Cholesterol metabolism in myelin and other subcellular fractions of rat brain

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Abstract For many years the bulk of myelin in adult brain was believed to be metabolically stable, although some metabolic activity of a small myelin fraction, especially in the gray matter of the brain, was recognized. We have attempted to compare the composition of myelin fractions isolated from two different areas of the brain. No differences in chemical composition were observed. We have also investigated the metabolism of cholesterol in myelin and other subcellular fractions from the two areas. Both young (16-day-old) and adult rats were used. Results show an uptake of radioactive cholesterol by all subcellular fractions of the brain, including myelin, in both young and adult animals, with ultimate uniform distribution of the radioactive sterol and its persistence in all uniformly labeled subcellular fractions of the brain. On the basis of these results we suggest that there is a pool of cholesterol in the brain from which all metabolizing structures, including myelin, draw their cholesterol supplies. There is continuous exchange of cholesterol between the brain pool and the blood. The rate of this exchange may be related to the rate of blood flow through the tissue.

Supplementary key words myelin composition · metabolism · cholesterol pools

For many years the myelin sheath has been regarded as being metabolically one of the most stable structures of the brain (1–4). This hypothesis was originally based on the findings that after injecting young animals with radioactive cholesterol, acetate, or other precursors, labeled cholesterol was found to persist in the brain, especially in the white matter, for very long periods It was therefore originally assumed that this cholestero was localized in myelin and that, in general, myelin was metabolically stable. Later work by Khan and Folch (5) suggested similar metabolic stability of cholesterol in the nuclei, microsomes, and mitochondria of adult rat brain. Further experiments on mature animals did show some uptake of lipid precursors into myelin, and this suggested that a portion of the myelin sheath may be metabolically more active (6, 7). This idea received support from the demonstration of unequal incorporation of [^{14}C]ethanolamine into large and small myelin fragments of adult rat brain (8, 9) and from other work in which metabolically active and stable components had been detected in myelin from adult rat brain (10–14).

In the first part of our investigations we have analyzed myelin fractions from two different regions of the brain to determine if any metabolic differences may be related to differences in chemical composition of myelin. Cholesterol metabolism in myelin and other subcellular fractions in two different areas of the brain has also been studied. In the metabolic experiments [U-^{14}C]acetate or [4-^{14}C]cholesterol was injected into young and adult rats. Our results have led us to postulate that the brain contains a pool of cholesterol with which all membrane structures, including myelin, may readily exchange. The hypothesis of such a cholesterol pool has been suggested for the peripheral nervous system by Hedley-Whyte and her coworkers (15, 16). Very recently, Banik and Davison (17) reported exchange of cholesterol between myelin and microsomal fractions of the developing rat brain in vitro.

MATERIALS AND METHODS

Wistar rats of both sexes were used. For metabolic studies we used 16-day-old animals injected intraper-

Abbreviations: C–M, chloroform–methanol; W, water; TLC, thin-layer chromatography; NANA, *N*-acetylneuraminic acid; PPO, 2,5-diphenyloxazole; POPOP, 1,4-di-2-(5-phenyloxazolyl)-benzene; SA, specific activity = (cpm/μCi injected)/μmole.

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itoneally and adult rats (300–350 g body wt) injected intravenously through the tail. Radioactive materials were obtained from Radiochemical Centre, Amersham, Buckinghamshire. [U-^{14}C]Acetate was made up in normal saline, and [4-^{14}C]cholesterol was in 60% aqueous ethanol.

Animals were anesthetized with chloroform and the brains were removed and cooled in ice-cold 0.32 M sucrose solution for 1–2 min. The chilled brains were blotted on damp filter paper, and whole cerebral cortexes were dissected out. Although care was taken to isolate anatomically comparable sections of the brain from each animal, contaminating white matter was inevitably present in cortical tissue, and gray matter, including cerebellum, was present in the remaining tissue of the brain. Brains were thus divided into area C, the cortical tissue containing predominantly gray matter, and area W, the remaining tissue, which also included cerebellum.

