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Isotopic studies of the biosynthesis of the cerebroside fatty acids in rats*

AMIYA K. HAJRA and NORMAN S. RADIN

Mental Health Research Institute,
University of Michigan,
Ann Arbor, Michigan

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SUMMARY

Groups of weanling rats were given a single injection of labeled acetate and were sacrificed at intervals up to 56 days. The saturated acids of brain cerebrosides and brain were isolated by gas-liquid chromatography, counted, and chemically degraded to measure the distribution of activity. The total activity in the cerebroside acids, except 18:0, increased with time over a period of 14 days or more, then slowly decreased. The latter period of decreasing activity shows that these acids undergo metabolic conversion. Degradation of the acids showed the carboxyl group had a maximal relative activity at around 4 hr, which declined with time toward values close to or below that expected for de novo synthesis from acetate. From these observations, it was concluded that the cerebroside acids are made by a chain-lengthening process from one or more shorter fatty acids. The relative activity in the carboxyl of 23h:0 decreased very rapidly with time and the activity in its α-carbon reached a high value; from this it is concluded that this acid is made by two enzyme systems, one being a 1-carbon degradation of a C_{24} acid. The later samples of cerebroside 18:0 showed uniform distribution of activity, suggesting that it is made directly from acetate. The 16:0 of total brain showed the same effect, while the 18:0 of whole brain resembled the cerebroside longer acids. The specific activity data suggested that the hydroxy and normal acids are made from a common precursor.

The cerebrosides in mammalian brain contain both saturated and unsaturated fatty acids, ranging from C_{16} to C_{26} (2, 3). More than half of these acids are present as 2-hydroxy acids, and odd-numbered acids are present in unusually large proportion, which increases with age (4). Like the more common fatty acids, the cerebroside acids are made from acetate (5–7) and propionate can serve as one of the precursors of the odd-numbered acids (6). In this work, we studied the in vivo metabolism of the cerebroside acids by using C^{14}- and H^3-labeled acetate. The total and specific activities of the saturated normal and hydroxy acids were determined at various time intervals after injecting the labeled precursor. Some of the radioactive fatty acids were chemically degraded to determine distribution of C^{14} in the chain. A similar examination was made of the saturated normal acids of whole brain.

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METHODS

Most of the materials and equipment were described before (6). Acetate-1-C^{14} (New England Nuclear Corp.) had a specific activity of 2 mc/mmole, and the acetate-2-H^3 had a specific activity of 98 mc/mmole.

Animals. Two series of male weanling rats of the Sprague-Dawley strain were given a single intraperitoneal injection of labeled acetate in 1.0–1.3 ml of isotonic saline. In Series A, 0.8 mc of acetate-H^3 was given each rat; in Series B, 0.1 mc of acetate-C^{14}. After various intervals, rats were sacrificed under CO_2 anesthesia, and the brains were immediately removed and pooled. The brains were stored 2–3 weeks at $-20°$ before processing. Seven to ten rats were killed at each time point.

Isolation of the Fatty Acids. The saturated acids were isolated as methyl esters by previously described

methods (2, 6). In the first series, the hydroxy esters were chromatographed as methyl ethers; in the second, because degradations were to be performed, the hydroxy esters were acetylated instead.

The acetylation was performed by heating the methyl esters in a screw-cap test tube at 60° for 30 min with 1 ml isopropenyl acetate and 2 μl sulfuric acid (8, 9). The acetates were freed of the catalyst by adding ether and water and purified by passing them through a 1.5 g Florisil column with 50 ml of Skellysolve B–absolute ether 99:1.

Where the esters were to be degraded, they were washed out of the collecting filters (10) with toluene, and aliquots were taken for counting and for decarboxylation. Duplicate gas-liquid chromatographic (GLC) runs were made for counting and determination of the individual acids. In the experiments with acetate-C^{14}, $^1/_{10}$ of the total lipids was set aside for isolation of the whole brain normal saturated fatty acids (6).

Degradation of the Normal Acids. After addition of about 5 mg carrier acid, the radioactive ester was saponified with ethanolic KOH. Some of the samples were recovered from scintillation vials, and here the esters were extracted from the dried mixture, diphenyl oxazole (PPO) and bis(oxazolyl)benzene (POPOP), with Skellysolve B. The remaining PPO in the extract was removed by extraction with Skellysolve after saponification.

The free acids were decarboxylated by the Schmidt degradation (11, 12) in a special apparatus (Fig. 1) made necessary by the severe foaming shown by the longer acids. After addition of the sample, sodium azide, and sulfuric acid, the absorption tube and stopper were inserted, and the apparatus was evacuated briefly through a hypodermic needle through the stopper. The stopper was coated with silicone grease to prevent leakage.

