Exchange of Sterols between Myelin and other Membranes of Developing Rat Brain

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(Received 29 December 1970)

1. The effect of inhibition of cholesterol synthesis by a hypocholesterolaemic drug (AY-9944) was studied in rat brain during development.
2. At 2 weeks after administration of AY-9944 to young rats 7-dehydrocholesterol accounted for half the total sterol of myelin and other subcellular components.
3. At 4 weeks after injection of the drug 7-dehydrocholesterol had disappeared whereas the cholesterol content of myelin had increased by an equivalent amount. Our studies show that purified myelin has low 7-dehydrocholesterol reductase activity and suggest that 7-dehydrocholesterol is largely converted into cholesterol outside the myelin sheath.
4. Resultant cholesterol may be re-incorporated into myelin by an exchange process.
5. The metabolism of sterols in developing brain is discussed.

For long it has been considered that nervous tissue cholesterol is metabolically stable (Waelsch, Sperry & Stoyanoff, 1940; Bloch, Berg & Rittenberg, 1943; Davison, 1970). Thus many months after uptake of [4-¹⁴C]cholesterol or incorporation of sterol precursors into developing animals, labelled cholesterol can be recovered from the nervous system with little alteration in radioactivity. More than a year after injecting [4-¹⁴C]cholesterol into developing chickens and rabbits little loss was found in the [4-¹⁴C]cholesterol incorporated into the brain and the ¹⁴C remained at the C-4 position of the sterol nucleus (Davison, Dobbing, Morgan & Payling-Wright, 1958; Davison & Wajda, 1959*a,b*). After injection of [¹⁴C]acetate into both young and mature rats the labelled cholesterol in brain and spinal cord has been shown to be metabolically stable (Smith & Eng, 1965; Smith, 1968). Differential centrifugation of brain tissue homogenate showed that most of the persisting radioactive sterol was localized in myelin (Smith & Eng, 1965; Cuzner, Davison & Gregson, 1966) and in mitochondria (Cuzner *et al.* 1966; Khan & Folch, 1967). It was therefore not expected that desmosterol (24-dehydrocholesterol) would be detected in isolated myelin from young animals for it is absent from the brain of mature animals (Banik & Davison, 1967; Smith, Fumagalli & Paoletti, 1967; Fumagalli, Smith, Urna & Paoletti, 1969). After injection of anticholesterolaeic drugs (Dvornik, Kraml, Dubuc, Givner & Gaudry, 1963; Scott & Barber, 1964; Niemiro & Fumagalli, 1965) into developing animals it has been shown that as much as 50% of the total sterol appears in the

brain as either 24-dehydro- or 7-dehydro-cholesterol whereas on discontinuing drug treatment these sterol precursors are soon replaced in myelin and subcellular fractions by cholesterol (Fumagalli *et al.* 1969). This suggested that contrary to current views myelin sterols in the developing rat are metabolically active and that either the myelin sterols could undergo exchange or that the cholesterol precursors could be reduced to cholesterol within the developing sheath. In this paper we report further studies in which a 7-dehydrocholesterol reductase inhibitor has been injected into newborn rats and amounts of 7-dehydrocholesterol have been subsequently measured in myelin and other subcellular fractions of the developing brain. To explain our results 7-dehydrocholesterol reductase activity has been measured in brain subcellular fractions and an attempt has been made to demonstrate the possibility of exchange reactions. A preliminary report of this work has been published elsewhere (Banik & Davison, 1968; Davison, 1969).

EXPERIMENTAL

Chemicals. AY-9944 [*trans*-1,4-bis-(2-chlorobenzyl-aminomethyl)cyclohexane dihydrochloride] was kindly supplied by Dr D. Dvornik (Ayerst Laboratories, Montreal, Quebec, Canada). NADPH and 7-dehydrocholesterol were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Sodium [1-¹⁴C]acetate was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Nicotinamide and cyclohexane (spectroscopy grade) were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. All other reagents used were A.R. grade.