Isolation of myelin

Myelin was isolated by the method of Eichberg, Whittaker and Dawson (18), as modified by Cuzner and Davison (19). The resulting "crude myelin" fractions were "water shocked" by mixing the suspensions with nine times their volume of ice-cold water and allowing the mixture to stand at 0–4°C overnight. "Purified myelin" was isolated from the dilute suspensions as a pellet by centrifugation at 12,000 *g* for 10 min (20). Further "water shocking" and layering of the purified myelin fractions from adult animals prepared by this method did not produce any additional subfractions. It was therefore concluded that the pellet represented a fairly homogeneous myelin fraction. For analytical purposes the pellets were suspended in water, and aliquots were removed for protein analysis and for extraction of lipids.

Protein assay

The method of Hess and Lewin (21) was employed after hydrolysis of the material for 18 hr at 37°C in 0.5 N NaOH. Freshly prepared bovine serum albumin was used as standard, and results are expressed as equivalent to BSA protein.

Lipid extraction and analysis

Aliquots of the aqueous suspensions of the original fractions were mixed with five times their volume of C–M 2:1 (v/v). The mixtures were allowed to stand at 0–4°C until the resulting two phases were clear, with insoluble residue at the interface. The upper (aqueous) phases were aspirated and discarded, leaving the insoluble residue floating over the chloroform-rich solution. These chloroform extracts were washed twice more with a "synthetic upper phase" containing citrate (C–M–0.1 M tripotassium citrate 3 : 48 : 47 [v/v/v]) in order to split the proteolipid proteins (22). Finally, the washed lipid extracts were filtered into measuring cylinders, made up to suitable volumes with C–M 2 : 1 (v/v), and analyzed.

When ganglioside values were required, the extraction procedure was altered. Aliquots of the original aqueous fractions were mixed with enough C–M 1 : 1 (v/v) to form a single phase. The mixtures were filtered and the residues were washed with C–M 2 : 1 (v/v). The composition of the combined filtrates and washings was adjusted to give final mixtures of 5 parts of C–M 2 : 1 (v/v) to 1 part of water. The resulting two phases were separated. The lower (chloroform) layers were washed once with synthetic upper phase containing citrate (as above) and once with synthetic upper phase made up of C–M–W 3 : 48 : 47 (v/v/v). The washed lipid extracts were filtered to remove proteolipid protein, made up to known volumes, and analyzed for lipids other than gangliosides.

The combined upper phases containing gangliosides together with nonlipid contaminants were concentrated to about one-third of their original volume on a rotary evaporator under reduced pressure at 45°C. The concentrated solutions were dialyzed at 0–4°C for 4 days with at least five daily changes of water. The volumes of the dialyzed solutions were measured, and aliquots were taken for estimation of NANA.

Isolation of cholesterol for isotope studies

Known volumes of total lipid extracts were evaporated to dryness and subjected to TLC on silica gel G plates (500 μm thick). Chromatograms were developed in C–M 85 : 15 (v/v), and bands were located with 0.04% (w/v) bromothymol blue in 0.01 N KOH. Cholesterol bands were scraped into tubes, and the lipids were eluted by shaking the powder with C–M–W 65 : 35 : 4 (v/v/v) and centrifuging. Aliquots of the clear solution were removed. Bromothymol blue was washed out by partitioning against water, and the colorless solutions were made up to known volumes. Portions were used for counting and for cholesterol assay.

Separation and analysis of individual phospholipids and neutral cerebrosides

Lipid samples were separated into individual lipids by TLC on silica gel G plates (500 μm thick) developed in C–M–W 24 : 7 : 1 (v/v/v). Bands were located with bromothymol blue and the plates were allowed to dry.

Phospholipid bands were scraped into tubes, and the lipids were eluted from the powder by shaking with 10 ml of C–M–W 7 : 7 : 1 (v/v/v) and centrifuging. Portions of the clear solutions were removed for phosphorus assay.

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Recovery of phosphorus from the plates ranged from 85 to 100%.

Cerebroside double bands were scraped into tubes, and lipids were eluted by the same method as described for cholesterol. After evaporation of the solvent from the washed solution, cerebroside was estimated by galactose assay.

Analytical methods

Cholesterol was determined by the method of Davison et al. (23). *Lipid phosphorus* was determined by the method of Martland and Robison (24) after ashing the dry lipid residues in a sand bath with a perchloric acid–H_2SO_4 mixture (equal volumes of 60% perchloric acid and 10 N H_2SO_4). *Galactose* content of neutral cerebrosides was determined by reaction with anthrone in H_2SO_4 as described by Radin (25). *Ganglioside NANA* was determined by the method of Svennerholm (26) as modified by Miettinen and Takki-Luukkainen (27).

