

## KETONE-BODY UTILIZATION AND LIPID SYNTHESIS BY DEVELOPING RAT BRAIN—A COMPARISON BETWEEN *IN VIVO* AND *IN VITRO* EXPERIMENTS

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**Abstract**—The distribution of ketone bodies between oxidation and lipid synthesis was analysed in homogenates of developing rat brain. The capacity for lipid synthesis of homogenized or minced brain preparations was compared with rates of lipid synthesis *in vivo*, assessed by incorporation of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  into fatty acids and cholesterol. Brain homogenates of suckling rats (but not those of adults) incorporated label from  $[3\text{-}^{14}\text{C}]$ ketone bodies into lipids, but this process was slow as compared to  $^{14}\text{CO}_2$  production ( $< 5\%$ ) and much slower than the total rate of ketone-body utilization ( $< 0.5\%$ ). Study of  $^3\text{H}_2\text{O}$  incorporation demonstrated that the rates of lipogenesis and cholesterogenesis are at least one order of magnitude higher *in vivo* than *in vitro*. Maximal rates of  $^3\text{H}$  incorporation into fatty acids ( $3\text{ }\mu\text{mol/g brain}\cdot\text{h}$ ) and into cholesterol ( $0.6\text{ }\mu\text{mol/g brain}\cdot\text{h}$ ) were found during the third postnatal week. Adult rats still incorporated  $^3\text{H}$  into brain fatty acids at an appreciable rate ( $1\text{ }\mu\text{mol/g brain}\cdot\text{h}$ ), whereas cholesterogenesis was very low. It is concluded that *in vitro* measurements of lipid synthesis severely underestimate the rates that occur in developing rat brain *in vivo*. The high rate of  $^3\text{H}$  incorporation into lipids by developing and adult rat brain as compared to the amounts of these lipids present in the brain suggests an important contribution of endogenous lipid synthesis during brain development and an appreciable rate of fatty acid turnover during brain growth, but also in the adult brain.

Ketone bodies are important metabolic substrates for the neonatal rat throughout the suckling period which coincides with the growth spurt of the brain (reviewed in Robinson and Williamson, 1980). Studies with isolated brain preparations demonstrate that ketone bodies can partly replace glucose both as an oxidative and as a lipogenic substrate (Yeh *et al.*, 1977; Patel and Clark, 1980; Patel and Owen, 1977; Lopes-Cardozo *et al.*, 1980). Moreover, in young rats *in vivo* ketone bodies are incorporated into brain lipids, with a preference for cholesterol (Webber and Edmond, 1979; Yeh, 1980; Koper *et al.*, 1981).

During the postnatal growth spurt of rat brain, membrane assembly is a very active process which requires an efficient supply of cholesterol and of fatty acids for the synthesis of complex lipids. Uptake from the blood (Dhopeswarkar and Mead, 1973; Lyles *et al.*, 1975) and synthesis *in situ* (Webber and Edmond, 1979; Yeh, 1980; Koper *et al.*, 1981; Dhopeswarkar and Subramanian, 1977; Bourre *et al.*, 1977; Cook, 1978; Murad and Kishimoto, 1978) both contribute; however, the relative importance of these processes is still a matter of debate (Bourre, 1980; Cook, 1982; Yeh *et al.*, 1983).

The present investigation addresses the following questions: (1) How are ketone bodies distributed between oxidative and synthetic pathways in brain preparations of developing rats? (2) How do rates of lipogenesis and cholesterogenesis observed *in vitro* relate to these rates *in vivo*? (3) Is it possible to estimate the fraction of brain lipids that is synthesized *in situ* during development?

### EXPERIMENTAL PROCEDURES

#### Materials

Radiochemicals were purchased from Amersham International Ltd. (U.K.).  $[3\text{-}^{14}\text{C}]$ Acetoacetate was prepared by hydrolysis of the ethyl-ester ( $37^\circ\text{C}$ , 1 h, 0.1 M KOH) and neutralization with 0.1 M HCl. Enzymes, substrates and cofactors were supplied by Boehringer (Mannheim, Germany) or by Sigma (St Louis, MO, U.S.A.). Wistar rats (14-day-pregnant dams, 200–250 g body weight) from the Central Institute for the Breeding of Laboratory Animals (Zeist, Netherlands) were caged individually. Their litters were trimmed to 10 pups and used without discrimination of sex. The mothers were separated from their pups at day 21 and used as adult controls in developmental studies (Table 1; Figs 1, 2 and 3).