After the reaction, 0.5 ml of 1 M Hyamine hydroxide (13) was injected into the absorption vial through the rubber stopper via a metal syringe stopcock, and the assembly was shaken for 3 hr. The absorption vial was then placed in a counting vial containing 15 ml of scintillation solvent (14).

The resultant fatty amines were recovered in good yield and found, by GLC, to be free from side products. The suitability of the degradation method for long-chain fatty acids was confirmed with lignoceric acid-1-C^{14}.

Decarboxylation of the Hydroxy Acids. The radioactive acid was dissolved in benzene–acetic acid 1:2 and stirred with $KMnO_4$ (5) for 2.5 hr at 24°. Inert gas carried the resultant CO_2 through a sulfuric acid wash and a dry-ice-cooled trap, then into a Vigreux

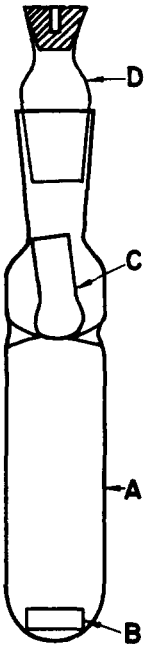

FIG. 1. Apparatus for azide decarboxylations. A: Reaction tube, lower section 10 x 2.5 cm, upper section made from 14/20 outer joint. Three or four indentations are made about 2 cm below upper section. B: Stirring magnet, Teflon coated. C: Absorption tube, about 2.3 cm long, made from 10 mm o.d. tubing blown out to form bulb about 13 mm o.d. D: Stopper, made from 14/20 inner joint by sealing on a short length of 7 mm o.d. tubing. A slight constriction at the juncture of the two tubes holds the rubber stopper, made by cutting short a micro stopper (Fisher Scientific Co. No. 21-381) and drilling a hole halfway down.

absorption tube (15) containing 0.5 ml 1 M Hyamine hydroxide and 4.5 ml toluene. The absorption tube was rinsed out with scintillation solvent into a counting vial.

Recoveries of the fatty acids resulting from the degradation were almost quantitative, and analysis by GLC showed there was very slight overdegradation.

RESULTS

The galactose contents (42) of the isolated cerebrosides were 20.2 to 21.7%; the theoretical value for the monohydrate is about 21.6%. Our values for the yields of normal and hydroxy fatty acid esters indicated the saturated acids comprise $^2/_3$ of the total cerebroside acids.

By means of thin-layer chromatography (16), the normal esters from some of the Series B rats were later found to contain a small amount of a nonpolar, nonradioactive impurity. The impurity, after elution from the plate, did not yield any visible peaks upon

TABLE 1. CONTENT OF THE NORMAL SATURATED CEREBROSIDE ACIDS IN THE TRITIUM RATS

Time of Sacrifice After Injection	Fatty Acid Carbon Number						
	18:0	20:0	22:0	23:0	24:0	25:0	26:0
days (or hours)	*μmoles/brain*						
4 h	0.44	0.14	0.35	0.06	0.87	0.03	0.07
8 h	0.43	0.14	0.33	0.06	0.85	0.03	0.06
12 h	0.51	0.14	0.35	0.07	0.87	0.04	0.07
1	0.45	0.14	0.34	0.06	0.85	0.03	0.06
2	0.41	0.15	0.34	0.06	0.88	0.04	0.06
4	0.48	0.16	0.39	0.08	1.02	0.04	0.08
8	0.38	0.18	0.39	0.09	0.98	0.04	0.08
14	0.50	0.23	0.44	0.11	1.21	0.05	0.09
21	0.55	0.27	0.52	0.14	1.43	0.07	0.10

examination by flame ionization GLC. Presumably, it was derived from one of the solvents and could not have affected the data for the *total* activities of the fatty acids. The derivatives of the hydroxy acids showed only very faint traces of impurities, which contained no detectable radioactivity.

Part of the cerebroside sample from the 28-day Series A rats was lost during isolation, so the total weights and activities could not be determined. However, this did not affect the determination of the *specific* activities, and the data thus obtained were combined with the extrapolated weight values to yield estimated values for the total activities (Figs. 2 and 3).

Content of the Normal Saturated Cerebroside Acids. Table 1 shows the distributions of the individual acids for the Series A rats, calculated from the weights of the normal acid fractions (7.0–11.2 mg/pool) and the GLC peak areas. Trace acids were omitted.

