thematic review

Thematic review series: Brain Lipids

Cholesterol metabolism in the central nervous system during early development and in the mature animal

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Abstract **Unesterified cholesterol is an essential structural component of the plasma membrane of every cell. During evolution, this membrane came to play an additional, highly specialized role in the central nervous system (CNS) as the major architectural component of compact myelin. As a consequence, in the human the mean concentration of unesterified cholesterol in the CNS is higher than in any other tissue (~23 mg/g). Furthermore, even though the CNS accounts for only 2.1% of body weight, it contains 23% of the sterol present in the whole body pool. In all animals, most growth and differentiation of the CNS occurs in the first few weeks or years after birth, and the cholesterol required for this growth apparently comes exclusively from de novo synthesis. Currently, there is no evidence for the net transfer of sterol from the blood into the brain or spinal cord. In adults, the rate of synthesis exceeds the need for new structural sterol, so that net movement of cholesterol out of the CNS must take place. At least two pathways are used for this excretory process, one of which involves the formation of 24(*S*)-hydroxycholesterol. Whether or not changes in the plasma cholesterol concentration alter sterol metabolism in the CNS or whether such changes affect cognitive function in the brain or the incidence of dementia remain uncertain at this time.**—Dietschy, J. M., and S. D. Turley. **Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J. Lipid Res.* 2004.** 45: **1375–1397.**

Supplementary key words Alzheimer's disease • amyloid precursor protein • oxysterols • dementia • amyloid β peptide • blood-brain barrier • apolipoprotein E • apolipoprotein A-I • lipoprotein transporters • ATP binding cassette transporters

In a study communicated to the Royal Society in London on February 11, 1909, benzoyl chloride was used to precipitate cholesterol from extracts of hens' eggs and newborn chicks. The amount of sterol in each egg was found to average 245 mg and the amount in each chick was 213 mg (1). This finding was later confirmed using digitonin to isolate the cholesterol (2). Furthermore, it was shown that cholesterol was disappearing from the yolk sac at the same time that the chick embryo was developing and accumulating increased amounts of sterol (3). Apparently, the cell membranes of the developing chick were being constructed from cholesterol derived from the hen and temporarily stored in the yolk sac, and not from sterol synthesized by the embryo itself. This result seemed to confirm the prejudice of early biochemists that "it is difficult to conceive how a [molecule] of the constitution of cholesterol [could] be synthesized in the organism from proteids, carbohydrate or fat . . . ." (1). In fact, this conclusion was further supported in 1937 once isotopically labeled precursors became available. No deuterium labeling was found in most of the cholesterol molecules of chicks taken from eggs enriched with deuterium oxide (4). However, the seminal observation was not made until 1969, when the sterol in the yolk sac was prelabeled by administering [^{14}C]cholesterol to laying hens. When the various organs of the newborn chick were examined, not surprisingly, the specific activity of the sterol isolated from nearly all of the tissues equaled 95–98% of the specific activity of the cholesterol in the yolk sac. These organs had indeed been largely constructed using preformed cholesterol from the hen. However, the specific activity of cholesterol isolated from the brain was only 11% of that found in the yolk sac (5). Clearly, cholesterol metabolism in the central nervous system (CNS) was very different from sterol metabolism in the rest of the body.

During the last 40 years, as the role of lipoproteins in the development of atherosclerosis was being defined,

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Abbreviations: ABC, ATP binding cassette; apoE, apolipoprotein E; CNS, central nervous system; kcal, kilocalories; LDL-C, cholesterol carried in low density lipoprotein; LDLR, LDL receptor; LRP, LDLR-related protein; LXRβ, liver X receptor-β; NPC1, Niemann-Pick C1; NPC2, Niemann-Pick C2; NPC1L1, Niemann-Pick C1-like 1; PNS, peripheral nervous system; SR-BI, scavenger receptor class B type I; SREBP, sterol-regulatory element binding protein; TC, total cholesterol.

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there was renewed interest in the possibility that the level of circulating cholesterol also affected, in some manner, the function of the CNS. It was postulated, for example, that the concentration of cholesterol circulating in the plasma of the newborn infant might affect brain development and even intelligence. In the adult, it was suggested that low levels of circulating cholesterol might be responsible for depression and violent, or even suicidal, behavior (6–8). Still more recently, the incidence of dementia has been related directly to the level of circulating cholesterol (9–13).