#### Brain preparations and incubation conditions

Whole-brain homogenates (40%, w/v, in incubation me-

Table 1. The utilization of ketone bodies by rat-brain homogenates as a function of postnatal age

Age (days)	Ketone-body utilization ( $\mu\text{mol/g brain/hr}$ )	
	Acetoacetate	D-3-Hydroxybutyrate
1	13.7 $\pm$ 1.1	7.4 $\pm$ 2.1
8	17.6 $\pm$ 0.4	16.2 $\pm$ 0.2
15	25.2 $\pm$ 1.9	19.6 $\pm$ 0.7
28	31.8 $\pm$ 0.7	25.1 $\pm$ 2.3
Adult	16.6 $\pm$ 0.4	9.5 $\pm$ 0.4

Homogenates, equivalent to 100 mg tissue, were incubated with 4 mM of either acetoacetate or D-3-hydroxybutyrate for 30 min at 30 °C in a medium containing 50 mM KCl, 150 mM sucrose, 2.5 mM Tris-EDTA, 10 mM Tris Cl, 1 mM L-malate, valinomycin (1  $\mu\text{g/ml}$ ) and 5 mM  $\text{MgCl}_2$ . The disappearance of the ketone bodies was corrected for their mutual interconversion (cf. Lopes-Cardozo and Klein, 1982). Mean  $\pm$  SE for triplicate incubations.

dium) were prepared with a loosely fitting, ground-glass Potter pestle operated by hand. Finely minced brain was added to the incubation flasks as a suspension (40%, w/v, incubation medium). Both preparations were added with a cell-pipette with a large orifice.

The incubation medium (high potassium Krebs-Henseleit-Ringer) contained 115 mM NaCl, 6 mM KCl, 1.2 mM  $\text{MgCl}_2$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , 1.2 mM  $\text{K}_2\text{SO}_4$ , 2.5 mM  $\text{CaCl}_2$  and 25 mM  $\text{KHCO}_3$  (pH 7.4) except in experiments described in Table 1. Brain preparations (equivalent to 200 mg brain per flask) were incubated in 25-ml Erlenmeyer flasks for 1 h at 37°C under  $\text{O}_2$ - $\text{CO}_2$  (95:5, v/v) unless indicated otherwise. Final volume, 1 ml. Reactions were stopped by injection of 1 ml 1 M  $\text{HClO}_4$  and the flasks were shaken for another 30 min to trap  $^{14}\text{C}$  quantitatively.

#### Ketone-body utilization

The utilization of ketone bodies was measured by enzymatic tests (Williamson *et al.*, 1962; Lopes-Cardozo and Klein, 1982) in samples from neutralized perchloric extracts of *in vitro* incubations and zero-time controls.  $^{14}\text{CO}_2$  was absorbed in an alkaline solution in a disposable center well attached to the rubber stopper (Kontes, Vineland, NJ, U.S.A.). Two-hundred  $\mu\text{l}$  Hyamine (1 M in methanol, Packard Instruments, Downers Grove, IL, U.S.A.) was injected into the center well 5 min before the end of the incubation period. After the incubation, the center wells were transferred to minivials with 3 ml Tritosol (Pande, 1976) and the  $^{14}\text{C}$  radioactivity was measured.

Total lipids were extracted from  $\text{HClO}_4$ -washed pellets (Bligh and Dyer, 1959). Control experiments indicated that less than 1% of the labeled lipids was in the supernatant and that more than 95% was recovered from the washed pellets. The distribution of label among lipid classes was measured after their separation by TLC (Koper *et al.*, 1981). Total lipids were fractionated after alkaline hydrolysis into a non-saponifiable fraction (sterols) and a saponifiable water-insoluble fraction (fatty acids) (Koper *et al.*, 1981).