Generally, the data agree with our previous analyses (4), which covered a somewhat longer time span and did not give the contents of the 18:0[1], 25:0, and 26:0 acids. As found before, there seems to be a tendency for deposition of some of the acids (24:0 and 26:0) to halt during the first week after weaning. The 20:0, 23:0, and 25:0 acids show the greatest relative increases with age.

Content of the Hydroxy Acids. Table 2 shows the corresponding data for the hydroxy acids of both series of rats. There is no sign of the plateau seen in some of the normal acids, and the 23h:0 and 25h:0 rise relatively rapidly with age. Unlike its normal analogue, the 26h:0 rises rather rapidly.

Radioactivity in the Normal Acids. Figure 2 shows

[1] The fatty acids are abbreviated with two numbers, the first showing the number of carbon atoms in the chain, the second showing the number of double bonds. An *h* indicates a 2-hydroxy acid. Thus, palmitic acid is 16:0 and cerebronic acid is 24h:0.

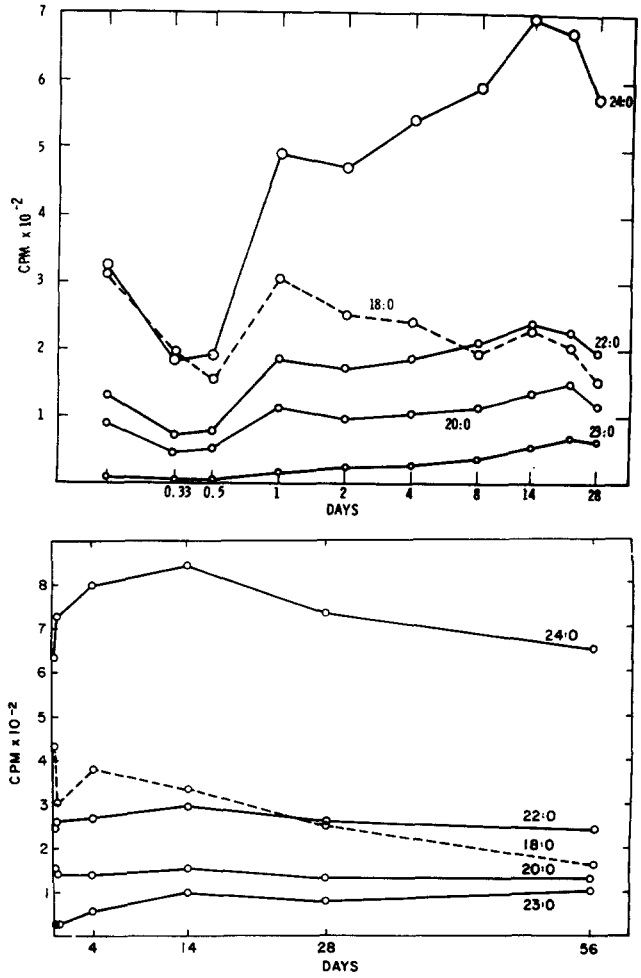

FIG. 2. Total activity per brain in each normal saturated cerebroside acid. Upper figure, Series A rats; lower figure Series B rats.

the *total* activities in the normal acids for both series of rats. The data for Series A are plotted on semi-logarithmic paper to show more clearly the data for the early time period. The partly synthetic points for the 28-day Series A rats referred to earlier seem to continue the trend set at 21 days and agree with the curves from Series B, so they were included in the graph.

The curves derived from acetate-H^3 show that the even-numbered acids reach a maximal activity near the 4-hr point. The peak is followed by a fairly rapid drop during the 4–8-hr period. This could indicate the presence of a cerebroside fraction having a high turnover rate. The curves for Series B show the drop in radioactivity only in the case of the 18:0 and 20:0 cerebrosides.

In both series of rats, there is a period of 14 days or more of rising total activity in all acids except 18:0. The 23:0 acid seems to rise for a longer period than the others.

TABLE 2. CONTENT OF THE HYDROXY SATURATED CEREBROSIDE ACIDS

Time of Sacrifice After Injection	Fatty Acid Carbon Number				
	22h:0	23h:0	24h:0	25h:0	26h:0
days (or hours)	*μmoles/brain*				
Series A Rats					
4 h	1.00	0.23	2.32	0.05	0.10
8 h	1.06	0.25	2.55	0.06	0.11
12 h	1.16	0.26	2.53	0.05	0.10
1	1.23	0.27	2.68	0.06	0.14
2	1.23	0.32	2.81	0.06	0.13
4	1.39	0.35	3.30	0.09	0.14
8	1.45	0.42	3.42	0.09	0.14
14	1.77	0.55	4.53	0.13	0.17
21	2.07	0.71	4.76	0.17	0.21
Series B Rats					
4 h	1.25	0.31	2.64	0.08	0.10
10 h	1.19	0.30	2.63	0.08	0.11
4	1.47	0.41	3.31	0.11	0.12
14	1.95	0.65	4.41	0.15	0.16
28	2.38	0.86	5.51	0.22	0.19
56	2.93	1.40	6.83	0.30	0.24