Radioactivity. Lipid samples were counted in a Beckman liquid scintillation counter, using a mixture of 0.5% PPO–0.03% dimethyl POPOP and 3% ethanol in toluene as the solvent. The samples were counted for 100 min or to a preset error of 1%. Counts lower than twice the background were ignored. Efficiency of counting was 96%.

RESULTS

Results of chemical analysis of myelin fractions prepared from cortical tissue, area C, and from the remaining noncortical area W of adult rat brain are expressed in Table 1. These results show the fractions to be similar in gross chemical composition, with myelin from the cortical area accounting for about one-third of whole brain myelin. This value for "cortical" myelin is much higher than that reported by Suzuki, Poduslo, and Poduslo (28); however, these authors concentrated on isolating pure gray matter from the cortex, whereas we were more concerned with isolating myelin from anatomically different parts of the brain in order to study metabolic differences. In calculating our results as percentages of dry weight of myelin, we assumed the molecular weight of cerebrosides and phospholipids to be 800 and we used Suzuki's (28) figures of 7.3% for sulfatide and 0.4% for insoluble residue, as we did not analyze these two components. TLC of the ganglioside fractions of myelin showed only one major component, the G_{M1} ganglioside, which is in agreement with the results reported by Suzuki, Poduslo, and Norton (20).

Metabolic experiments

All subcellular fractions were monitored by protein assay. Routine TLC of total lipid extracts, in which 20 μg of total lipid phosphorus was applied per spot, showed only traces, if any, of cerebrosides in all fractions except the purified myelin and the nuclear pellet fractions, indicating that the remaining fractions were not significantly contaminated by myelin. The amounts of cerebroside in these fractions were below the levels of sensitivity of our method of assay, and therefore their degree of contamination could not be assessed.

Experiment 1. Uptake and distribution of [4-^{14}C]cholesterol into subcellular fractions of adult rat brain

In this experiment adult rats were injected intravenously with 2.9 μCi of [4-^{14}C]cholesterol and killed after various time intervals. Tissue from area C and area W was fractionated into subcellular components, and lipids were extracted. Two animals were used per time period. Radioactive cholesterol accounted for 85–100% of total lipid activity of the fractions, suggesting that there was direct uptake of the lipid from the blood by the adult brain.

TABLE 1. Composition of purified myelin fractions from divided rat brain

	Area C			Area W			Whole Brain[a]
	μmoles of Lipid/ mg of Myelin Protein	Molar Ratio of Lipids	Calculated % in Dry Wt of Myelin	μmoles of Lipid/ mg of Myelin Protein	Molar Ratio of Lipids	Calculated % in Dry Wt of Myelin	% in Dry Wt of Myelin
Cholesterol	1.35 ± 0.05 (13)	100	14.7	1.49 ± 0.05 (15)	100	15.3	19.4
Neutral cerebroside	0.61 ± 0.02 (7)	45	13.6	0.74 ± 0.01 (5)	50	15.7	20.1
Total lipid phosphorus	1.60 ± 0.03 (8)	118	35.9	1.64 ± 0.04 (8)	110	34.8	27.8
Lecithin	0.53 ± 0.01 (3)	39	11.9	0.63 (2)	42	13.3	6.8
Ethanolamine phospholipid	0.41 ± 0.05 (3)	30	9.3	0.51 (2)	34	13.4	10.5
Ganglioside (μmoles)	0.03 (2)	0.02		0.01	0.01		
Protein (% dry wt myelin)			28.1			26.5	26.9
Protein (mg/g original tissue)	3.3 ± 0.6 (16)			8.5 ± 1.7 (17)			
Ganglioside (μg/brain)			14.6 (2)			30.9 (2)	19.9–61.8
Weight of original tissue/ brain (mg)	765 ± 79 (18)			983 ± 89 (17)			

[a] Suzuki et al. (28). The ganglioside values are a range for ages 144–425 days.