This possibility that the differentiation and function of the CNS was in some way influenced by the concentration of cholesterol circulating in low density (LDL-C) or high density lipoproteins in the plasma received further support from recent developments in our understanding of the molecular events involved in transmembrane cholesterol movement. It was recognized, for example, that the brain contained or expressed a number of different apolipoproteins, e.g., apolipoprotein E (apoE) and apoA-I; lipoprotein receptors, e.g., the low density lipoprotein receptor (LDLR) and scavenger receptor class B type I (SR-BI); and members of the ATP binding cassette (ABC) family of transporters, e.g., ABCA1 and ABCG1 (14–19). Because several of these proteins have well-established roles in cholesterol metabolism in many organs elsewhere in the body, it was assumed that they played similar roles in the CNS. However, direct experimental evidence that this is the case remains elusive. This review, therefore, was undertaken to summarize our current knowledge of the processes that result in the accumulation of cholesterol in the CNS and that are responsible for the net movement of sterol among cells of the brain and between the brain and circulating lipoproteins in plasma.

NET MOVEMENT OF CHOLESTEROL ACROSS THE PLASMA MEMBRANE AND THROUGH THE WHOLE ANIMAL

Before reviewing cholesterol metabolism in the brain, however, it is useful to consider the major pathways that function in other tissues of the body to maintain sterol homeostasis, because many of these same pathways conceivably also function in the CNS. This brief review will be limited to those processes that have been shown to affect the mass movement of cholesterol in one or more tissues of the body where rates of sterol movement have actually been quantified in appropriate mutant or knockout animals.

Major proteins involved in cellular cholesterol transport

As illustrated in **Fig. 1**, in the prototypic cell most cholesterol is unesterified and is located in either the outer (red) or inner (green) leaflet of the plasma membrane. Only a small portion of the pool of unesterified cholesterol in most tissues is located in intracellular structures such as the endoplasmic reticulum, Golgi apparatus, or nucleus. Nevertheless, this small, metabolically active pool of sterol plays a critical role in regulating the content of cholesterol in the cell. Cholesterol accounts for ~20–25% of the lipid molecules in the plasma membrane of most cells, whereas various phospholipids, sphingomyelin, and glycolipids make up the remainder. The distribution of these different lipids within the leaflets of the plasma membrane, however, is not uniform. The glycolipids and, to a lesser extent, sphingomyelin are found predominantly in the outer leaflet, and the various phospholipids are predominantly located in the inner leaflet. In many cell membranes, portions of these leaflets are organized into microdomains that are enriched with cholesterol, sphingomyelin, and glycolipids and therefore are resistant to solubilization in detergents (20–22). Although not shown in Fig. 1, embedded within the plasma membranes are a variety of transporters and signaling molecules as well as various aquaporins (23). These latter proteins are responsible for the observation that plasma membranes, in general, are highly permeable to water but relatively impermeable to ions and protons. Obviously, the concentration of unesterified cholesterol in these leaflets must be tightly regulated because this molecule plays an important role in determining the fluidity and permeability characteristics of the membrane as well as the function of both the transporters and signaling proteins.

Because cholesterol is continuously shed from the outer leaflet into the surrounding lymph, every cell requires a continuous supply of new sterol to maintain constant this critical concentration in the plasma membrane. As shown diagrammatically in Fig. 1, there are at least four mechanisms for acquiring cholesterol: de novo synthesis within the cell (1) and uptake of unesterified or esterified cholesterol from the external environment using the LDLR (2), SR-BI (3), or the Niemann-Pick C1-like protein (NPC1L1) (4). In most cells, the major source of new sterol is endogenous synthesis from acetyl-CoA (24). In general, the higher the basal metabolic rate in a particular species, the higher the rate of synthesis in its different organs (25). Cholesteryl ester also can be brought into the cell by taking up various lipoproteins from the external environment. The LDLR, for example, binds particles that contain either apoE or apoB-100, and these include the remnants of chylomicrons and very low density lipoproteins as well as LDL (26, 27). These particles are then processed through the clathrin-coated pit pathway to late endosomes and lysosomes, where acidification and hydrolysis of the cholesteryl esters take place (26, 27). Although still poorly understood, two other proteins, Niemann-Pick type C1 and C2 (NPC1 and NPC2), are then required to move this unesterified cholesterol to the metabolically active pool, where the molecule can be transferred to the plasma membrane, metabolized to other products, or act as a regulator of cell sterol metabolism (28, 29). In a similar manner, HDL particles containing apoA-I can be bound by SR-BI in cells like hepatocytes and endocrine cells (30, 31). However, unlike the clathrin-coated pit pathway, apparently only the cholesteryl ester from the particle is transferred into the cell (32). This molecule then enters an as yet unidentified compartment where