Radioactivity in the Hydroxy Acids. Figure 3 shows the total activities found in the saturated 2-hydroxy acids. The curves are qualitatively similar to the corresponding normal acids but differ quantitatively: (1) the hydroxy acids contain much more activity, (2) the duration of the rise period is longer, and (3) the decline is slower. Like the normal analogue, the 23h:0 acid may not undergo detectable metabolic conversion even in 56 days.

Comparisons of Specific Activities. Figure 4 shows the specific activities found in the *whole brain* fatty acids of the C^{14} rats. All the acids reach a maximum at about 4 hr and, like the total activities of the H^3-cerebroside acids, show a subsequent drop. With the exception of 16:0, all the acids show a second maximum at approximately 4 days. This maximum is to be expected if 16:0 is the precursor of the longer acids. The descending arm of the 16:0 curve does come close to intersecting the second maximum and we have rounded off the experimental curves to strengthen the classical analysis of precursor/product turnover data (17, 18).

Examination of the later values for the specific activities suggests that the turnover rate of each acid is inversely related to its chain length.

The specific-activity curves for the major cerebroside hydroxy acids are shown in Fig. 5. The curves for the

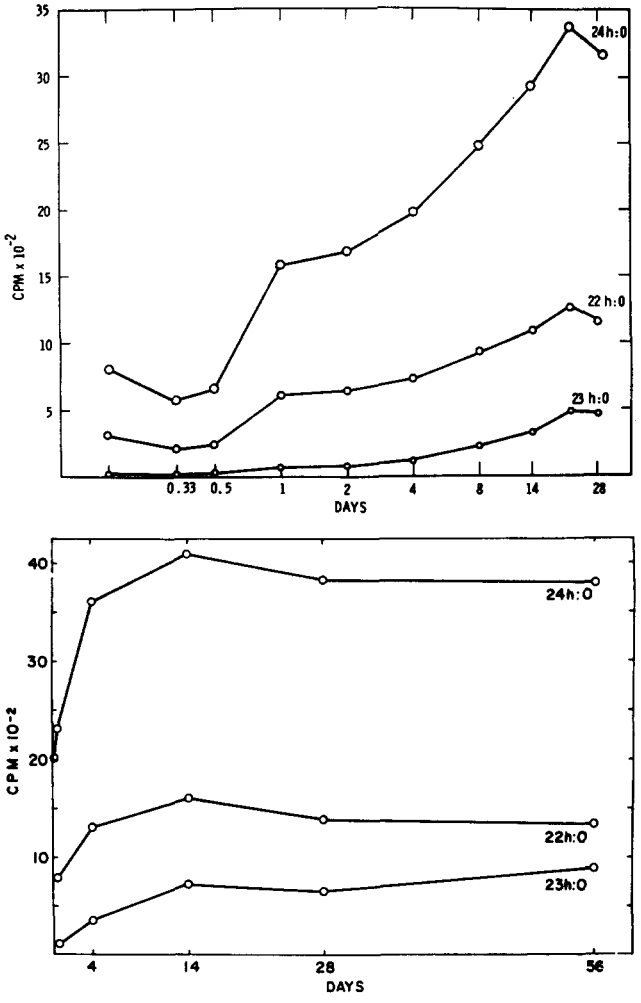

FIG. 3. Total activity per brain in each hydroxy saturated cerebroside acid. Upper figure, Series A; lower figure, Series B rats.

C^{14} rats are rather similar to those for the acids longer than 16:0 in Fig. 4 in that both types of acids have maxima near the same 4-day time point.

The tritium curves are more complex, as each acid also shows a maximal value at the early time point. This is to be expected if a rapidly metabolizing cerebroside fraction exists. The curves for the 25h:0 and 26h:0 acids, not shown in Fig. 5, follow the general form of the 23h:0 and 24h:0 acids, but with higher specific activities.

The specific activity curves for the tritium-derived normal cerebroside acids (not shown) resemble those of the hydroxy acids.