#### Tritium incorporation

Lipogenesis in the brain *in vivo* was measured by incorporation of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$ , essentially as described by Stansbie *et al.* (1976). Briefly,  $^3\text{H}_2\text{O}$  was injected (0.1–0.2 mCi/g body weight, i.p.). Two pups were decapitated after 1 h and 2 pups after 2 h. The brain hemispheres

were digested separately in alkaline. Saponifiable and non-saponifiable lipid extracts were prepared and 3- $\beta$ -hydroxysterols were isolated from the non-saponifiable fraction with digitonin (Jeske and Dietschy, 1980) with a recovery of 94%. The specific activity of  $^3\text{H}_2\text{O}$  in the blood plasma was determined and used to calculate the rates of fatty acid and sterol synthesis in the brain. Ratios of 0.9 and 0.7 of  $^3\text{H}_2\text{O}$  per acetyl-group incorporated into fatty acids (Lowenstein *et al.*, 1975) and into sterols (Andersen and Dietschy, 1979), respectively, were adopted. Control experiments showed that  $^3\text{H}_2\text{O}$  equilibrated in less than 5 min over the body fluids and into the brain. The rate of  $^3\text{H}$  incorporation into fatty acids and into cholesterol was linear over a period of at least 24 h.

#### Other analytical procedures

Cholesterol (Zlatkis and Zak, 1969) and total fatty acids (Metz *et al.*, 1973) were assayed in aliquots of the non-saponifiable and saponifiable lipid extracts, respectively.

#### Calculations

The specific activity (dpm/nmol) of  $^{14}\text{C}$ -labelled substrates added to *in vitro* incubations was determined by measurement of their radioactivity and by enzymatic assay of their final concentration.

All values shown are means of duplicate or triplicate measurements differing less than 10%, unless indicated otherwise. Results were verified in at least two independent experiments.

Incorporation of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  *in vivo*: Duplicate values (for two brain hemispheres of 1 rat), differing less than 10%, were averaged. No difference was observed in the rates of incorporation of 1-h and 2-h experiments. The standard deviation of the mean result of 4 rats, therefore, mainly indicates biological variations within 1 litter.

## RESULTS

### Ketone-body utilization by preparations from developing rat brain

Figure 1 shows that the oxidation of [3- $^{14}\text{C}$ ]acetoacetate to  $^{14}\text{CO}_2$  by rat-brain homogenates increased until day 20, followed by a gradual decrease to an adult level which was equal to the rate found just after birth (approx 1  $\mu\text{mol/g brain/h}$ ). The oxidation of [U- $^{14}\text{C}$ ]glucose was low after birth and increased to an adult rate of 0.7  $\mu\text{mol/g brain/h}$ . The incorporation of [3- $^{14}\text{C}$ ]acetoacetate into lipids by homogenates of developing rat brain was slightly higher (60 nmol/g/h) for the second week than for the first week after birth and decreased during the third week and post-weaning period down to very low rates in adult rats (<10 nmol/g/h; Fig. 1). D-3-Hydroxybutyrate is the main circulating ketone body in the suckling rat. The developmental pattern of incorporation of this substrate into lipids was very similar to acetoacetate, though the rate was approx 30% lower (not shown). Glucose slightly ( $\leq 10\%$ ) stimulated lipogenesis from acetoacetate whereas

NADPH had no effect under our reaction conditions (see, however, Patel and Clark, 1980), indicating that lipogenesis was not limited by the availability of reducing equivalents.

The utilization of D-3-hydroxybutyrate followed a similar developmental pattern as that of acetoacetate (Table 1). The rate of acetoacetate utilization (14–32  $\mu\text{mol/g/h}$ ), however, was much higher than its oxidation to  $^{14}\text{CO}_2$  (Fig. 1); most of the label accumulated in acid-soluble intermediates (not shown, cf. Lopes-Cardozo and Klein, 1982).