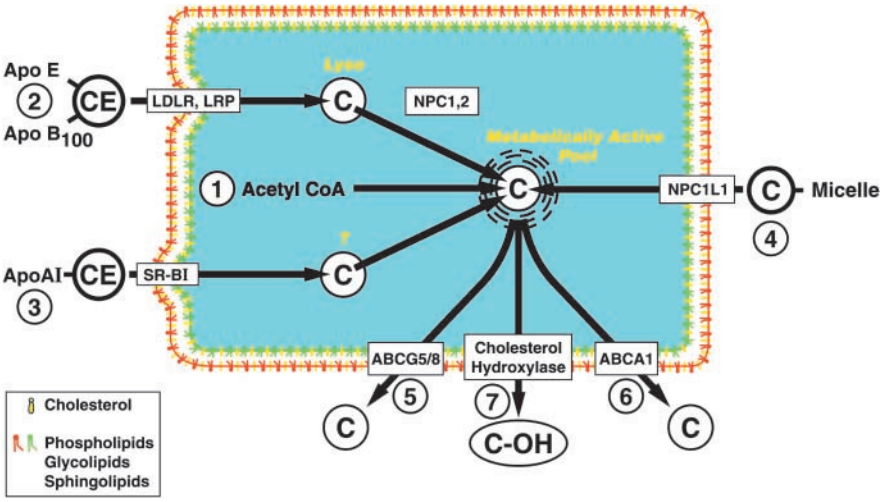

Fig. 1. The major proteins that have been shown to transfer cholesterol across the plasma membrane of different cells in the body. Each transport pathway is numbered and is described in detail in the text. The abbreviations C, CE, and C-OH represent unesterified cholesterol, cholesteryl ester, and hydroxylated cholesterol, respectively. The two particles numbered 2 and 3 represent lipoproteins that contain either apolipoprotein E (apoE) and/or apoB-100 or apoA-I, respectively. The particle numbered 4 represents unesterified cholesterol contained in a mixed micelle of bile acid and phospholipid. The outer leaflet of the plasma membrane is represented by the red line, and the inner leaflet is shown in green. The metabolically active pool of cholesterol in the cytosol is that pool of cholesterol in the cell that regulates these enzymatic and transport processes and that can be used as a substrate for these various processes. This diagram is based upon a number of publications cited in the text. LDLR, LDL receptor; LRP, LDLR-related protein; NPC1,2, Niemann-Pick C1 and C2; NPC1L1, Niemann-Pick C1-like 1; SR-BI, scavenger receptor class B type I.

acidification and hydrolysis also take place before the unesterified cholesterol is delivered into the metabolically active pool (33). It should be noted that mutations in NPC1 lead to the retention of unesterified cholesterol in late endosomes and lysosomes after uptake of lipoproteins through the clathrin-coated pit pathway but have no effect on the uptake and processing of cholesteryl ester from the HDL particle (34). Finally, recently, a third transporter has been reported, NPC1L1, that takes up unesterified cholesterol from mixed micelles in the intestine and delivers it into the metabolically active pool within the enterocyte (35, 36).

Cholesterol is continuously shed from the outer leaflet of the plasma membrane, and much of this movement may simply be driven by chemical gradients between the leaflet and lipoprotein receptors in the plasma (37, 38). However, at least three other mechanisms have also been described that bring about net excretion of cholesterol out of the cell. These include the transporters ABCG5/8 (5) and ABCA1 (6) and various enzymes capable of hydroxylating cholesterol (7). ABCG5/8 is made up of two half transporters that can move unesterified cholesterol from the cell membrane or cytosol to the external environment (39). Similarly, ABCA1 also apparently transports unesterified cholesterol out of the plasma membrane in certain cells (37, 40). In an entirely different mechanism, many cells contain enzymes capable of hydroxylating the cholesterol molecule in the 7α, 24, 25, and 27 positions, and these hydroxylated sterol molecules, in turn, can diffuse out of the cell across the plasma membrane [reviewed in ref. (41)]. Although not shown in Fig. 1, a few types of cells, such as hepatocytes and enterocytes, can also incorporate cholesteryl esters, along with triacylglycerol, into nascent lipoprotein particles and export these packaged lipids to the surrounding aqueous phase.

Cholesterol transport in the whole animal

As illustrated in **Fig. 2**, these various transport mechanisms are expressed in specific organs in such a way that there is essentially net unidirectional movement of cholesterol from the peripheral organs to the intestinal lumen. Apparently, every cell in the periphery, including the CNS, can synthesize cholesterol and move it up to the leaflets of the plasma membrane. Because the concentration of sterol in these leaflets remains constant, cholesterol must be continuously removed and becomes associated with circulating apoA-I. In cells such as macrophages, this desorption process may be under the control of ABCA1, but this seems not to be the case with most other cell types (40). Within the plasma space, the HDL particle enlarges as cholesteryl ester is formed under the influence of LCAT. Depending upon the species, a portion of this cholesteryl ester may then be transferred by cholesteryl ester transfer protein to apoB-containing lipoproteins and taken up into the liver by means of the LDLR and the clathrin-coated pit pathway. Alternatively, the cholesteryl ester may be selectively transported by SR-BI directly into the hepatocyte. In either case, the cholesteryl ester is hydrolyzed and the unesterified cholesterol is mixed with other pools of sterol that come from de novo synthesis or