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TABLE 2. Protein and cholesterol levels of subcellular fractions isolated from brains of adult rats used in the isotope experiments

	Area C[a]		Area W[b]	
	Protein	Cholesterol	Protein	Cholesterol
	mg/brain	*μmoles/brain*	*mg/brain*	*μmoles/brain*
Nuclear pellet	11.9 ± 3.1 (4)	3.7 ± 0.3 (4)	28.9 ± 4.8 (5)	11.1 ± 3.4 (5)
Microsomes + supernate	19.3 ± 6.6 (4)	2.7 ± 0.4 (4)	28.0 ± 2.9 (5)	4.3 ± 1.7 (5)
Nerve ending particles	21.1 ± 6.6 (4)	4.5 ± 1.1 (4)	20.9 ± 6.9 (5)	4.7 ± 1.7 (5)
Mitochondria	4.4 ± 1.0 (3)	0.8 ± 0.5 (4)	8.2 ± 2.7 (5)	0.9 ± 0.4 (5)
Purified myelin	3.8 ± 0.7 (5)	3.4 ± 0.6 (5)	7.9 ± 2.0 (5)	10.9 ± 3.7 (6)

[a] Area C, 723 ± 140 mg/brain (6).
[b] Area W, 1093 ± 182 mg/brain (6).

Results of chemical analysis of subcellular fractions from this group of animals are given in Table 2. Table 3 shows that at 18 hr after injection only traces of radioactivity are in myelin, although the absolute level of radioactive cholesterol in the brain is of the same order of magnitude as that after longer time intervals. After 5 days there is more radioactive cholesterol in myelin. The proportion of radioactive cholesterol in myelin increases steadily up to 71 days (the longest time period investigated). During this time the specific activity of cholesterol of myelin from both area C and area W increases from a very low value and approaches the specific activity of the lipid in the other subcellular fractions from the corresponding parts of the brain (Table 4). It is interesting to note that, in contrast to the experiments in which 16-day-old rats were injected (experiments 3 and 4), the specific activity of cholesterol in all subcellular fractions from area C is higher than the specific activity of the lipid in the corresponding fractions from area W. This difference is most striking for the first three time periods, i.e., up to 21 days after injection. In the myelin fraction it remains high even after 63 days. Whether this difference in specific activities in the myelin fractions from the different parts of the brain decreases with time remains to be established.

TABLE 3. Distribution of radioactive cholesterol among different subcellular fractions of adult rat brains at time intervals after injection

	18 hr	5 days	10 days	21 days	71 days
Area C					
Wt of tissue/brain (mg)	805	605	630	640	970
Total lipid activity/brain (cpm/μCi injected)	288	134	321	161	
	% total lipid radioactivity in the tissue				
Nuclear pellet	57.5	27.1	30.7	18.2	
Microsomes + supernate	13.2	25.6	23.0	20.3	
Nerve ending particles	17.3	25.6	27.7	36.8	
Mitochondria	6.2	3.3	6.3	2.8	
Purified myelin	1.4	8.2	10.3	7.1	
% Recovery	95.6	89.8	98.0	85.2	
Area W					
Wt of tissue/brain (mg)	825	1320	1220	950	1170
Total lipid activity/brain (cpm/μCi injected)	264	200	412	229	581
	% total lipid radioactivity in the tissue				
Nuclear pellet	56.0	26.9	30.8	34.3	35.9
Microsomes + supernate	5.4	15.5	19.5	17.3	14.3
Nerve ending particles	13.7	12.9	29.1	20.7	9.5
Mitochondria	3.9	3.8	6.2	1.7	2.0
Purified myelin	2.2	12.6	19.8	20.6	35.0
% Recovery	81.2	71.7	105.4	94.6	96.7

Adult rats given 2.9 μCi of [4-^{14}C]cholesterol intravenously (experiment 1).