Optimal doses of pyruvate and acetate were compared with ketone bodies as precursors for lipid synthesis by brain homogenates of 2-week old rats (Table 2). The relative rates of incorporation into lipids (sum of saponifiables and non-saponifiables) of acetyl-groups derived from the various substrates decreased in the following order: Acetoacetate (100%), D-3-hydroxybutyrate (82%), pyruvate (57%), acetate (33%), glucose (8%). About equal amounts of label from  $[\text{U-}^{14}\text{C}]\text{glucose}$  and from  $[\text{3-}^{14}\text{C}]\text{acetoacetate}$  were recovered in the total lipid fraction (before saponification). However, more than 90% of the label from  $[\text{U-}^{14}\text{C}]\text{glucose}$  was recovered in the glycerol-moiety of complex lipids whereas this was only 10% in case of  $[\text{3-}^{14}\text{C}]\text{acetoacetate}$ . The high incorporation of ketone bodies into lipids was even more marked at younger ages.

#### *Incorporation of tritium as a measure of lipid synthesis in vitro and in vivo*

The rate of lipogenesis was also estimated by measuring incorporation of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  (Stansbie *et al.*, 1976, Table 3). This rate was about equal to the rate of lipogenesis from  $[\text{3-}^{14}\text{C}]\text{acetoacetate}$  in the case of a brain homogenate, taking into account that acetoacetate yields two acetyl-groups. On the other hand, with a minced brain preparation only 60% of newly synthesized lipid carbon was derived from added acetoacetate indicating an important contribution from endogenous substrates.

A striking difference in composition was observed between the  $^3\text{H}$ -labeled lipids synthesized *in vitro* by homogenates as compared to the situation *in vivo*. *In vitro*  $^3\text{H}$  was about equally partitioned between cholesterol, free fatty acids and acyl-groups of phospholipids (approx. 0.03  $\mu\text{mol/g/h}$  to each category). On the other hand, *in vivo* almost no incorporation in free fatty acids was observed while  $^3\text{H}$  in the acyl-groups of phosphatidylcholine and phosphatidylethanolamine accounted for more than 90% of the saponifiable lipid fraction (results not shown).

Figure 2 shows the  $^3\text{H}$  incorporation into fatty

acids and into 3- $\beta$ -hydroxysterols by developing rat brain *in vivo* as a function of age. A large variation was observed, even when pups from the same litter were used. This variation must have a biological origin because  $^3\text{H}$  incorporation into the two separate brain hemispheres of the same pup varied by less than 10% (cf. Experimental Procedures). The amount of medulla spinalis per dissected brain is a possible source of variation because spinal cord and medulla were much more active in lipid synthesis than cortex areas, especially between day 10 and day 30 (unpublished results).  $^3\text{H}$  incorporation into fatty acids was maximal between days 10 and 20 (about 3  $\mu\text{mol/g/h}$ ) and decreased slowly to approx. 1  $\mu\text{mol/g/h}$  in adult rats. The  $^3\text{H}$  incorporation into cholesterol varied only little during development (about 0.5  $\mu\text{mol/g/h}$ ) but dropped to very low rates in adult rats.

Data on the weight and on the total fatty acid cholesterol content of developing rat brain (Fig. 3) were obtained from littermates of the pups used for the measurements of  $^3\text{H}$  incorporation (Fig. 2). The total amounts of fatty acids and of cholesterol increased though at a slightly slower rate than the increase in brain weight during the phase of rapid growth (day 0  $\rightarrow$  20). After day 20, when myelination proceeds, the rate of lipid deposition becomes higher in the brain than its gain in weight.

## DISCUSSION

### *Ketone-body utilization in vitro: Distribution between oxidation and lipid synthesis*

Only a small fraction (<10%) of the carbon derived from ketone bodies which were utilized by brain homogenates (Table 1) was recovered in metabolic end-products ( $\text{CO}_2$  and lipids, Fig. 1). This phenomenon was observed previously with homogenates from adult rat brain (Lopes-Cardozo and Klein, 1982) and could be explained by an extensive accumulation of labeled, acid-soluble intermediates.