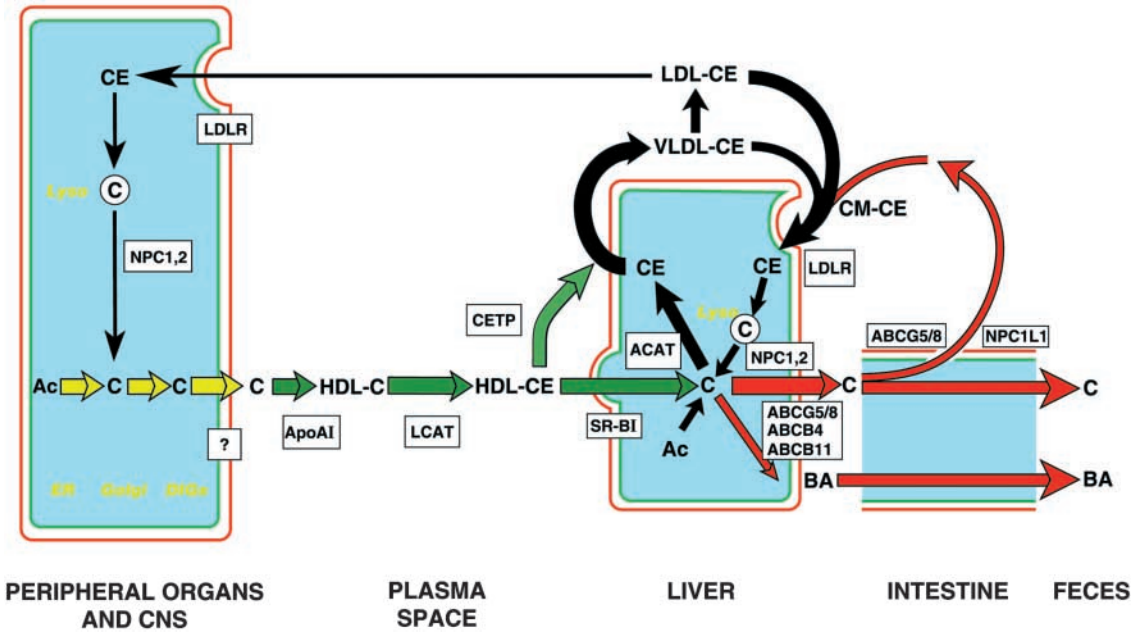

Fig. 2. The flow of cholesterol through the major organs of an animal or human. In this formulation, all of the peripheral organs of the body, including the central nervous system (CNS), are grouped together in one compartment, whereas the liver and intestine are shown separately to reflect their unique roles in sterol metabolism in the whole animal. The specific proteins involved in the movement of cholesterol across cell membranes or through the plasma space are identified in boxes. The abbreviations C and CE refer to unesterified cholesterol and cholesteryl ester, respectively, and BA represents bile acid. Ac, acetyl CoA; ACAT, acyl-CoA:cholesterol acyltransferase; CM, chylomicron; DIGs, deterent-insoluble glycosphingolipid-enriched complexes; ER, endoplasmic reticulum. This figure has been modified and redrawn, with permission of the publisher, from ref. (43).

from the intestine (42). After a portion of this pool is metabolized to bile acid, these sterols are transported across the canalicular membrane of the hepatocyte by a complex process that requires three separate transport proteins. These three proteins bring about the movement of phospholipid (ABCB4), bile acid (ABCB11), and cholesterol (ABCG5/8) into the bile and, ultimately, into the intestinal lumen. Although both the cholesterol and bile acid molecules are partially reabsorbed and recycled back to the liver through an enterohepatic circulation, ultimately there is net excretion of both of these molecules from the body as fecal neutral and acidic sterols. It should be emphasized that the rate-limiting step in this unidirectional transport system appears to reside within the individual cells of the peripheral organs. Functional deletion of ABCA1, apoA-I, LCAT, SR-BI, or LDLR does not significantly alter the overall flux of cholesterol from these peripheral tissues to the intestinal lumen (43, 44).