Table 1. Cholesterol and 7-dehydrocholesterol in the brain myelin and subcellular fractions of rat treated with 7-dehydrocholesterol reductase inhibitor

Several litters of 5-day-old rats were injected with AY-9944 (1mg/10g body wt.) and killed at intervals up to 25 days thereafter. Myelin and subcellular fractions were isolated from brain suspensions as described by Banik & Davison (1969). Cholesterol and 7-dehydrocholesterol were separated by g.l.c. and by methods described in the text.

No. examined Mean wet wt. of brain ...		Content (μmol/whole brain)									
		10-day-old 2 0.85 g	15-day-old 2 1.10 g	21-day-old 3 1.41 g	25-day-old 4 1.41 g	30-day-old 4 1.49 g	35-day-old 1 1.44 g				
Fraction	Sterol	Cholesterol	7-Dehydro- cholesterol	Cholesterol	7-Dehydro- cholesterol	Cholesterol	7-Dehydro- cholesterol	Cholesterol	7-Dehydro- cholesterol	Cholesterol	7-Dehydro- cholesterol
Whole brain		4.97	7.85	16.53	22.2	33.20	10.4	42.1	Trace	49.0	0
Nuclei		0.25	0.22	1.38	3.04	0.55	0.55	4.92	0.31	—	—
Mitochondria		0.14	0.15	0.34	0.54	1.30	0.22	1.70	0	—	—
Nerve-ending particles		0.78	0.86	1.62	3.02	5.65	0.75	12.40	0	—	—
Myelin		0.50	0.50	2.25	4.29	4.14	1.79	7.37	0.49	8.9	0
Myelin-like		0.19	0.15	0.41	0.97	1.17	0.29	0.51	0	0.39	0
Microsomes		1.58	1.81	3.50	4.65	7.80	1.41	7.53	Trace	9.0	0
Supernatant		0.55	0.66	0.72	1.32	1.60	0.33	—	0	—	—

Table 2. Cholesterol and 7-dehydrocholesterol in the myelin and subcellular fractions of rats treated with 7-dehydrocholesterol reductase inhibitor

Rats (14 days old) were given AY-9944 (1 mg/10g body wt.). They were re-injected at the age of 21 and 31 days. Subcellular fractions were isolated according to Banik & Davison (1969). Sterols were separated by g.l.c. as described in the text.

No. examined ... Mean wet wt. of brain ...		Content (μmol/whole brain)									
		35-day-old 2 1.47 g	49-day-old 1 1.46 g	98-day-old 1 1.98 g							
Fraction		Cholesterol (A)	7-Dehydro- cholesterol (B)	Ratio A/B	Cholesterol (A)	7-Dehydro- cholesterol (B)	Ratio A/B	Cholesterol (A)	7-Dehydro- cholesterol (B)	Ratio A/B	
Whole brain		28.2	29.6	0.99	34.4	19.9	1.73	74.0	Trace	—	—
Myelin		7.7	10.3	0.75	12.4	7.6	1.63	19.6	0.91	21.5	—
Myelin-like		0.80	0.72	1.1	—	1.2	—	1.27	0	—	—
Microsomes		4.4	4.4	1.0	3.9	2.02	1.93	—	—	—	—

Animals. Wistar rats of either sex were used throughout this investigation. AY-9944 was dissolved in water and given by intraperitoneal injection (1 mg/10 g of body wt.). A 10 μ Ci sample of [1-¹⁴C]acetate (50 μ Ci/ml in 0.9% NaCl) was given by intraperitoneal injection as stated.

Preparation of subcellular fractions. Young rats up to 15-day-old were decapitated and older rats were lightly anaesthetized with chloroform before bleeding. Brains were quickly removed into pre-cooled beakers, weighed and cooled in ice. Subcellular fractions were immediately prepared by the method described by Banik & Davison (1969).