The developmental patterns of ketone-body utilization (Table 1) and of the conversion of  $[\text{3-}^{13}\text{C}]\text{acetoacetate}$  into  $^{14}\text{CO}_2$  (Fig. 1) reflect changes in the activities of enzymes involved in ketone-body metabolism in developing rat brain. The activities of 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) and of 3-oxoacid CoA-transferase (EC 2.8.3.5) increase until the end of the third postnatal week and decrease after weaning to low values in the adult (Page *et al.*, 1971). Our data differ from a report by Yeh *et al.* (1977) who observed maximal  $^{14}\text{CO}_2$  production from

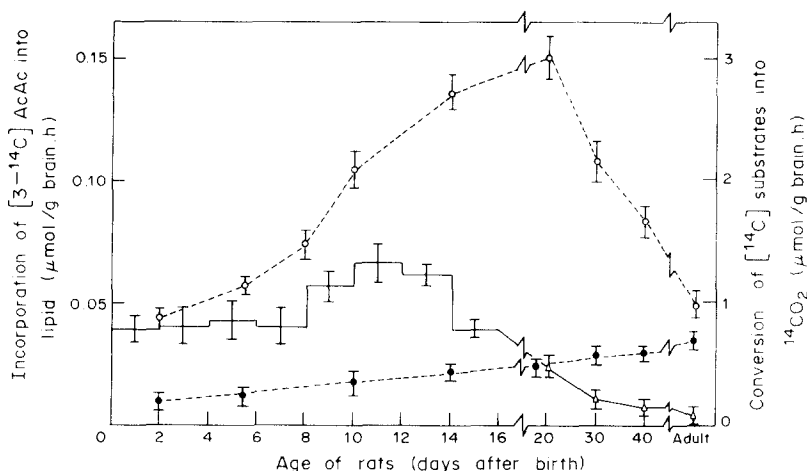


Fig. 1. Oxidation and lipid synthesis by brain homogenates of developing rats.  $^{14}\text{C}$  Incorporation from  $[3\text{-}^{14}\text{C}]\text{acetoacetate}$  (4 mM, 0.1 Ci/mol) into  $\text{CO}_2$  ( $\bigcirc$ ----- $\bigcirc$ ) and into lipids ( $\triangle$ — $\triangle$ ) was compared with  $^{14}\text{CO}_2$  formation from D- $[U\text{-}^{14}\text{C}]\text{glucose}$  (10 mM, 0.1 Ci/mol) ( $\bullet$ ----- $\bullet$ ). The values for  $^{14}\text{C}$  incorporation into lipids up to day 16 were obtained from pups of 16 separate litters (day 1 to day 16). The results were averaged over 3-day periods (day 0–2, 2–4 etc.). Bars indicate the SD.

$[3\text{-}^{14}\text{C}]\text{acetoacetate}$  by brain homogenates from 5-day old rat pups.

Ketone bodies were also used for lipid synthesis by homogenates of young rat brain (Fig. 1; Table 2), in contrast to brain homogenates of adult rats (Fig. 1 and Lopes-Cardozo and Klein, 1982; see however Yeh *et al.*, 1977). Higher rates of lipid synthesis by homogenates of developing rat brain have been re-

ported by other groups (Yeh *et al.*, 1977; Patel and Clark, 1980). We found higher rates only with minced (Table 3) or sliced (not shown) brain preparations, indicating that cellular integrity is important for the incorporation of ketone bodies into lipids. The preferential use of ketone bodies compared to glucose-derived carbon for the synthesis of cholesterol (Table 2, last column) is expressed more clearly in the brain

Table 2. The conversion of various  $[^{14}\text{C}]\text{labelled}$  substrates to  $^{14}\text{CO}_2$  and the incorporation of  $^{14}\text{C}$  into lipids by brain homogenates from 2-week old rats

