aminobutyrate transaminase peaks were clearly separated (Reijnierse *et al.*, 1975).

Short-chain fatty acid activation in brain

The activity of acetyl-CoA synthase in adult rat brain was about 17 μmol/h per g wet wt., higher than reported previously (Schuberth, 1965; Neidle *et al.*, 1969; Tencati & Rosenberg, 1973). The rate of operation of the tricarboxylic acid cycle is about 90 μmol/h per g wet wt. in mouse brain (Van den Berg & Garfinkel, 1971) and of the same order in rat brain (Hawkins *et al.*, 1974). The activity of acetyl-CoA synthase is therefore low compared with the actual rate of formation of acetyl-CoA. When rat brain slices were incubated with acetate as carbon source, no increase in oxygen uptake due to the acetate was found (Quastel & Wheatley, 1932; Gonda & Quastel, 1966); radioactive acetate was, however, converted into amino acids and labelled carbon (Gonda & Quastel, 1966).

Acetate may be produced in brain by various reactions. It was observed that brain slices incubated with pyruvate form a fair amount of acetate (Krebs & Johnson, 1937). Whether this acetate production was completely the result of an artifact or partly physiological is still not clear. A fairly active acetyl-CoA hydrolase with an activity of about 50 μmol/h per g wet wt. has been found in rat brain, mainly in the postmitochondrial supernatant (Anderson & Erwin, 1971). Finally, acetate is produced by the hydrolysis of acetylcholine, occurring *in vivo* at a rate of the order of 0.6 μmol/h per g wet wt. (Jenden *et al.*, 1974). The acetyl-CoA synthase present in brain is certainly active enough to remove acetate from this source. The degradation of acetylcholine into choline and acetate must occur largely outside the acetylcholine-containing neurons, since nerve-ending acetylcholine is stable in the absence of cholinesterase inhibitor (Whittaker, 1965).

Our observation that acetyl-CoA synthase is not localized to any significant extent in the nerve-endings, as follows from the low activity found for the enzyme in the region of the gradient where most of the lactate dehydrogenase is present, would be consistent with a role for acetyl-CoA synthase in the re-utilization of acetate liberated from acetylcholine.

The short-chain fatty acids have probably also a role in the synthesis of long-chain fatty acids in brain. Labelled acetate is rapidly incorporated into long-chain fatty acids in brain (for review see Dhopeshwarkar & Mead, 1973) and labelled propionate is rapidly incorporated in odd-numbered long-chain fatty acids (Kishimoto & Radin, 1966). If these short-chain fatty acid synthases play a role in the synthesis of long-chain fatty acids, this seems to occur mainly within the mitochondria, and probably in only a limited subpopulation of these mitochondria. Further, the developmental changes observed suggest that the rate of synthesis of fatty acids could increase with development, which contrasts with the viewpoint that the rate of lipid (fatty acid) synthesis is very high at the time of growth of the brain, and slows down when growth is complete (i.e. in the adult).

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References

Aas, M. (1971) *Biochim. Biophys. Acta* **231**, 32–47
Anderson, A. D. & Erwin, V. G. (1971) *J. Neurochem.* **18**, 1179–1186
Barth, C., Sladek, M. & Decker, K. (1971) *Biochim. Biophys. Acta* **248**, 24–33
Berl, S. (1965) *J. Biol. Chem.* **240**, 2047–2054
Blokhuis, G. G. D. (1970) Ph.D. Thesis, University of Leiden
Blokhuis, G. G. D. & Veldstra, H. (1970) *FEBS Lett.* **11**, 197–199
Buckley, B. M. & Williamson, D. H. (1973) *Biochem. J.* **132**, 653–656
Cremer, J. E. & Lucas, H. M. (1971) *Brain Res.* **35**, 619–621
Dhopeshwarkar, G. A. & Mead, J. F. (1973) *Adv. Lipid Res.* **11**, 109–142
Gonda, O. & Quastel, J. H. (1966) *Biochem. J.* **100**, 83–94
Hawkins, R. A., Miller, A. L., Cremer, J. E. & Veech, R. L. (1974) *J. Neurochem.* **23**, 917–923
Jenden, D. J., Chol, L. & Silverman, R. W. (1974) *Life Sci.* **14**, 55–63
Kishimoto, Y. & Radin, N. S. (1966) *Lipids* **1**, 47–61
Krebs, H. A. & Johnson, W. A. (1937) *Biochem. J.* **31**, 645–660
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Neidle, A., Van den Berg, C. J. & Grynbaum, A. (1969) *J. Neurochem.* **16**, 225–234
O'Neal, R. M. & Koeppe, R. E. (1966) *J. Neurochem.* **13**, 835–847
O'Neal, R. M., Koeppe, R. E. & Williams, E. I. (1966) *Biochem. J.* **101**, 591–597
Patel, A. J. & Balázs, R. (1970) *J. Neurochem.* **17**, 955–971
Quastel, J. H. & Wheatley, A. H. M. (1932) *Biochem. J.* **26**, 725–744
Reijnierse, G. L. A., Veldstra, H. & Van den Berg, C. J. (1975) *Biochem. J.* **152**, 469–475
Salganicoff, L. & Koeppe, R. E. (1968) *J. Biol. Chem.* **243**, 3416–3420

Scholte, H. R., Wit-Peeters, E. M. & Bakker, J. C. (1971) *Biochim. Biophys. Acta* **231**, 479–486

Schuberth, J. (1965) *Biochim. Biophys. Acta* **98**, 1–7

Tencati, J. R. & Rosenberg, R. N. (1973) *Biochim. Biophys. Acta* **293**, 542–551

Tucek, S. (1967) *J. Neurochem.* **14**, 531–545

Van den Berg, C. J. (1970) *J. Neurochem.* **17**, 955–971

Van den Berg, C. J. (1973) in *Metabolic Compartmentation in the Brain* (Balázs, R. & Cremer, J. E., eds.), pp. 137–166, Macmillan, London

Van den Berg, C. J. (1974) in *Biochemistry of the Developing Brain* (Himwich, W. A., ed.), vol. 2, pp. 149–198, Marcel Dekker, New York

Van den Berg, C. J. & Garfinkel, D. (1971) *Biochem. J.* **123**, 211–218

Van den Berg, C. J., Krzalic, Lj., Mela, P. & Waelsch, H. (1969) *Biochem. J.* **113**, 281–290

Van Kempen, G. M. J., Van den Berg, C. J., Van der Helm, H. J. & Veldstra, H. (1965) *J. Neurochem.* **12**, 581–588

Whittaker, V. P. (1965) *Prog. Biophys. Mol. Biol.* **15**, 39–96