Decarboxylation Data. Table 3 gives the results of the decarboxylation of certain C^{14} acids from cerebrosides and whole brain lipids. The bottom line in the table gives the percentage of activity to be expected in

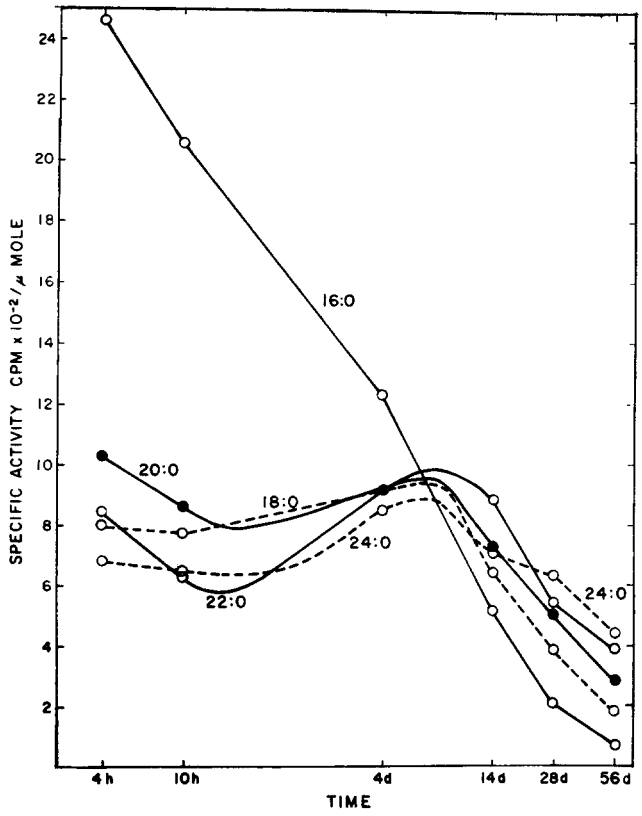

FIG. 4. Specific activities of major normal saturated acids of whole brain, from Series B rats.

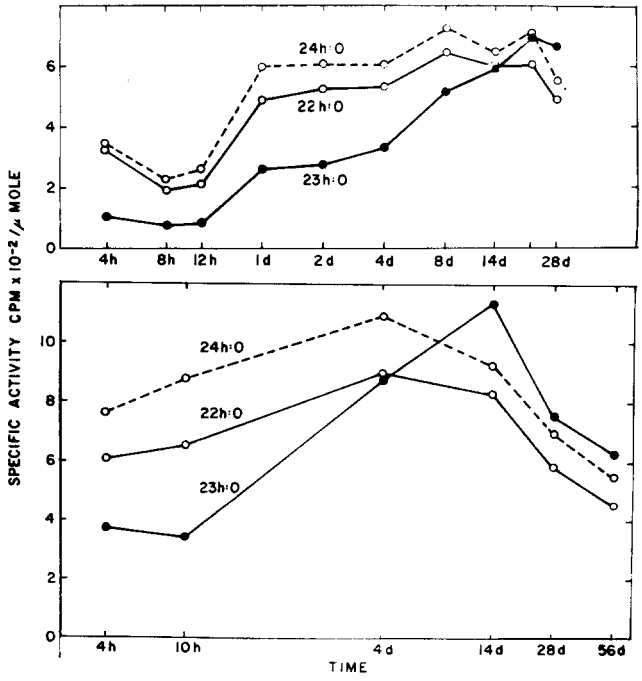

FIG. 5. Specific activities of hydroxy saturated cerebroside acids. Upper figure, Series A; lower figure, Series B rats.

the carboxyl group if each acid were made by a de novo acetate condensation process. These findings have been reported in preliminary form (19).

The 16:0 acid of whole brain shows a strikingly consistent value throughout the period studied, even though the total activity per brain drops by a factor of 31. In contrast, the 18:0 of whole brain shows a high relative carboxyl activity during the first few days, later dropping below the value calculated for "total-acetate" synthesis.

The data for the cerebroside acids show an analogous situation, in which 18:0 resembles total brain 16:0, and the longer acids resemble total brain 18:0. The longer acids, except 23h:0, do not show as rapid a fall-off in relative COOH activity, but this is to be expected since the turnover data indicate these acids have a somewhat slower turnover rate.

Distribution of Activity in the Odd-Numbered Acids. The decarboxylation data in Table 3 for the 23:0 and 23h:0 acids are particularly interesting. While these two acids show many similarities in the other aspects of this study, the carboxyl groups are markedly different. The normal acid shows only a minor drop in carboxyl relative activity, consistent with its very slow turnover shown in Fig. 2. (The high value of 31.7% at 28 days is derived from a low-activity sample and is not supported by other data, so its significance is not clear.) The 23h:0 acid, however, despite similar indications of a low turnover rate, shows a very large drop in relative carboxyl activity. To investigate this phenomenon further, we measured the activity in the α-carbon atom of several hydroxy acid samples by performing a second degradation by means of the azide method.