Added $[^{14}\text{C}]\text{substrate}$	Conversion into $[^{14}\text{C}]\text{products}$ ( $\mu\text{mol/g brain/hr}$ )		Sterol
	$\text{CO}_2$	Lipids	Fatty acid
$[3\text{-}^{14}\text{C}]\text{Acetoacetate}$ (4 mM, 0.1 Ci/mol)	$2.85 \pm 0.22$	$0.038 \pm 0.003$	0.82
$[3\text{-}^{14}\text{C}]\text{D-3-Hydroxybutyrate}$ (4 mM, 0.1 Ci/mol)	$2.52 \pm 0.61$	$0.031 \pm 0.005$	0.72
$[U\text{-}^{14}\text{C}]\text{Glucose}$ (0.1 Ci/mol)	$0.42 \pm 0.04$	$0.003 \pm 0.001$	—†
$[2\text{-}^{14}\text{C}]\text{Pyruvate}$ (5 mM, 0.1 Ci/mol)	$3.49 \pm 0.24$	$0.043 \pm 0.007$	0.56
$[1\text{-}^{14}\text{C}]\text{Acetate}$ (5 mM, 0.1 Ci/mol)	$0.88 \pm 0.13$	$0.025 \pm 0.002$	0.95

†Incorporation into sterols was below the detection level ( $<0.001 \mu\text{mol/g/h}$ ).

The incubation medium (Experimental Procedures) was supplemented with 10 mM D-glucose and various  $[^{14}\text{C}]\text{labelled}$  substrates indicated in the Table. The radioactivity in  $\text{CO}_2$  and in the saponifiable and non-saponifiable lipid-fractions was measured (the sum of these fractions is tabulated under Lipids). Mean  $\pm$  SE; for acetoacetate  $n = 7$ , for other substrates  $n = 3$ . The last column gives the ratio of  $^{14}\text{C}$  incorporation into the non-saponifiable lipid fraction over the saponifiable lipid fraction.

Table 3. The incorporation of label from  $^3\text{H}_2\text{O}$  and from  $[3\text{-}^{14}\text{C}]\text{acetoacetate}$  into the saponifiable and non-saponifiable lipid fractions by 10-day old rat brain *in vitro* and *in vivo*

	Incorporation into lipids ( $\mu\text{mol/g brain/hr}$ )			
	$^3\text{H}$ from $^3\text{H}_2\text{O}$		$^{14}\text{C}$ from $[3\text{-}^{14}\text{C}]\text{acetoacetate}$	
	Saponifiable lipids	Non-saponifiable lipids	Saponifiable lipids	Non-saponifiable lipids
Brain homogenates	0.05	0.04	0.03	0.02
Brain mince	0.31	0.10	0.10	0.04
Brain <i>in vivo</i>	2.5	0.5	—	—

For *in vitro* experiments  $18\text{ mCi } ^3\text{H}_2\text{O}$ ,  $4\text{ mM } [3\text{-}^{14}\text{C}]\text{acetoacetate}$  ( $0.1\text{ Ci/mol}$ ) and  $10\text{ mM D-glucose}$  were added to the incubation medium.

*in vivo* (Webber and Edmond, 1979; Yeh, 1980; Koper *et al.*, 1981).

#### Lipid synthesis by developing rat brain *in vivo*; comparison with *in vitro* results

The developmental patterns of lipid synthesis *in vitro* (Fig. 1) and *in vivo* (Fig. 2) show some striking differences. The utilization of acetoacetate for lipid synthesis by brain homogenates (Fig. 1) remained almost constant throughout the suckling period and dropped to a very low level after weaning. Lipogenesis *in vivo* (Fig. 2) on the other hand increased after birth until day 15 and decreased slowly afterwards to a rate in adult rats which was still about 50% of the maximal rate.

These results suggest that ketone bodies are important precursors for synthesis of brain lipids only during the suckling period in which they are indeed

among the physiological substrates (Robinson and Williamson, 1980).

Using the incorporation of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  as an index for lipogenic rate, we found that this process is at least 8-fold more active *in vivo* than *in vitro* (Table 3). The distribution of the  $^3\text{H}$  label among lipid classes was also quite different.