As also shown in Fig. 2, there is a second pathway that is important in the movement of both cholesterol and triacylglycerol through the body. The enterohepatic circulation of cholesterol apparently depends upon NPC1L1, whereas ABCG5/8 acts to limit the uptake of sterols of plant origin (45). This newly absorbed cholesterol is delivered essentially entirely to the liver, where it mixes with other sterol in the metabolically active pool. A portion of this pool is esterified and incorporated into VLDL and, ultimately, into LDL. The majority of the cholesterol carried in VLDL and LDL is then returned to the liver. In most species, less than 20% of the LDL-C is taken up by cells of the peripheral organs, where synthesis is the major source for cholesterol (25, 43).

Many of these same proteins involved in the movement of cholesterol throughout the body are also expressed in the CNS. These include many members of the LDLR family of receptors as well as SR-BI (15, 46). The apolipoproteins apoE, apoA-I, apoA-IV, apoD, and apoJ have also been detected in cerebrospinal fluid, and various members of the ABC family of transporters are expressed in specific cells of the CNS (16, 47). The crucial question, then, is which if any of these proteins is involved in the net movement of cholesterol across the blood-brain barrier into the CNS, between individual cells within the brain and from the CNS back into the plasma.

Concentration of cholesterol in various organs in the steady state

This flow of cholesterol from the sites of synthesis in the cells of the peripheral organs to the liver and intestine is so tightly regulated that in the steady state, the concentration of sterol in cell membranes is kept remarkably constant at a level that is characteristic of each particular tissue. As illustrated in **Fig. 3**, the pool of cholesterol in the whole animal is ~2,200 mg/kg body weight. This is true for essentially all species from the mouse to the primate and indicates that the average concentration of cholesterol in the whole animal is ~2.2 mg/g fresh tissue (48).

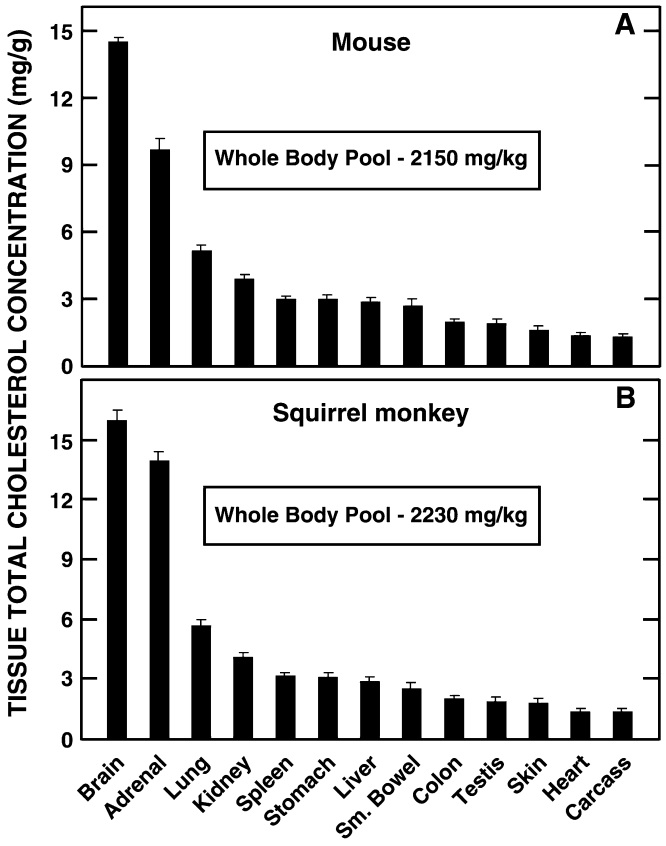

Fig. 3. Concentration of cholesterol in all major tissues of the male mouse and squirrel monkey. These data show the total concentration of cholesterol, both unesterified and esterified, in each organ, expressed as milligrams of sterol per gram wet weight of tissue. These animals had been maintained on a low dietary cholesterol intake before these measurements. Also shown are the whole-body cholesterol pools, expressed as milligrams of total cholesterol (TC) present per kilogram of body weight. These values come from both published and unpublished data from this laboratory (48, 126). Error bars represent ±1 SEM.

However, as also shown in this figure, this steady-state concentration varies markedly among the different organs.

In the brain, for example, the mean sterol concentration is 15–20 mg/g in many species. Although small amounts of desmosterol and cholesteryl esters have been found in the CNS of very young animals (49–53), in the adult essentially all sterol in the CNS is unesterified cholesterol that is present predominantly in the myelin and plasma membranes of various cellular elements. Cholesteryl esters apparently are found only with diseases such as multiple sclerosis (54). The adrenal gland and other endocrine tissues of most species also have high concentrations of cholesterol, but in contrast to the CNS, most of this sterol is stored in the cytosol as cholesteryl esters, and the concentration of unesterified cholesterol in cell membranes averages only 2–4 mg/g. In all of the remaining organs, from the lung and kidney (4–5 mg/g) to the striated muscle of carcass (1.4 mg/g), nearly all tissue sterol is unesterified cholesterol present predominantly in the plasma membranes of parenchymal cells. These latter tissues contain little or no cholesteryl esters (55). Only if the animal is challenged with exogenous, dietary cholesterol does the level of cholesteryl esters increase, and this increase occurs only in the liver and, to a much lesser degree, the intestine (55–57).