The percentages of total fatty acid activity found in the individual carbon atoms are shown below:

28-day group: C_{21}—$\overset{8.5}{C}$HOH—$\overset{4.3}{C}$OOH

56-day group: C_{20}—$\overset{1.4}{C}$HOH—$\overset{9.1}{C}$OOH

C_{21}—$\overset{8.3}{C}$HOH—$\overset{2.9}{C}$OOH

C_{22}—$\overset{0.8}{C}$HOH—$\overset{8.4}{C}$OOH

The high ratio of activity in the COOH vs α-carbon atoms in the 24h:0 and 22h:0 acids is to be expected if these acids are made by chain-lengthening with acetate residues. In contrast, the two 23h:0 samples show an unexpectedly large fraction of their activity in the α-carbon.

The effect is greater in the 56-day rats, in which the contribution of the old radioactive 23h:0 molecules is less significant. It would appear that part of the 23h:0 is derived from 24h:0 by a 1-carbon degradation step

TABLE 3. ACTIVITY IN THE CARBOXYL GROUP AS PERCENTAGE OF ACTIVITY IN TOTAL ACID SAMPLE

Time of Sacrifice	Fatty Acid Carbon Number								
	Whole Brain		Brain Cerebrosides						
	16:0	18:0	18:0	22:0	23:0	24:0	22h:0	23h:0	24h:0
days (or hours)									
4 h	12.9	20.7	18.2	34.8†	—	15.7	20.2	—	17.9
10 h	12.7	18.2	10.9	—	26.9†, ‡	15.6	—	18.9†, ‡	15.6
4	12.3	11.7	11.2	—	—	13.1	—	—	12.3
14	—	—	—	—	—	—	—	—	11.4
28	12.2	8.0	10.9	—	31.7†	8.1	—	4.3†	8.8
56	12.9	6.0	10.2	—	22.2†	7.8	9.1	2.9	8.3
de novo value*	12.5	11.1	11.1	9.1	10.0	8.3	9.1	10.0	8.3

* Value to be expected if the acid were made directly from acetate-1-C^{14}. In the case of 23:0 and 23h:0, the first three carbons are assumed to come from unlabeled propionate.

† Ester recovered from counting vials before degradation.

‡ Derived from samples pooled from the first three groups of rats.

in which the α-carbon of the 24h:0 becomes the COOH of the 23h:0. The α-carbon of the 23h:0 is then derived from the β-carbon of the 24h:0, which presumably has a high relative activity similar to that of the 24h:0 COOH.

Cholesterol Turnover. In the course of isolating the cerebrosides from the Series A rats, we collected the ether effluent from the Florisil columns and used this to isolate cholesterol as the tomatinide (20). The tomatinide was converted to toluene-soluble form for liquid scintillation counting by refluxing for 3 hr with acetic anhydride, then evaporating to dryness in vacuo with the oil pump swirler (21). The total activities per brain followed a course similar to that of the cerebroside acids (Figs. 2A and 3A). The activity at the start was high (48,200 cpm), then dropped at the 8- and 12-hr points by nearly half. The activities between 1 and 21 days were fairly steady at about 56,000 cpm, then dropped to 49,200 cpm by the 28-day point.

DISCUSSION

Comparison of C^{14}-and H^3-Labeling. The differences in the early time points of Figs. 2 and 3 between the tritium and C^{14} rats deserve comment. Injected acetate is metabolized very rapidly (22, 23), so that the specific activity of brain acetate must reach its maximum shortly after injection. Thus, the fatty acids made in brain in the first hour or so must be made largely from labeled acetate, but the fatty acids made later derive their radioactivity from the fatty acids in the high-turnover group (C^{14} and H^3) as well as from water (H^3 only). The high-turnover fatty acids yield acetate and possibly longer fragments, which are reutilized for cerebroside acid synthesis.

This recycling of labeled acetate, which has been observed in our Fig. 4 and in other studies (24–26), acts to obscure the existence of fatty acids having a high turnover rate. In the case of acetate-H^3, the recycling is minor because much or all of the tritium is lost to water during fatty acid oxidation. In an additional study with a group of rats injected with acetate-H^3, we showed that the body water as a source of tritium could account for only a minor part of the activity in the cerebroside acids.