Experiment 2. Uptake of [^{14}C]acetate into cholesterol of myelin fractions from area C and area W of adult rat brain

The different rate of uptake of radioactive label into myelin fractions from area C and area W of adult rats found in experiment 1 was confirmed by an experiment in which nine adult rats were injected intravenously with 25 μCi of [^{14}C]acetate and killed 25 hr after injection. Sets of three animals were used for isolation of

TABLE 4. Specific activity[a] of cholesterol at time intervals after injection

	18 hr	5 days	10 days	21 days	63 days	71 days
Area C						
Nuclear pellet	35.5	11.0	27.0	9.0	13.9	
Microsomes + supernate	12.2	15.4	28.9	12.2	14.7	
Nerve ending particles	8.9	8.8	27.7	11.7	13.7	
Mitochondria	13.1	12.4	21.4	10.0	10.3	
Purified myelin	1.6	7.9	17.5	9.9	16.5	
Area W						
Nuclear pellet	21.3	4.6	13.5	6.3	12.9	13.4
Microsomes + supernate	9.0	6.3	20.1	7.9	10.9	13.4
Nerve ending particles	8.0	7.6	21.6	6.6	13.6	18.1
Mitochondria	9.7	11.5	16.5	10.0	11.5	16.2
Purified myelin	0.8	2.1	10.3	5.8	7.6	12.2
SA of whole blood cholesterol	2629	539	366	89	20	10

Adult rats given 2.9 μCi of [4-^{14}C]cholesterol intravenously (experiment 1).
[a] cpm/μCi injected/μmole.

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TABLE 5. Protein and cholesterol levels and specific activity of cholesterol in myelin fractions isolated from different parts of the brain 25 hr after injection

	Area C			Area W			Ratio SA (C)/ SA (W)
	Protein	Cholesterol	SA[a]	Protein	Cholesterol	SA[a]	
	mg/brain	*μmoles/brain*		*mg/brain*	*μmoles/brain*		
Purified myelin (a)	3.3	5.0	1.11	8.6	13.2	0.71	1.55
Purified myelin (b)	3.7	4.9	1.35	9.0	11.8	0.83	1.63
Myelin[b]	3.8	4.7	1.12	8.9	12.5	0.60	1.87

Adult rats given 25 μCi of [^{14}C]acetate intravenously (experiment 2).
[a] Specific activity, cpm/μCi injected/μmole.
[b] Purified by the method of Suzuki et al. (20).

myelin. Two myelin fractions were prepared by the method used throughout the course of our work and one myelin fraction by a method developed by Suzuki et al. (20). The uptake of radioactive label into cholesterol of the myelin fractions of area C was found to be higher than into the cholesterol of the myelin fraction from the corresponding area W of the brain in all samples investigated (Table 5).

Experiment 3. Persistence of radioactive cholesterol in subcellular fractions of the brain following injection of [^{14}C]acetate into 16-day-old rats

In this experiment, 16-day-old rats were injected intraperitoneally with 25 μCi of [^{14}C]acetate. Whole brain tissue was investigated 10 min, 24 hr, and 7 months after injection, and tissue from area C and area W 6 months after injection. Results in Table 6 show that, using the 10 min time period as a point of reference (100%), only 8.6% of total lipid activity remains in the whole brain after 6 months (6.7% for the 7 month time point). The activity remaining in the myelin fraction after this time (6 and 7 months) is 31.8 and 28.8%, respectively, of the original total lipid radioactivity of myelin. There also appears to be a loss of a comparable amount of radioactive cholesterol from the myelin fraction. The specific activity of cholesterol (Table 7) in all subcellular fractions from cortical area, area C, and from the remaining tissue, area W, after 6 months, and from the whole brain after 7 months, was found to be fairly uniform. The results of this experiment show that during 6 months following injection of 16-day-old rats with [^{14}C]acetate there is a considerable loss of radioactive lipids from the brain and from the myelin fraction and that radioactive cholesterol remaining in the brain is distributed throughout the different membranes in such a way as to result in uniform specific activity of the lipid throughout the organ. In a similar experiment on 6-wk-old rats, Khan and Folch (5) found a loss of about 50% of the original cholesterol activity of whole homogenate after approximately 190 days following intraperitoneal injection of [U-^{14}C]glucose.

Experiment 4. Uptake and distribution of radioactive cholesterol in the brains of young rats

16-day-old rats were injected intraperitoneally with 0.97 μCi of [4-^{14}C]cholesterol. Tissue from area C and area W was used for fractionation into subcellular components at various time intervals. The specific activity of cholesterol in these fractions was determined; two animals per time period were used. Here again, in all fractions examined, radioactive cholesterol accounted for 85–100% of total radioactivity in the lipids.