Cholesterol exchange in vitro. Myelin and microsomal fractions were isolated from 29- and 38-day-old rats after prior injection with [1-¹⁴C]acetate (10 μ Ci/rat) when they were 15-days old. Labelled myelin suspension (2 ml) in 0.32 M-sucrose (375 mg original wet wt. of tissue/ml) was incubated at 37°C for 30 min with 5 ml of unlabelled 20% (w/v) whole-brain homogenate in 0.32 M-sucrose prepared from young rats (29- or 38-day-old). During the incubation all tubes were gently shaken. As a control, unlabelled whole-brain homogenate was incubated either alone or after addition of labelled myelin with storage at 0°C for the 30 min incubation period. After incubation the tubes were chilled in ice and the contents were adjusted to the equivalent of 10% (w/v) of original wet wt. of brain by addition of 0.32 M-sucrose solution. Microsomal and water-shocked myelin fractions were then prepared as described before and cholesterol radioactivity was determined. The same design of experiment was repeated with labelled microsomes incubated with non-radioactive whole-brain homogenate. Radioactivity in cholesterol was determined in the myelin and microsomal fractions re-isolated after incubation. Similar experiments were performed with 7-dehydrocholesterol containing myelin incubated with whole-brain homogenate.

Preparation of lipid extracts. Lipid extracts of whole-brain sample and subcellular fractions were prepared by extracting with 19 vol. of chloroform-methanol (2:1, v/v)/g fresh wet wt. (Folch, Lees & Sloane-Stanley, 1957). Lipids were separated by t.l.c. for the determination of

radioactivity (Cuzner & Davison, 1967). Cholesterol and 7-dehydrocholesterol separation was by t.l.c. according to the method of Morris (1964). Radioactivity was determined in a Beckman liquid-scintillation counter at an efficiency of 91%. The scintillator used was 0.5% 2,5-diphenyloxazole, 0.03% 1,4-bis-(5-phenyloxazol-2-yl)-benzene and 3% (v/v) ethanol in toluene.

Gas-liquid chromatography. 7-Dehydrocholesterol was separated from other sterols by g.l.c. by using a dual glass column (2 m $\times$ 6.4 mm) with nitrile silicone polymer 2.5% (w/w) XE-60 on AW-DMCS Chromosorb G 60-80 mesh (Perkin-Elmer model F11). The column was run at 225°C, with N₂, H₂ and air at 20, 15 and 20 lb/in² respectively.

Sterols were identified and determined from known standard samples of recrystallized cholesterol and 7-dehydrocholesterol. 7-Dehydrocholesterol in chloroform-methanol (2:1, v/v) was also determined by a u.v. spectrophotometric method (Kandutsch, 1962). The content of 7-dehydrocholesterol in chloroform-methanol (2:1, v/v) extracts was determined from the maximum extinction coefficient obtained at 282.5 nm. The molar extinction coefficient maximum for 7-dehydrocholesterol when extracted with chloroform-methanol (2:1, v/v) was $8.9 \times 10^3 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 282.5 nm, when extracted with cyclohexane the molar extinction coefficient maximum was $9.6 \times 10^3 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 281.5 nm. Total sterol and free cholesterol were determined colorimetrically by the Liebermann-Burchard reaction (Sperry & Webb, 1950).

Enzyme assay. 7-Dehydrocholesterol reductase activity was assayed by the method of Kandutsch (1962) as modified by Niemi & Fumagalli (1965). Enzyme activity is expressed as μ mol of 7-dehydrocholesterol converted into cholesterol/h per g wet wt. of tissue. Before use the 7-dehydrocholesterol was recrystallized several times from methanol. Cyclic 2':3'-nucleotide 3'-phosphohydrolase activity was assayed by the method of Kurihara & Tsukada (1967) as described with slight alteration by Banik & Davison (1969). Tissue samples were treated with an equal volume of 1% (v/v) Triton X-100 instead of by sonication. Triton X-100 treatment was carried out in

Table 3. *Radioactivity of lipids in the myelin and subcellular fractions of the developing rat brain*

Rats (9-day-old) were given [1-¹⁴C]acetate (10 μ Ci/rat) intraperitoneally. Methods of isolation of subcellular structures and analyses of lipids are described in the text. Results are shown as radioactivity in the equivalent of whole brain.