The Existence of Rapidly Metabolizing Lipids in Brain. Our data for the whole brain fatty acids and cerebroside acids indicate there are lipids having a half-life of only a few hours, as well as many weeks. Our earlier turnover study of cerebroside metabolism with labeled galactose (27) yielded suggestive evidence for a fraction with a high turnover rate; another, covering a shorter time period (28), also pointed toward a rapidly metabolizing fraction. A study with labeled serine (29), which mainly covered the much later time points, suggested there is a fraction that has a very low turnover rate.

Cholesterol, on the basis of our acetate-H^3 experiments, also shows a fast and a very slow fraction. Both types of cholesterol have been reported in brain (29–33). Some of these studies only covered the later time points or used less precise modes of injection or used few animals, so the failure to observe a fast-turnover fraction does not rule out its existence. As with the cerebroside acids, acetate-H^3 is more suited than acetate-C^{14} to showing rapid turnover.

It is reasonable to postulate that the two groups of lipids have separate roles: the slowly metabolizing fraction may be "structural", going to form myelin, while the rapidly metabolizing fraction may be "functional", serving a role in brain metabolism. The former fraction should show little incorporation of radioactivity in experiments with mature animals, whose myelin is presumably entirely laid down, while the latter fraction should be detectable at any age.

The Chain-Lengthening System. The cerebroside acids could conceivably be synthesized in either of two ways: (*a*) by total synthesis from acetate by a single enzyme complex, such as the one that appears to synthesize 16:0 (34), or (*b*) by chain elongation of fatty acids (such as 16:0 and 18:0) by addition of acetate residues. Several pieces of evidence in this paper point toward the latter mechanism.

(1) The long period of rising total activity in the cerebroside acids (Figs. 2 and 3), after the disappearance of labeled acetate from the brain, indicates there is a reservoir of labeled precursors available to the cerebroside synthesizing cells. While this reservoir must include a certain amount of carbohydrate and protein derived from labeled acetate, the most likely form is shorter fatty acids.

(2) The turnover data (Fig. 4) for total brain *normal* acids show the expected precursor/product relationship between 16:0 and the longer acids, up to 24:0. This directly confirms the in vitro work in nonnervous enzyme preparations, which indicates 18:0 is made from 16:0 by a chain-lengthening step (34). Other in vivo work with labeled fatty acids has demonstrated chain lengthening can occur (35–37).

(3) The cerebroside *hydroxy* acids, 22h:0 and 24h:0, also show maximal specific activity at around 4 days (Fig. 5B). Comparison with the specific activity curve for total brain 16:0 (Fig. 4) shows the two curves are intersected by the 16:0 curve at points close to their maxima and, therefore, also exhibit the classical precursor/product relationship.

(4) The relative activity in the COOH group of total brain 16:0 (Table 3) is that to be expected if this acid were made directly from acetate. The changes in relative activity seen in brain 18:0 can be interpreted to mean that this acid is made by chain lengthening of 16:0. The first molecules of 18:0 are made from nonradioactive 16:0 and highly radioactive acetate and yield a high relative activity in the COOH group. The molecules formed much later are made from moderately radioactive 16:0 and nonradioactive acetate.

A similar interpretation can be made of the data for the cerebroside acids. Here, if we disregard the first time point for 18:0, it would appear that this acid is made by a de novo total synthesis from acetate. The high value for the first time point may mean that the 18:0 in the rapidly metabolizing cerebroside fraction is made by chain elongation. The longer acids are apparently made by chain elongation of 18:0.

(5) It is possible to estimate the specific activity of cerebroside acids laid down during any time interval by assuming that metabolic degradation is negligible. The calculation is made by dividing the increase in total activity by the increase in the total weight. Table 4 lists the calculated values for the four most common acids from the rats given acetate-H^3. Allowing for the variability to be expected from the many measurements involved, particularly for the small amounts of 22:0, one sees appreciable similarity in the specific activities of all four acids synthesized during any single time period. During the periods chosen, the amount of labeled acetate in the brain is extremely low, so the specific activities must refer to the precursor fatty acid that is being elongated. It would appear that the normal and hydroxy acids that are incorporated into the cerebrosides are derived from a common precursor, and that the precursor has a fairly low turnover rate. The specific activities of the cerebroside-bound C_{22} and C_{24} acids, the mixture of old and newly deposited molecules, range between 500 and 690 cpm/μmole during the periods shown in Table 4. Since these values are somewhat lower than those for the newly deposited molecules, one may conclude that the bound acids are not interconverted (hydroxylated or reduced). In subsequent experiments with labeled 24:0 and 24h:0 acids, we found (38) that intracranially injected 24:0 is converted to cerebroside-bound 24:0 and 24h:0, while 24h:0 is incorporated into cerebrosides but is not reduced.

