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METABOLISM OF 1-¹⁴C PALMITIC ACID IN THE DEVELOPING BRAIN:
PERSISTENCE OF RADIOACTIVITY IN THE CARBOXYL CARBON

by

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SUMMARY

$1-^{14}\text{C}$ Palmitic acid was injected intraperitoneally into young rats (12-13 days old) to study the label distribution over an extended period of 2 months during which time rapid growth and active myelination occurs. It was found that, 24 hours after injection, brain palmitate still had 78% RCA. It was also noted that label persisted in the carboxyl carbon of brain palmitate even after an extended period of 2 months.

Radioactivity distribution and specific activities of 18:0, 18:1, 20:4 and 22:6 conclusively showed that these fatty acids were synthesized by chain elongation. Similarly, radioactivity in the cholesterol fraction indicated that injected palmitate was degraded to form radioactive acetate; thus it was surprising to find that radioactivity in brain palmitate was not redistributed by de novo synthesis from radioactive acetate. Hypothetical possibilities for this finding are discussed.

INTRODUCTION

Earlier work⁽¹⁾ from this laboratory revealed that for periods ranging from 1 to 24 hours after feeding 1-¹⁴C palmitic acid to adult rats, the radioactivity of brain total lipids as well as the brain palmitate remained at about the same level. Further, when palmitic acid from the brain was decarboxylated, it was found that at all time periods, the carboxyl carbon retained most of the activity. Later work⁽²⁾ showed that when palmitate was injected intracarotidly, there was a rapid uptake followed by persistence of the label. No information is available as to whether the label from 1-¹⁴C long-chain fatty acids injected into young rats of premyelinating age would be redistributed or remain in the carboxyl carbon during the period of myelination and rapid lipid synthesis. Similar data were obtained for 4-¹⁴C cholesterol by Davison and Wajda⁽³⁾. However, the nucleus of the cholesterol molecule is not readily degradable in the mammalian system and hence the persistence of radioactivity in brain cholesterol is not unexpected. In the case of carboxy-labeled fatty acid administered to an animal, randomization of the label is a probability since the fatty acids are known to be degraded to acetate and resynthesized. Long term studies on the fate of carboxy-labeled fatty acids by Hajra and Radin⁽⁴⁾ were restricted to a maximum of 9 days. Sun and Horrocks⁽⁵⁾ have carried the experiment over much longer periods using uniformly labeled palmitate, but uniformly labeled palmitate is not suitable to provide information regarding oxidation to acetate and resynthesis.

The present study was undertaken to study the metabolic transformation of 1-¹⁴C palmitic acid, administered to young animals of premyelinating

age, over an extended period of two months after injection, during which active myelination and rapid synthesis occur.

MATERIALS AND METHODS

Animals: 18 Baby albino Wistar rats, 12-13 days old, weighing approximately 32 g were used in the study. They were given an I.P. injection of $1\text{-}^{14}\text{C}$ palmitate and returned to their mothers. After reaching an age of 21-24 days, they were separated from their mothers. During the entire period, the rats had free access to food (Purina Lab Chow) and water.

Tracer: 10 μC of $1\text{-}^{14}\text{C}$ palmitic acid (57.7 mC/mM, Radiochemical Centre, Amersham, U.K.) was injected I.P. as an albumin complex.

5 Baby rats were sacrificed 24 hours after the tracer dose. One month after the tracer dose, 5 of the surviving rats were sacrificed. Finally, 2 months after the dose, 6 surviving rats were sacrificed.

Lipid extraction and fractionation: Blood was collected by heart puncture; the brain and liver were excised and the total lipids were extracted as described for previous studies⁽¹⁾. The total lipids were fractionated according to the method described by Rouser *et al.*⁽⁵⁾. Methanolysis of the total lipids, isolation of the fatty acids, fractionation and decarboxylation of the purified fatty acid have been described previously⁽⁷⁾.

RESULTS

Table I shows the incorporation of radioactivity from $1\text{-}^{14}\text{C}$ palmitic

← TABLE I

acid into the blood, brain and liver total lipids. The radioactivity of blood lipids (cpm/ml plasma) decreased from 9856 cpm to 69 cpm within a period of one month and only traces remained 2 months after injection. Similarly, the radioactivity of liver lipids (cpm/g tissue), which was high 24 hours after the dose, decreased within a period of 1 month, resulting in retention of only 1% of the original radioactivity. Two months after the injection there was no detectable radioactivity in the liver lipids.