Age (days)	Lipid	Radioactivity (d.p.m./whole brain)			
		Whole brain	Purified myelin	Myelin-like	Microsomes
12	Cholesterol	30566	2580	948	7470
	Cerebroside	2800	286	41	179
	Total phospholipid	76510	5911	1970	19100
20	Cholesterol	28187	2830	1440	9500
	Cerebroside	2790	820	199	410
	Total phospholipid	51803	3645	1968	15805
26	Cholesterol	38510	7752	630	11891
	Cerebroside	5465	1435	73	600
	Total phospholipid	47497	7649	570	15458
30	Cholesterol	25276	5348	566	7472
	Cerebroside	4825	2100	93	792
	Total phospholipid	37100	7100	501	10945

ice for 60 min with occasional stirring. Protein content was determined by the method of Gornall, Bardawill & David (1949).

RESULTS

After a single injection of AY-9944 into 5-day-old rats 7-dehydrocholesterol appeared in large amounts in the brain although it is normally present only in traces (Table 1; Fumagalli, Niemiro & Paoletti, 1965). Maximum accumulation of the sterol precursor occurred in the brain during the period from 10 to 15 days after birth when it accounted for as much as 70% of the total sterol. However, after rats were 21 days old, 7-dehydrocholesterol gradually disappeared from the brain and was slowly replaced by cholesterol, until rats were 35 days old when 7-dehydrocholesterol could no longer be detected in the brain. During the experimental period there were only small differences in the ratio of 7-dehydrocholesterol to cholesterol in each subcellular fraction of the brain at a given age and this was true even for the myelin fractions. When several injections of the drug AY-9944 were given at intervals to young rats (Table 2) 18 days after discontinuing treatment 7-dehydrocholesterol was found to account for about 40% of total brain sterol but traces of 7-dehydrocholesterol were present 67 days after injection. Although the proportion of 7-dehydrocholesterol in the myelin was lower than found in the first experiment (Table 1) the sterol remained for longer than in the animals injected when 5 days old.

Experiments with radioactive acetate. When [^{14}C]acetate was injected into control 9-day-old rats, the amount of labelled cholesterol recovered from whole brain and the subcellular fractions examined showed little change (Table 3); whereas ^{14}C incorporated into phospholipid of whole brain showed slow turnover, phospholipid in myelin remained stable. Radioactivity in cerebrosides increased in the whole brain and myelin. In particular a sevenfold increase was observed in myelin cerebroside radioactivity in the period from 12 to 30 days after birth (Table 3). Relatively little change in radioactivity was seen in the sterol and cerebroside of the myelin-like fraction but turnover of [^{14}C]phospholipid occurred. The distribution of radioactivity in 7-dehydrocholesterol and cholesterol in subcellular fractions isolated from developing brain from animals at different times after treatment with AY-9944 and [^{14}C]acetate is given in Table 4. In the 12-day-old rat brain about 85% of the total sterol radioactivity is found in 7-dehydrocholesterol whereas at 30 days almost all of the equivalent of the 7-dehydrocholesterol radioactivity has been recovered in labelled cholesterol. These results suggest that 7-dehydrocholesterol after

Table 4. *Incorporation and persistence of 7-dehydrocholesterol in the myelin and subcellular fractions of the developing brain*

Rats (5-day-old) were given a single intraperitoneal injection of AY-9944 (1 mg/10 g body wt.) followed by an injection of [^{14}C]acetate ($10\mu\text{Ci/rat}$) at the age of 9 days. Brain fractionation and sterol analyses are described in the text. Results are shown as radioactivity in the equivalent of whole brain.

Age (days)	Sterol	Radioactivity (d.p.m./whole brain)							Recovery (%)
		Whole brain	Nuclei	Mitochondria	Nerve-ending particles	Myelin	Myelin-like fraction	Microsomes Supernatant	
12	Cholesterol	5360	330	228	914	223	384	189	95.5
	7-Dehydrocholesterol	31879	1605	1188	4931	2615	2154	440	72.5
	Total sterol	37239	1935	1416	5845	2838	2538	629	—
20	Cholesterol	11750	1185	1062	1308	1185	860	447	77.0
	7-Dehydrocholesterol	13505	1168	630	1489	1682	580	326	69.0
	Total sterol	25255	2353	1692	2797	2867	1440	773	—
26	Cholesterol	23800	5349	1188	6470	5071	2540	790	109.0
	7-Dehydrocholesterol	3980	690	459	459	790	309	99	95.5
	Total sterol	27780	6039	1878	6929	5861	2849	889	—
30	Cholesterol	29400	—	1430	7672	6671	1440	—	76.0
	7-Dehydrocholesterol	2895	380	715	314	612	310	—	96.0
	Total sterol	32385	—	2145	7986	7283	1750	—	—