TABLE 4. SPECIFIC ACTIVITIES OF NEWLY DEPOSITED CEREBROSIDE ACIDS (SERIES A RATS)

Time Interval*	Cerebroside Acid			
	24h:0	24:0	22h:0	22:0
	cpm/μmole			
14h—4d	1720	2330	2110	2750
2d—8d	1300	1190	1310	800
8d—14d	414	465	447	600

* h represents hours, d represents days.

Comparison of the specific activities of newly deposited C_{22} and C_{24} with the shorter cerebroside-bound acids shows that the latter have much lower specific activities. This indicates the latter cannot act to an appreciable extent as substrates for the chain-lengthening reaction.

Data from other sources also point to chain lengthening as the mode of synthesis of the cerebroside acids. Bernhard et al. (39) have injected labeled acetate intracranially and found a long period of rising activity like ours (point 1 above). Fulco and Mead (5) have offered evidence that nervonate (24:1) is formed by chain lengthening of oleate. A structural study of the unsaturated sphingolipid acids indicates these acids are made by chain lengthening of several shorter unsaturated acids.[2] Finally, we have found by intracranial injection of labeled 16:0 and 18:0 that these are both elongated to 24:0 (38).

Fulco and Mead (5) have concluded 24:0 and 24h:0 are made from acetate "with little dilution along the way from fatty acids of intermediate chain length." Their data were obtained with rats given four daily injections of labeled acetate, then killed shortly afterward. Under these conditions, as our data show, one would find all brain fatty acids to be uniformly labeled, and one could not conclude whether or not chain extension took place.

The 1-Carbon Fatty Acid Degradation System. Our degradation data showing that 23h:0 acid is formed from 24h:0 could be interpreted also to mean that the normal acid, 24:0, is also a precursor. The specificity of the enzyme system may be such that other cerebroside acids undergo the reaction. The relatively high activity found in the α-carbon of 22h:0 (as compared with 24h:0) suggests that some of this acid is formed from 23h:0. If the 25h:0 is formed by chain elongation of an odd-numbered acid, its β-carbon should be rather radioactive, and degradation to 24h:0 would yield molecules labeled to an appreciable extent in the α-position. This mechanism explains the activity found there.

Mead and Levis (40) have furnished similar degradation data for 24h:0 and 23h:0. The relative activity in their 23h:0 carboxyl group was even lower than ours, apparently because their rats were sacrificed after a somewhat longer interval. They additionally found that the β-carbon of their 24h:0 was less radioactive than the carboxyl carbon, which could be interpreted to mean that some of the 24h:0 is made by elongation of a C_{22} acid (and that *all* the acids in the cerebroside-synthesizing cells can act as building blocks).

The presence of a 1-carbon degradation system in brain raises several questions.

(1) Are the odd-numbered cerebroside acids made only by this route? We have shown that propionate can act as a precursor for these acids (6), but propionate may not normally occur in brain. The presence of C^{14} in the COOH of 23h:0 and 23:0 shows these acids are made in part by chain elongation of a shorter odd-numbered acid. Odd-numbered acids occur in most diets, and it is possible that they are oxidized to propionate, which might reach the brain.

(2) Does the 1-carbon degradation system exist outside of the brain? We have found that 23h:0 and 25h:0 acids occur in several rat tissues, although in small concentrations (41). It is therefore possible that these enzymes are fairly widespread.

(3) Are the more common odd-numbered acids formed by the same enzyme system? We found some 15:0 and 17:0 in brain, but the total amount per brain *decreased* with time, whereas every other acid increased. This may mean that propionate and acetate are the only precursors of these acids, and that propionate is normally available only to young, nursing animals (possibly from the relatively plentiful odd-numbered acids of milk).

Our finding of a rapidly decreasing relative activity in the 23h:0 carboxyl does not mean that propionate-derived 23h:0 has a high turnover rate, but only that the 23h:0 formed from 24h:0 becomes more dominant as the latter increases in specific activity. The total amount of activity per rat in the 23h:0 carboxyl carbon was 35 cpm in the pooled sample (4-hr, 10-hr, 4-day groups), 28 cpm in the 28-day group, and 26 cpm in the 56-day group. If this drop is significant, 23h:0 must then undergo some metabolic conversion. The fact that the total activity in 23h:0 rises for a longer period than any other acid is a reflection of its biosynthesis from a large pool of 24h:0.

[2] Y. Kishimoto and N. S. Radin, paper in preparation.

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