Turnover of cholesterol in various organs and in the whole animal in the steady state

Thus, in essentially all tissues there is a continuous flow of cholesterol from the endoplasmic reticulum to the cell membrane, and from this plasma membrane to the liver and intestine. During this process, the concentration of sterol in the plasma membranes of each organ (Fig. 3) and the size of the pool of cholesterol in the whole animal remain essentially constant. However, the rate of movement of sterol through these pathways, and therefore the rate of plasma membrane cholesterol turnover, is very different in animals with different basal metabolic rates. As shown in **Fig. 4**, for example, the basal metabolic rate in the mouse is ~170 kilocalories (kcal)/day/kg, and the flow of cholesterol from all peripheral organs to the liver is greater than 100 mg/day/kg (43, 58). In contrast, the basal metabolic rate in the human is only 25 kcal/day/kg, and the flow of sterol from the periphery is only ~10 mg/day/kg. Because the size of the steady-state pool of sterol is nearly the same in these various species, these data indicate that ~8% of the cholesterol in plasma membranes of the mouse is replaced each day and that only 0.7% of that found in the membranes of humans is turned over (43, 59).

This same relationship is seen in individual organs in any particular species. The organ-specific basal metabolic

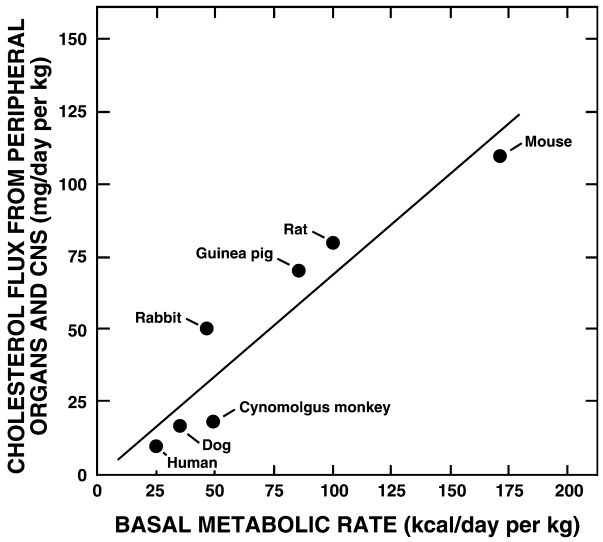

Fig. 4. Relationship between the flow of cholesterol from all peripheral organs, including the CNS, to the liver and the basal metabolic rate found in different animal species. This rate of movement of sterol is expressed as milligrams of TC moving out of these peripheral tissues each day per kilogram of body weight. The line was fitted by eye to illustrate this apparent relationship. kcal, kilocalories. These data were calculated from a variety of sources (24, 25, 58, 126).

rate in the intestine, for example, is very high, and the rate of cholesterol turnover in the plasma membranes of this tissue is also very high (24, 60). In contrast, the basal metabolic rate of striated muscle is very low, as is the rate of sterol turnover. However, the CNS appears to be an exception to this rule. Despite the fact that the organ-specific basal metabolic rate is very high in the brain, the rate of turnover of the sterol pool in the CNS of the mouse equals only 0.4% per day (compared with a whole-body turnover of 8% per day) and in the human brain is only 0.03% per day (compared with a whole-body turnover of 0.7% per day) (61). This apparent discrepancy between the high basal metabolic rate of the CNS and the slow rate of cholesterol turnover is addressed later in this review.