Table 5. 7-Dehydrocholesterol reductase activity in rat brain subcellular fractions

Subcellular fractions were prepared by the method described in the text. The microsomal fraction was prepared by centrifuging the post-mitochondrial supernatant at 100000g for 60 min. Results are the average of several determinations and expressed as μmol of 7-dehydrocholesterol reduced/h per g wet wt. tissue. Standard deviations are recorded where more than two experiments were carried out. 6-day-old rats were injected with AY-9944 (1 mg/10g body wt.).

Fraction	No. of observations ...	Enzyme activity ($\mu\text{mol/h}$ per g wet wt.)	
		21-day-old AY-9944-treated (2)	21-day-old normal (5)
Whole brain		0.925, 0.800	0.210 ± 0.020
Microsomes		0.470, 0.190	0.129 ± 0.007
Crude myelin		0.180, —	0.037 ± 0.0014
Purified myelin		0, 0.080	0.021 ± 0.0036

incorporation is slowly reduced to cholesterol and possibly re-utilized. The distribution of radioactivity was similar in 7-dehydrocholesterol and cholesterol of all the subcellular fractions of the 12-day-old rat. After 30 days most of the sterol radioactivity of each subcellular fraction was recovered in cholesterol; small amounts of labelled 7-dehydrocholesterol still remained, particularly in the mitochondrial fraction where there was little change in radioactivity. Loss of radioactivity from total labelled brain sterol was slight, there was steady accumulation of [^{14}C]cholesterol into myelin and at the same time a decrease in the amount of labelled sterol in the microsomal fraction. This suggested a possible transference of cholesterol from the microsomes to the developing myelin sheath.

7-Dehydrocholesterol reductase activity. The activity of the enzyme 7-dehydrocholesterol reductase responsible for the reduction of 7-dehydrocholesterol to cholesterol, (Dempsey, 1965; Kandutsch, 1962; Niemi & Fumagalli, 1965) was determined in rat brain and its subcellular fractions during the period of maximum conversion of the precursor into cholesterol (Table 5). Reductase activity was increased in the brain of rats pretreated with AY-9944; about 50% of the total enzyme activity was found to be localized in the microsomal fraction and 15–20% of the activity in unshocked crude myelin. However, when the crude myelin fraction was subjected to osmotic shock and recentrifuged, only about 5% of total reductase activity was found, suggesting that the presence of high activity of this enzyme may be due to microsomal contamination or cytoplasm trapped within the myelin vesicles.

Exchange experiments. When myelin isolated from rats pre-injected with [^{14}C]acetate was incubated with unlabelled whole-brain homogenate *in vitro*, an exchange of radioactive lipids between myelin and microsomes apparently occurred. This exchange from labelled myelin to unlabelled micro-

somes accounted for 10–12% of the total radioactive cholesterol, 10% for total phospholipids, and 6–7% for cerebroside. Somewhat less exchange has been observed from labelled microsomes to myelin (Table 6). Similar results were obtained when experiments were carried out with myelin isolated from AY-9944-treated rats, when exchange of 7-dehydrocholesterol from myelin to microsomes was observed. Incubation experiments of this type are clearly full of potential artifact and difficult to control; moreover on re-isolation of myelin considerable losses occur (see Table 6). As a means of monitoring contamination by myelin, cyclic 2':3'-nucleotide 3'-phosphohydrolase activity in microsomes isolated after incubation at 37°C for 30 min was determined. There was no increased phosphohydrolase activity in the recovered microsomes (Table 6). In another experiment myelin (containing labelled protein) was incubated with microsomes and on re-isolation only traces of radioactivity were found in the microsomal protein and in no other incubation experiments did total microsomal protein increase, as might have been expected if there was extensive myelin contamination.