The first time point investigated was 30 min after injection, when no activity was found in the brain. Specific activities of cholesterol after longer times in the different subcellular fractions of the brain together with cholesterol content of the fractions are given in Table 8.

TABLE 6. Total lipid activity and cholesterol activity in whole brains and in myelin fractions

No. of Animals	Time after Injection	Whole Brain Total Lipids	Myelin Total Lipids	Myelin Cholesterol
		cpm	*cpm*	*cpm*
18	10 min	14,034 (100%)	784 (100%)	295 (100%)
10	24 hr	11,616	715	
4	6 months	1,200 (8.6%)	249 (31.8%)	71 (24.1%)
1	7 months	946 (6.7%)	226 (28.8%)	73 (24.7%)

16-day-old rats given 25 μCi of [^{14}C]acetate intraperitoneally (experiment 3). Results are cpm/brain/μCi injected.

TABLE 7. Specific activity[a] of cholesterol in subcellular fractions of rat brain 6 and 7 months after injection

	6 months Area C	6 months Area W	7 months Whole Brain
Nuclear pellet	5.2	6.5	5.0
Microsomes + supernate	5.4	7.3	4.7
Nerve ending particles	4.7	5.7	3.9
Mitochondria			4.9
Purified myelin	6.0	5.2	5.3

16-day-old rats given 25 μCi of [U-^{14}C]acetate intraperitoneally (experiment 3).
[a] cpm/μCi injected/μmole.

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TABLE 8. Cholesterol levels and specific activity of cholesterol in subcellular fractions from area C and area W

	6 days				35 days				91 days			
	Area C		Area W		Area C		Area W		Area C		Area W	
	µmoles/ brain	*SA*[a]	*µmoles/ brain*	*SA*[a]	*µmoles/ brain*	*SA*[a]	*µmoles/ brain*	*SA*[a]	*µmoles/ brain*	*SA*[a]	*µmoles/ brain*	*SA*[a]
Nuclear pellet	0.75	485	2.19	287	2.22	131	6.65	109	2.01	62	7.74	58
Microsomes + supernate	2.70	209	3.30	201	2.14	123	3.76	104	2.29	58	4.21	56
Nerve ending particles	3.12	204	3.22	213	4.59	112	5.21	129	3.71	63	5.28	57
Mitochondria	0.32	263	0.62	379	0.40	65	0.54	89	0.52	60	0.49	67
Purified myelin	0.34	219	3.52	98	1.40	125	6.82	97	2.04	67	7.75	54
Wt of fresh tissue/brain (mg)	500		650		500		880		660		1090	
SA of cholesterol in whole blood	4363				110				3			

16-day-old rats given 0.97 µCi of [4-^{14}C]cholesterol intraperitoneally (experiment 4).
[a] Specific activity, cpm/µCi injected/µmole.

The weight of fresh tissue and specific activity of cholesterol in the blood are also recorded. 6 days after the injection, radioactive cholesterol was distributed throughout all subcellular fractions, but not uniformly. The nuclear pellet, especially from the cortical tissue (area C), appeared to contain more radioactivity than the remaining fractions, whereas the purified myelin fraction from area W was the least radioactive. Specific activity of blood cholesterol was very high, and blood contaminating the brain may well have contributed to the high specific activity of cholesterol of the "nuclear pellet," which contained cell debris and other impurities that sediment at low speed. 35 days after injection the specific activity of cholesterol appeared to be fairly uniform throughout all subcellular fractions of the brain. No consistent differences in specific activities were observed between the fractions isolated from area C and from the corresponding area W. The fractions remained uniformly labeled for the duration of the experiment, i.e., 91 days after injection.

Both experiments 3 and 4 on 16-day-old rats suggest that radioactive cholesterol is taken up by the brain from the blood stream and that once in the brain the lipid becomes uniformly distributed throughout all membranes. It remains uniformly distributed for a very long time, although with an overall loss of the lipid from the brain, as shown in experiment 3.