Various factors could be involved in lowering the lipogenic capacity of *in vitro* preparations like: (1) dilution of cofactors (2) induction of a catabolic rather than anabolic state by cellular damage (3) a block in the synthesis of complex lipids. As NADPH, ATP and CoASH did not stimulate lipogenesis by homogenates, we have no evidence for (1), (2) and (3)

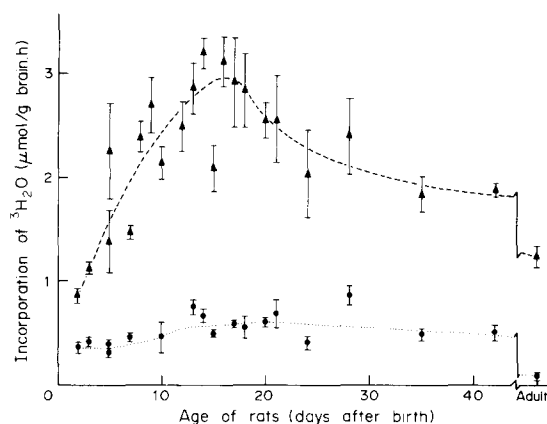


Fig. 2. Incorporation of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  into total fatty acids and into  $3\text{-}\beta\text{-hydroxysterols}$  by developing rat brain *in vivo*—( $\Delta$ — $\Delta$ ), incorporation into total fatty acids (saponifiable fraction) ( $\bullet$ ..... $\bullet$ ), incorporation into  $3\text{-}\beta\text{-hydroxysterols}$  (non-saponifiable, digitonin-precipitable). Mean  $\pm$  SD.

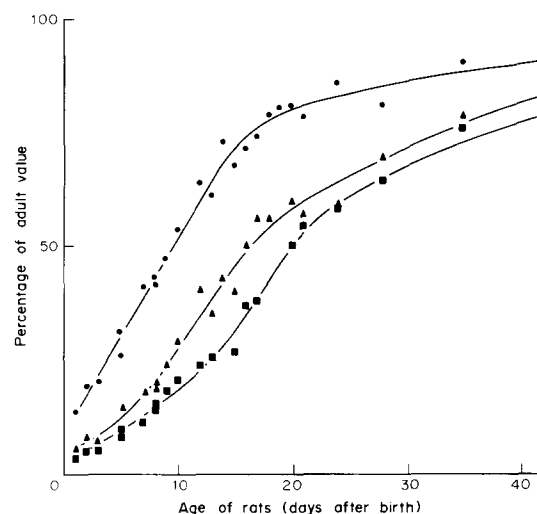


Fig. 3. Increase in wet weight and in total fatty acid and cholesterol content during development of rat brain. ( $\bullet$ — $\bullet$ ), brain weight. ( $\Delta$ — $\Delta$ ), total fatty acids. ( $\blacksquare$ — $\blacksquare$ ), cholesterol. Values from adult rats ( $\text{♀}$ ,  $225\text{ g}$  body weight) were:  $1.54 \pm 0.03\text{ g}$  brain wet weight,  $143.3 \pm 6.0\text{ }\mu\text{mol}$  total fatty acids/brain,  $76.4 \pm 4.5\text{ }\mu\text{mol}$  cholesterol/brain.

Table 4. Comparison of the increase in brain lipids during postnatal growth and the rate of  $^3\text{H}$  incorporation from  $^3\text{H}_2\text{O}$  in these lipids

Postnatal period, day	Cholesterol		Fatty acids	
	10 → 20	30 → 40	10 → 20	30 → 40
Increase ( $\mu\text{mol}/\text{brain}$ )	22	6	52	15
(acetyl- $\mu\text{eq.}/\text{brain}$ )	396	108	416	120
Incorporation of $^3\text{H}_2\text{O}$ ( $\mu\text{mol}/\text{brain}/\text{h}$ )*	0.66	0.84	2.97	3.08
( $\mu\text{mol}/\text{brain}/10\text{ days}$ )*	158	202	713	739

\*Average rates.

The increase in brain cholesterol and total fatty acids were taken from the data in Fig. 3 and converted into acetyl-equivalents assuming that 18 and 8 of these equivalents are used per mol of cholesterol and fatty acid, respectively. The incorporation rates of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  were taken from Fig. 2 and multiplied by the average brain weight for the two periods (Fig. 3).

appear more likely because we observed an accumulation of labeled free fatty acids *in vitro* whereas *in vivo de novo* synthesized fatty acids were found predominantly in the phospholipid fraction.