DISCUSSION

In this paper we have confirmed many previous observations indicating a high degree of apparent metabolic stability of cholesterol in the nervous system and we found persisting [^{14}C]cholesterol to be especially concentrated in myelin (Table 3). After pretreatment of 5-day-old rats with AY-9944, large amounts of 7-dehydrocholesterol continue to accumulate in the brain and myelin, for the drug persists in nervous tissue for some time (Rawlins & Uzman, 1969). The 7-dehydrocholesterol content of the brain reaches a maximum 17 days after injection. Subsequently the sterol precursor concentration decreases in myelin and other subcellular fractions to be replaced by an equivalent amount of cholesterol (Table 1; Fumagalli *et al.* 1969).

Table 6. *Exchange of lipids between myelin and microsomes isolated from developing rat brain*

Experimental procedures are described in the text. In Expts. 1 and 2 ^{14}C -labelled myelin isolated from 29- and 38-day-old brain was incubated with unlabelled 29- and 38-day-old rat whole-brain homogenate respectively. In Expt. 3 ^{14}C -labelled 29-day-old rat brain microsomal preparation was incubated with the same age of unlabelled rat whole-brain homogenate and in Expt. 4 7-dehydrocholesterol containing myelin isolated from 24-day-old brain was incubated with unlabelled 24-day-old rat whole-brain homogenate. Incubation was carried out at 37°C for 30 min and after incubation, myelin and microsomes were isolated by the method described in the text. Cyclic 2':3'-nucleotide 5'-phosphohydrolase activity was determined in Expts. 1 and 2 and used as a measure of contamination.

Marker	Sample	Expt. no.	Cholesterol		Total phospholipids		Cerebrosides		Cyclic 2':3'-nucleotide 5'-phosphohydrolase sp. activity ($\mu\text{mol/h}$ per g wet wt.)
			Radioactivity (d.p.m.)	Exchange (%)	Radioactivity (d.p.m.)	Exchange (%)	Radioactivity (d.p.m.)	Exchange (%)	
[1- ^{14}C]Acetate	Myelin, before incubation	1	1950	—	3180	—	1398	—	3813
	Myelin, after incubation	2	1457	—	2815	—	1150	—	4700
	Myelin, before incubation	1	715, 1108	—	902, 1661	—	456, 880	—	4000
	Microsomes, after incubation	2	625	—	1025	—	383	—	4250
Unlabelled	Microsomes, before incubation	1	205, 256	10.5, 13.1	307, 127	9.7, 4.0	89, 89	6.6, 6.6	726
	Microsomes, before incubation	2	147	10	261	9.3	75	6.5	852
	Microsomes, before incubation	2	—	—	—	—	—	—	715
	Microsomes, before incubation	3	1795	—	3112	—	212	—	825
[1- ^{14}C]Acetate	Microsomes, after incubation	3	697	—	1147	—	51	—	—
	Myelin, after incubation	3	97	5.4	131	4.2	21	10	—
	Myelin, after incubation	3	—	—	—	—	—	—	—
	Myelin, after incubation	3	—	—	—	—	—	—	—
7-Dehydrocholesterol labelled	Myelin, before incubation	4	Content (μmol) 1.71	Exchange (%) —	—	—	—	—	—
	Myelin, after incubation	4	0.93	—	—	—	—	—	—
	Microsomes, after incubation	4	0.23	13.5	—	—	—	—	—
	Microsomes, after incubation	4	—	—	—	—	—	—	—