In the brain, however, considerable radioactivity was retained even after two months. The lipid content of the brain per g of tissue almost doubled during the growth period of 13 to 73 days. The radioactivity per g of brain tissue is calculated to include the increase in lipid concentration. It was found that at the end of 2 months after injection 64% of the original radioactivity (taken up by the brain 24 hours after injection) was still retained.

The incorporation of radioactivity in various lipid fractions obtained from the brain and liver total lipids is shown in Table II.

TABLE II

The liver phospholipid fraction had higher specific activity than either the triglycerides or cholesterol fraction, 24 hours after the tracer dose. However, at the end of one month the triglycerides retained more radioactivity than the other fractions. At the end of 2 months no detectable radioactivity remained in liver total lipids.

Among the various components of brain lipids, phosphatidyl choline had the highest specific activity 24 hours after the injection. Cholesterol, phosphatidyl serine and cerebroside had very similar specific

activities. Phosphatidyl ethanolamine had the lowest specific activity. One month after the tracer dose, the specific activity of cholesterol remained almost unchanged whereas phosphatidyl choline showed a 60% decrease in specific activity. There was a marginal increase in the specific activity of phosphatidyl ethanolamine, sphingomyelin and cerebroside. Two months after the tracer, cholesterol was the most radioactive component whereas phosphatidyl choline and serine had the lowest specific activities. Cerebroside seems to have retained a considerable amount of radioactivity.

Table III shows the distribution of radioactivity in fatty acids isolated from the brain polar lipids. Palmitic acid showed a steady drop in specific activity as the interval between injection of the tracer and sacrifice of the animal increased. The drop in specific activity of stearate during the period between 24 hours and 1 month was only marginal as compared to palmitate, whereas, there was an increase in specific activities of oleic, arachidonic and docosahexaenoic acids. At the end of 2 months, the specific activity of all fatty acids decreased with the exception of that of the docosahexaenoic acid. Oleic acid had the highest specific activity at this time.

← TABLE III

An unexpected observation was that the carboxyl carbon of palmitic acid, which retained 78% of the radioactivity after 24 hours, did not lose all its activity even 2 months after injection. The % RCA of stearic and oleic acids was low after the initial 24-hour period and during the remaining 2 months there was very little change. In contrast, the % RCA of both 20:4 and 22:6 was fairly high in the initial

period of 24 hours and remained so during the entire period of 2 months after injection.

Table IV shows the distribution of radioactivity in fatty acids isolated from brain PC, PE and cerebroside compared to those obtained from total polar lipids. This study was restricted to brain samples obtained 2 months after the tracer injection. Although the % RCA of stearate and arachidonate obtained from total lipids were different from those obtained from phosphatidyl choline or phosphatidyl ethanolamine, there were no outstanding differences in the % RCA of fatty acids from the individual phospholipids. One exception to this is the fatty acids obtained from cerebroside which include rather substantial amounts of 24:0 and 24:1. Palmitic acid obtained from the cerebroside fraction had a % RCA of 48, which is the lowest for palmitic acid obtained from any fraction. Both 18:0 and 18:1 from cerebroside contained considerable activity in the carboxyl carbon.

← TABLE IV

Table V shows the distribution of radioactivity in the liver fatty acids. Palmitate retained almost all its activity in the carboxyl carbon even after 2 months irrespective of whether it was obtained from triglycerides or phospholipids. The % RCA of stearate from the phospholipid fraction was very low (5.5%) after the initial period of 24 hours but stearate associated with the triglyceride fraction had a % RCA over twice as high. During the period of one month after the dose, there was only a marginal change in the % RCA of palmitate or stearate. The % RCA of oleic acid was much higher than that of stearic acid and arachidonic acid contained most of its activity in the carboxyl carbon.

← TABLE V

DISCUSSION

Rapid brain growth during the active myelination period of rats, 13 to 25 days after birth, resulting in increased mass, decreased water content^(7,8) and increased rate of lipid synthesis has been noted by many investigators^(8,9). This explains the rapid incorporation of radioactivity into brain lipids during the first 24 hour period after the tracer dose. The persistence of radioactivity in brain lipids thereafter may be explained on the basis of recycling and utilization of the degradation products for resynthesis, as in the closed system proposed by Sun and Horrocks⁽¹⁰⁾.

At the end of 24 hours, PC was the most radioactive fraction; of all the brain polar lipid components, however, at the end of 1 month more than half of the radioactivity was lost and 2 months after the injection there had been a 7-fold decrease in radioactivity. In contrast, both the sphingomyelin and cerebroside fractions retained considerable amounts of radioactivity. Cholesterol also retained most of the activity derived from radioactive acetate, itself a degradation product of the injected radioactive palmitate. These results agree with the turnover rates of brain polar lipids observed by others^(11,12,13).