CONCENTRATION OF CHOLESTEROL IN THE PLASMA AND DISORDERS OF THE CNS

Although the concentration of cholesterol in the plasma membranes of most organs in the body is held very constant (Fig. 3), the concentration of sterol circulating in lipoproteins varies markedly during those periods of development when brain size, degree of myelination, and cholesterol content are also rapidly changing. The changes seen in the circulating total cholesterol (TC) concentration in several species during the fetal, suckling, and adult periods of development are shown in **Fig. 5**. Typically, during fetal development both the TC and LDL-C levels decrease in late gestation (62–65). In the lamb, for example, the TC declines from ~40 mg/dl to ~25 mg/dl and the LDL-C decreases from ~25 mg/dl to only ~10 mg/dl over the course of fetal development (62). Similar declines are seen in both the pig and human fetuses, with TC and LDL-C concentrations reaching ~55 and ~30 mg/dl, respectively, at birth in humans (63, 65). With the onset of suckling, however, the plasma sterol levels rapidly increase 2- to 4-fold in all species, including sheep, pig, and human. The breast-fed human newborn, for example, ingests ~18 mg cholesterol/day/kg body weight under circumstances in which the infant is synthesizing ~25 mg/day/kg (dietary cholesterol intake is 72% of endogenous synthesis) (66, 67). Because of this relatively large dietary cholesterol (and triacylglycerol) load, the TC promptly increases to ~170 mg/dl, the LDL-C increases to ~90 mg/dl, and there is suppression of endogenous sterol synthesis (67, 68). However, as also shown in Fig. 5C, if the infant is placed on a low-cholesterol synthetic formula, dietary cholesterol intake is reduced to only ~2 mg/day/kg (8% of endogenous synthesis), the TC and LDL-C levels increase to only ~95 and ~35 mg/dl, respectively, and there is much less suppression of endogenous sterol synthesis (67, 69, 70). Thus, all species show a marked increase in the plasma cholesterol concentration during the suckling period, although, in the human, this physiological hypercholesterolemia can be partly abrogated by feeding a low-cholesterol formula diet (Fig. 5C).

Similarly, in virtually all species, both the TC and LDL-C

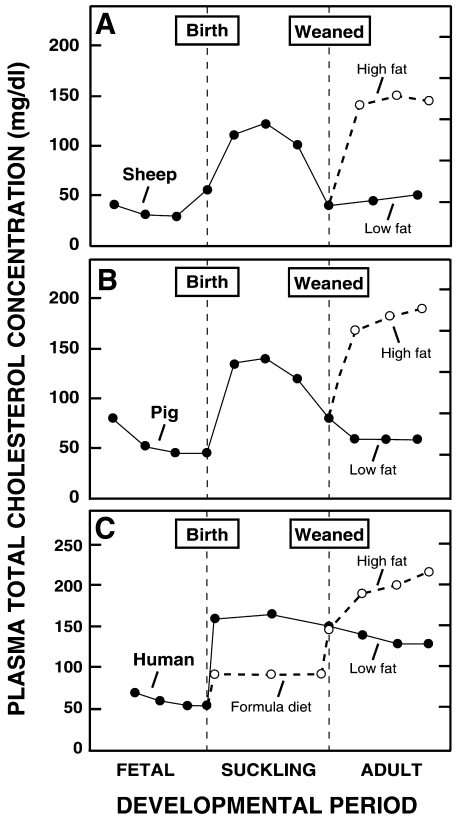

Fig. 5. Concentration of TC in the plasma of the fetus, suckling animal, and adult of several species. These data illustrate the profound differences that exist in the circulating cholesterol levels at different times of development and under circumstances in which the suckling or adult animal or human was placed on a diet either high or low in cholesterol and triacylglycerol. The duration of each of these periods of development has been normalized to the same interval for purposes of this illustration. The values plotted for the different species come from a variety of different sources, both published and unpublished (62–65, 68–72).

concentrations promptly decrease once the young animals are weaned onto diets that typically are very low in both cholesterol and triacylglycerol content. Such steep declines are seen in calves, lambs (Fig. 5A), piglets (Fig. 5B), and, to a lesser degree, humans (Fig. 5C) (71, 72). However, as also shown in Fig. 5, these same species can be made hypercholesterolemic by weaning the young animals onto diets containing large quantities of both cholesterol and triacylglycerol. This finding is particularly important with respect to human populations. There are large groups of people, including Amerindians of Meso and Central America, highland tribes of Papua New Guinea, and rural Chinese, who typically consume diets containing <100 mg cholesterol/day (<14% of endogenous synthesis) and in whom <11% of calories come from triacylglycerol. Such people typically have TC and LDL-C concentrations of ~135 and ~70 mg/dl, respectively, in adults of all ages, and these groups have virtually no coronary artery disease (71, 73–78). In contrast, humans who are weaned onto the usual Western diets containing 300–500 mg cholesterol/day (43–71% of endogenous synthesis) and in

whom >40% of calories come from triacylglycerol typically have TC and LDL-C levels of >200 and >110 mg/dl, respectively, and these populations have a very high incidence of coronary artery disease (79–81). These differences in plasma lipid levels in the adults are not attributable to genetic differences in these groups of people; rather, they are primarily the result of differences in intake of dietary lipids. When people of Amerindian genetic background, for example, are placed on Western diets, their TC and LDL-C values promptly increase and become indistinguishable from those values found in people of Caucasian or African genetic backgrounds eating the same Western diets (78, 82).