Our experiments with [^{14}C]acetate suggest that this increase in labelled cholesterol originates from the reduction of 7-dehydrocholesterol for there is no loss of total labelled sterol during the course of the experiment. At first it appeared that accumulation of myelin ^{14}C -labelled cholesterol may result from the reduction *in situ* of radioactive 7-dehydrocholesterol in the membrane but the low 7-dehydrocholesterol reductase activity of purified myelin cannot account for the rate of conversion into cholesterol (Table 5). An alternative explanation of our results is that 7-dehydrocholesterol in myelin and other membranes can exchange for cholesterol and that the released 7-dehydrocholesterol is reduced by microsomal reductase before re-incorporation. Such a possibility implies that in the developing brain sterol is readily exchangeable and our preliminary experiments *in vitro* support this suggestion (Table 6). Graham & Green (1967) have already demonstrated a 57% exchange of cholesterol from adult myelin with plasma lipoproteins after 24h incubation at 37°C. It is also well established that cholesterol of erythrocyte stroma undergoes ready exchange with plasma sterols (Ashworth & Green, 1964; Hagerman & Gould, 1951; Rouser, Nelson, Fleischer & Simon, 1968). In our experiments enhanced exchange occurred with whole-brain suspensions rather than myelin plus isolated microsomes. It has been demonstrated that exchange *in vitro* requires a heat-labile supernatant factor, presumably a lipoprotein carrier (Wirtz & Zilversmit, 1968; McMurray & Dawson, 1969; Beattie, 1969), for the exchange of phospholipid molecules between rat liver mitochondria and microsomes (Jungalwala & Dawson, 1970); hence a similar protein carrier may be important in the exchange of cerebral lipid.

In a series of papers, Hedley-Whyte and her colleagues (Hedley-Whyte, Rawlins, Salpeter & Uzman, 1969a,b; Rawlins, Hedley-Whyte, Villegas & Uzman, 1969) have described studies in which [$1,2\text{-}^3\text{H}$]- and [$4\text{-}^{14}\text{C}$]-cholesterol have been injected into developing mice and persistence and distribution of sterol traced by using radioautography in peripheral nerve up to 75 days after injection. Labelled cholesterol was found by light- and electron-microscopic techniques (Hedley-Whyte, Darrah, Stendler & Uzman, 1968) to be localized primarily in the myelin sheath but even after 7 weeks radioactive cholesterol was found in Schwann cells, axons and other tissue components of the sciatic nerve. These results were taken to indicate the presence of a 'bound' cholesterol pool available for re-utilization and possibly continuous circulation within the peripheral nerve (Hedley-Whyte *et al.* 1969b; Rawlins *et al.* 1969). In the brain it is known that cholesterol-catabolizing enzymes are of low activity (Davison, 1970) and that [$4\text{-}^{14}\text{C}$]-

cholesterol incorporated into the developing central nervous system remains unchanged for more than a year (Davison & Wajda, 1959a). Although some radioautographic evidence suggests that exchange of cholesterol is less in the central than peripheral nervous systems (Torvik & Sidman, 1965), it seems likely that the interpretation of the previous demonstration of metabolic stability of cholesterol is complicated by possible re-utilization of labelled sterol especially during development.

Re-utilization was previously suggested as an explanation of the unexpected lack of turnover of brain mitochondrial cholesterol (Cuzner *et al.* 1966; Khan & Folch, 1967; Smith, 1968) for we found other mitochondrial lipids were metabolized with a mean half-life of about 40 days. After the administration of hypocholesterolaemic drugs to 21-day-old rats Fumagalli *et al.* (1969) showed longer persistence of desmosterol and 7-dehydrocholesterol in brain myelin and mitochondria. It appears that ready exchange of sterol in developing myelin may well occur before compact lamellae are laid down but we have yet to establish if similar exchange of cholesterol can occur in brain membrane systems of mature animals. The experiments of Dvornik & Hill (1968) suggest that slow exchange of 7-dehydrocholesterol for cholesterol is possible in the adult brain, for they fed AY-9944 to adult rats for 6 and 12 months and found 30% of the total brain sterols as 7-dehydrocholesterol to be replaced by cholesterol after 6 months on normal diet. It therefore seems from the work cited in this paper that cholesterol is loosely bound in the myelin lamella and that the overall stability of this structure is more accurately reflected by isotope studies on the more tightly bound lipids (e.g. cerebroside) and the proteolipid protein matrix (Davison, 1961; Wood & King, 1971; Martenson & Gaitonde, 1969).

We are grateful to the Multiple Sclerosis Society of Great Britain for financial support.

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