It has been clearly established by earlier studies^(7,14) that brain palmitate is synthesized in situ from acetate and irrespective of the interval between injection and sacrifice, the % RCA of brain palmitate is always close to 12.5, which corresponds to the theoretical de novo synthesis value. However, in the present study, palmitic acid isolated from the brain of rats given 1-¹⁴C palmitate never reached this theoretical

value even at the end of 2 months after injection. The brain palmitate had 78% of the total activity in the carboxyl carbon 24 hours after the injection and even more unexpected was the fact that 2 months after injection it still had 60% of the activity in the carboxyl carbon. In other words only 40% of the palmitate was derived by de novo synthesis from acetate. This would, at first glance, suggest that the injected palmitate was not degraded to provide the radioactive acetate for resynthesis. However, examination of the chain elongation process and cholesterol synthesis does indeed show that radioactive acetate was being formed. Distribution of radioactivity in stearate, arachidonate and docosahexaenoate corresponds to predicted values assuming these fatty acids to be formed by a chain elongation process⁽⁹⁾. There is also an increase in the specific activities of these fatty acids as well as that of cholesterol.

This paradoxical observation, concerning formation of radioactive acetate but a lack of de novo synthesis, involves complex possibilities. It is known that the injected palmitate is oxidized to acetate in the mitochondria⁽¹⁵⁾ and that the radioactive acetate can theoretically be used for many synthetic reactions. The acetate can be utilized for chain elongation of saturated and monounsaturated fatty acids in the mitochondria themselves, as shown by Boone and Wakil⁽¹⁶⁾; it can combine with oxalacetate to form citrate, which is transported out of the mitochondria⁽¹⁷⁾ and, after splitting by the citrate cleavage enzyme, can be utilized by the microsomes to form polyunsaturated fatty acids by chain elongation of 18:2 and 18:3. The acetate may be utilized for cholesterol synthesis via several enzymatic steps. Finally the fatty acid synthetase of the cytosol can utilize

acetyl CoA and malonyl CoA for de novo synthesis of fatty acids. Palmitate is the major end product of this reaction^(18,19). Thus the observed label distribution in brain palmitate or liver palmitate should have been predominantly that postulated for the de novo synthetic reaction.

There are several possibilities to explain the apparent lack of this otherwise dominant process. First, the radioactive acetate formed in the mitochondria might be diluted not only by the acetate from carbohydrate but from the rather large amount of glutamate occurring in the brain. Because of this dilution, although the de novo synthesis of brain palmitate is proceeding at normal rate, in the overall process the resynthesis of palmitate, as indicated by label distribution, did not manifest itself. This argument is in contrast to the data dealing with chain elongation and cholesterol synthesis. A second possibility is the assumption that the acetyl CoA pool is nonhomogenous, as suggested by Yates and Garland⁽²⁰⁾. If there are compartmentalized pools with different fates, it is possible that radioactive acetate obtained from oxidative degradation of the injected palmitate is utilized more for chain elongation or cholesterol synthesis than for de novo synthesis of palmitate.

A third possibility stems from the finding that the enzyme acetyl-CoA carnitine acetyltransferase (EC 2.3.1.7), proposed as a mechanism for transporting acetyl groups into different mitochondrial compartments^(21,22) had only 10% of its adult concentration in rabbits 4 days after birth. The bulk of the increase in enzyme activity occurred at 32 days of age⁽²³⁾. If the same changes occur in the young rats, although palmitate was β -oxidized to acetate, the transfer of acetyl groups out of the mitochondrial

compartment might have slowed down leading to reduced resynthesis of palmitate in the cytosol. However, recent evidence by Watson and Lowenstein⁽¹⁷⁾ giving evidence that citrate is the major carrier in the transfer of acetate from the mitochondrial matrix to the cytoplasm would minimize the role of the enzyme acetyltransferase.

Finally, the injected palmitate may be rapidly incorporated into complex lipids⁽²⁴⁾, part of this could be associated with the myelin being laid down during the actively myelinating period. The turnover of such a component would be rather slow, as shown by Smith⁽¹¹⁾ and others^(12,13). This in turn would explain the persistence of radioactivity in the carboxyl carbon of brain palmitate. The portion associated with the grey matter may very well be the one that has a relatively rapid turnover resulting in disappearance of the label from the palmitic acid as seen in the decreased specific activity of the brain palmitate. In order to find out if fatty acids associated with PC, PE and cerebrosides have different turnover rates resulting in widely different % RCA and specific activities, the data shown in Table IV were obtained. It appears that the fatty acids of the cerebrosides did have a different radioactive distribution. The specific activities of both 16:0 and 18:1 were high and the % RCA of 18:0 and 18:1 were also higher than those of the corresponding acids obtained from either the PC or PE fraction.