#### Contribution of endogenous synthesis to brain lipids during development

The relatively low metabolic activity of brain preparations *in vitro* has led to an underestimation of the capacity of developing rat brain to synthesize fatty acids and cholesterol. An intriguing question is how this endogenous synthesis relates quantitatively to the uptake of lipids from the blood and to the over-all deposition of lipids in the brain during growth; a problem that has been discussed previously by others (Bourre, 1980; Cook, 1982; Yeh *et al.*, 1983). Our data shed some light on this problem: The rates of lipogenesis and cholesterologenesis can be calculated from the  $^3\text{H}$  incorporation (Fig. 2) and compared with the rates of fatty acid and cholesterol deposition during development of the rat (Fig. 3). Such calculations have been carried out in Table 4 for two 10-days periods: Period I (days 10–20), when the

growth rate of the brain is maximal. Period II (days 30–40), after weaning when the brain weight increases at a much slower rate (Fig. 3). It should be noted that the results of Table 4 must be interpreted with caution because: (i)  $^3\text{H}$  incorporation was taken from the experiment in Fig. 2 and extrapolated (and averaged) over a 10-day period. Linearity of  $^3\text{H}$  incorporation over a 1-day period was observed, however (Experimental Procedures). (ii) The stoichiometry of  $^3\text{H}$  incorporation into brain lipids is not known (cf. Cenedella, 1982). Ratios of 0.9 and 0.7 of  $^3\text{H}_2\text{O}$  per acetyl-group incorporated into fatty acids (Lowenstein *et al.*, 1975) and into sterols (Andersen and Dietschy, 1979) are generally adopted.

The following conclusions can be drawn from the results in Table 4: (a) In period I more cholesterol is deposited than synthesized in the brain, indicating an exogenous contribution. (b) In period II the situation is reversed:  $^3\text{H}$  incorporation into cholesterol greatly exceeds the rate of cholesterol deposition. This may result from turnover of brain cholesterol, which is unlikely (Dhopeswarkar and Subramanian, 1981), or from export of cholesterol from the brain. (c)

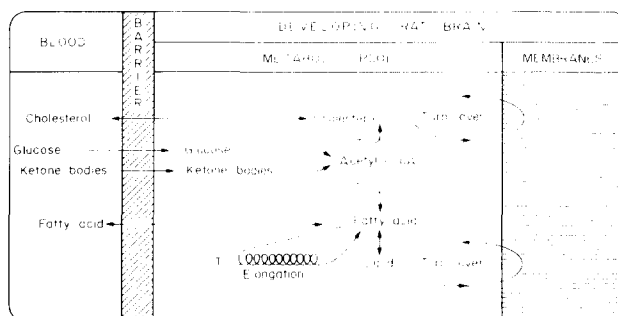


Fig. 4. Scheme of fatty acid and cholesterol metabolism in developing rat brain. T, entry of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$ .

Incorporation of  $^3\text{H}$  into fatty acids greatly exceeds the deposition of fatty acids in developing brain in period I and even more so in period II. This discrepancy between  $^3\text{H}$  incorporation and deposition of fatty acid is even more pronounced in view of the high content of polyunsaturated fatty acids in brain (approx 30%) (Bourre, 1980) which require linoleic and linolenic acids as precursors (Sinclair, 1975). Figure 4 tries to fit these conclusions into a conceptual framework. Cholesterol and fatty acids can be synthesized *de novo* by developing rat brain or can be imported from the blood. Part of the fatty acids are processed (elongation, desaturation) before their incorporation into complex lipids. The contribution of ketone bodies as lipid precursors is difficult to quantify *in vivo* (cf. Koper *et al.*, 1981). Recent data indicate, however, that ketone bodies contribute 33 and 50% to the synthesis of fatty acids and cholesterol, respectively, in the brains of 18-day old rats (Bergstrom *et al.*, 1981).

Our  $^3\text{H}$ -incorporation data indicate that turnover and/or export of cholesterol and fatty acids are quantitatively important processes during brain development. Even in adult rats  $^3\text{H}$  is incorporated into brain fatty acids at a rate of about  $1\ \mu\text{mol } ^3\text{H}_2\text{O/g/h}$  (Fig. 2) which is equivalent to  $40\ \mu\text{mol}$  acetyl-groups (about 2% of its total fatty acid content) per brain per day.

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