DISCUSSION

The original concept that myelin is a highly stable metabolic structure was first questioned when it was shown that lipid precursors could be incorporated into myelin lipids of adult animals (6–11). Further experiments by Smith and Eng (12, 13) gave evidence for metabolic activity of myelin with different half-lives of individual components of the fraction. The half-life of myelin cholesterol was calculated by these authors to be more than 8 months (13). Chevallier and Petit (14) observed incorporation of radioactive cholesterol into the brain of adult rats and noted its deposition in areas of myelinated fibres. Dvornik and Hill (29) demonstrated the appearance of 7-dehydrocholesterol in brains of adult animals treated with AY-9944, indicating active sterol metabolism at a time when cholesterol synthesis had been thought minimal. Recently, Ramsey, Jones, and Nicholas (30) reported a marked and early uptake of ^{14}C-labeled digitonin-precipitable material into myelin fractions after injecting adult animals intracerebrally with [2-^{14}C]mevalonic acid. Lapetina and coworkers (31) reported fairly uniform labeling of phospholipids of all subcellular fractions, including myelin, after administration of [^{32}P]P_i into adult animals. Jungalwala and Dawson (32) demonstrated ready exchangeability of a substantial part of phospholipid molecules in adult myelin membranes, presumably with those of other membranes. All these findings are difficult to interpret in terms of the suggested metabolic stability of myelin, and yet the demonstration of long persistence of cholesterol in the myelin fraction, as also in other subcellular fractions of the brain (5), has been repeatedly confirmed and needs to be explained. We propose that, as for phospholipids, there is a pool of cholesterol in the brain from which all structures, including myelin, metabolizing at their own individual rates draw their cholesterol supplies. Evidence for the exchangeability of cholesterol in the myelin sheath of young rat brain has been reported (17). The present hypothesis is compatible with previous results of Davison and his coworkers (1–4) and also with those of other workers (5, 11–14) and is supported by results of our present work. Once radioactive cholesterol enters the brain of young or adult animals it becomes distributed throughout all subcellular structures to give uniform specific activity of the lipid throughout the brain. The process of distribution takes some time, the time taken being related to the particular region of the brain and to the age of the animals. In the young animal the subcellular fractions become

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uniformly labeled in 21 days; noncortical tissue, area W, is labeled to the same extent as tissue from area C. In adult animals, tissue from the cortical area originally becomes more highly labeled than the corresponding area W. Uniform distribution of the label among the different subcellular structures takes much longer in the adult than in the young animal in both parts of the brain. Once the cholesterol of the different subcellular components of the brain becomes uniformly labeled, it remains so for a prolonged period, although there is an overall loss of activity from the brain, as shown in experiment 3, Table 6. These results can be explained by the hypothesis that there is a constant slow exchange of cholesterol between the pool and blood. Kennedy et al. (33) have recently shown that, in the dog, blood flow through the different parts of the brain varies from birth to maturity. Blood flow through the "white matter" was found to be very much higher during maturation than at maturity, and at all ages it was found to be lower than the flow through "cortical tissue." If the same is true for all species, then the different rates of uptake of radioactive cholesterol from blood by the different parts of the rat brain at different ages are easily explained by supposing that the rate of exchange of cholesterol for any region of the brain between the brain pool and blood is related to the rate of blood flow through the tissue. The specific activity of blood cholesterol is very high immediately after injection and remains high for a considerable length of time (Table 4). This results in continuous entry of radioactive cholesterol into the brain pool, which supplies the lipid to all metabolizing structures. Since the rates of turnover of the different membrane systems vary, these structures take up the highly radioactive lipid to different extents. As the specific activity of blood cholesterol falls to the level of brain pool cholesterol or below, the exchange of cholesterol between brain pool and blood results in an overall slow loss of radioactivity from the brain, while at the same time there is constant redistribution of the radioactive lipid remaining in the brain among the different subcellular structures. The overall net result is uniform distribution of radioactive cholesterol throughout all the membrane systems of the brain. The actual time taken for the uniform distribution of the lipid among the different membrane systems or the time taken for myelin, the "slowest" fraction, to become labeled to the same extent as the other subfractions of the brain cannot be determined from these experiments because of the constant supply of radioactive cholesterol from the highly radioactive blood to the pool for a long time after injection. The above experiments, however, do show that in both young and adult animals myelin cholesterol undergoes active turnover, although the rate of this process lags behind that of the other subcellular fractions.

Skillful technical assistance of Mr. D. Leigh is gratefully acknowledged.

Manuscript received 3 December 1970 and in revised form 29 October 1971; accepted 7 April 1972.

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