Additional work with brain preparations capable of β -oxidation and resynthesis of fatty acids in vitro is needed to clarify the compartmentalization suggested to explain the label distribution of brain palmitate. This is the direction of our present studies.

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TABLE I

INCORPORATION OF RADIOACTIVITY FROM 1-¹⁴C PALMITIC ACID INTO BLOOD, BRAIN AND LIVER LIPIDS

Tracer 10 μ c 1- ¹⁴ C palmitic acid	Body weight g	Blood Lipids		Brain Lipids			Liver Lipids		
		Sp. act cpm/mg	Cpm/ml (plasma)	Mg/g wet weight	Sp. act cpm/mg	Cpm/g wet tissue	Mg/g wet tissue	Sp act cpm/mg	Cpm/g wet tissue
5 Baby rats sacri- ficed 24 hours after dose	32 $\pm$ 3	3532	9856	56.7 $\pm$ 2.9	395 $\pm$ 75	22,305 $\pm$ 3727	45.6 $\pm$ 2.1	7330 $\pm$ 2606	335,374 $\pm$ 123,977
5 Surviving rats sacrificed 1 month after dose	130 $\pm$ 18	28	69	88.5 $\pm$ 5.4	283 $\pm$ 53	24,980 $\pm$ 4,732	44.8 $\pm$ 5.8	41 $\pm$ 10	1782 $\pm$ 332
6 Surviving rats sacrificed 2 months after dose	293 $\pm$ 36	Trace	Trace	99.4 $\pm$ 5.1	145 $\pm$ 33	14,263 $\pm$ 2,618		Trace	Trace

TABLE II

INCORPORATION OF RADIOACTIVITY FROM 1-¹⁴C PALMITIC ACID INTO
VARIOUS COMPONENTS OF LIVER AND BRAIN TOTAL LIPIDS

	Liver Lipids			Brain Lipids						
	Sp. Act. cpm/mg			Sp. Act. cpm/mg						
	Chol.	TG	PL	Total PL	Chol.	PC	PE	PS	Cereb.	Sph.
24 Hours after tracer	1306	5575	7090	387	334	562	264	320	305	276
1 Month after tracer	33	89	39	295	329	233	331	270	363	332
2 Months after tracer	No activity			—	207	86	107	80	188	140

TABLE III

RADIOACTIVITY IN FATTY ACIDS ISOLATED FROM BRAIN TOTAL POLAR LIPIDS

	Palmitic Acid		Stearic Acid		Oleic Acid		Arachidonic Acid		Docosahexaenoic Acid	
	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA
24 Hours after tracer dose	1212	78.4	627	9.4	371	11.2	52	57.1	44	69.5
1 Month after tracer dose	448	70.4	540	4.6	697	4.8	97	55.4	96	57.7
2 Months after tracer dose	71	60.3	145	6.6	212	6.0	32	47.3	96	76.2

TABLE IV

RADIOACTIVITY IN FATTY ACIDS ISOLATED FROM BRAIN POLAR LIPIDS 2 MONTHS
AFTER INJECTION OF 1-¹⁴C PALMITIC ACID

	16:0		18:0		18:1		20:4		22:6		24:0		24:1	
	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA
Fatty acids from TL	71	60.3	145	6.6	212	6.0	32	47.3	96	76.2	—	—	—	—
Fatty acids from PC	60	72.6	122	15.8	165	5.6	184	100	66	100	—	—	—	—
Fatty acids from PE	50	62.8	96	17.3	252	5.8	31	89	51	88.5	—	—	—	—
Fatty acids from cere- broside	219	48.0	150	36.7	338	43.3	—	—	—	—	392	26.8	253	11.3

TABLE V

DISTRIBUTION OF RADIOACTIVITY IN FATTY ACIDS ISOLATED FROM LIVER LIPIDS

		16:0		18:0		18:1	20:4
		Sp. Act. Cpm/mg	% RCA	Sp. Act. Cpm/mg	% RCA	% RCA	% RCA
13-Day-old baby rats, sacrificed 24 hours after dose (5)	PL	36,474	100	12,006	5.5	—	—
	TG	—	99.6	—	15.4	41.7	83.9
Surviving rats, sacrificed 1 month after dose (5)	PL	104	93.4	85	7.2	38.6	75.5
	TG	192	96.3	124	14.4	35.1	54.4