Thus, there are wide fluctuations in the TC and LDL-C concentrations at different times during the development of the animal or human. Furthermore, during the suckling and adult periods, additional variation in the circulating lipid levels can be induced by either dietary or pharmacological manipulations. Assuming that cholesterol metabolism in the CNS is in some manner affected by the concentration of sterol in the blood, some investigators have raised the possibility that these manipulations might have a detrimental effect on brain function. Certainly, children with the Smith-Lemli-Opitz syndrome who cannot convert 7-dehydrocholesterol to cholesterol in the blood or CNS have profound abnormalities in brain development and function (83). However, more subtle effects of fluctuations in circulating cholesterol levels on brain function have been postulated over the years in at least four areas. First, because the physiological hypercholesterolemia seen during suckling (Fig. 5) coincides with the period of major growth and myelination in the brain, it was suggested that formula feeding or limitations on dietary cholesterol intake might be deleterious to the differentiation of the CNS or even to the level of intelligence (84, 85). Second, in several studies in adults, aggressive, suicidal, or even criminal behavior was reported to be more common in individuals with low circulating cholesterol levels (6–8, 86–88). It was postulated that these low plasma levels might lead to a loss of cholesterol from membranes in the brain and, hence, to a reduction in the number of serotonin receptors (87, 89). Indeed, the concentration of serotonin in the blood was found to be lower in men with persistently low plasma cholesterol levels (90). Third, a number of epidemiological studies suggested that the relationship between all-cause mortality rates and the plasma cholesterol concentration was U-shaped. That is, mortality rates were increased in people with high cholesterol levels, presumably because of coronary artery disease and strokes; however, these rates were also increased in individuals with very low plasma cholesterol concentrations, for reasons that were not immediately obvious but that might be related to emotional changes (91–94). Finally, it was suggested that there was a direct correlation between the plasma cholesterol level and the incidence of dementias, including Alzheimer's disease (10, 95–97). Furthermore, treatment of older individuals with pharmaceutical agents that decreased the plasma cholesterol concentration appeared to be associated with a lower incidence of such dementias (11, 12).

It should be emphasized that many of these associations have not been confirmed by more recent and rigorous epidemiological studies. Nevertheless, these observations raise the possibility that there is a relationship between the plasma level of cholesterol carried in LDL, HDL, or other lipoproteins and the growth, myelination, and function of the CNS. If this were true, these lipoproteins might make important contributions to the cholesterol pools within the brain and spinal cord and possibly affect, directly or indirectly, the processing of proteins such as serotonin receptors or amyloid precursor protein (98).

CHOLESTEROL METABOLISM AND EVOLUTION OF THE BRAIN

Before reviewing the role of the plasma lipoproteins on CNS function, it is important to outline the critical functions played by the cholesterol molecule during the evolution of the complex vertebrate brain [reviewed in ref. (99)]. Even in primitive animals like jellyfish, neurons evolved to collect incoming information on the branched dendrites at one end of the cell and to convey this information through the axon to the effector sites at the other end of the cell. Generation of the action potential that transmits this information critically depends upon the permeability characteristics of the plasma membrane surrounding the axon. These characteristics are determined, in part, by the cholesterol and other lipid molecules making up this membrane. As animals evolved in size and complexity, three problems related to the conduction of the action potential had to be solved (100). First, the velocity of movement of the action potential down the axon had to be increased as much as possible. Second, the transverse diameter of the axons had to be kept as small as possible so that the complex "wiring" of the neurons in the brain could be accommodated within a relatively small volume. Third, the energy requirements to maintain the potential differences across the plasma membranes of axons had to be minimized. Cholesterol came to play a crucial role in the solution of these problems.

The problem of nerve conduction velocity

Although the permeability characteristics of the cholesterol/phospholipid bilayer allow potential differences to be maintained across the plasma membranes of virtually all cells, only neurons (and muscle cells) propagate electrical signals over long distances. The velocity of movement of this electrical signal down the axon is inversely proportional to both the electrical resistance encountered in the axon (r_a) and the capacitance of the plasma membrane surrounding the axon (c_m) (100). Thus, conduction velocity in nerves could be greatly increased by reducing the term $r_a c_m$. One approach to this problem was to reduce axonal resistance by evolving neurons with axons of very large diameter (~0.5 mm), as did some cephalopods like the giant squid (100, 101). However, such a so-

lution could not be used in the more complex brains of higher vertebrates. The alternative approach was to greatly reduce the capacitance of each neuron by increasing the thickness of the cholesterol/phospholipid membranes surrounding each axon.

This was accomplished by evolving two new types of cells, the oligodendrocyte in the CNS and the Schwann cell in the peripheral nervous system (PNS) (101). As shown diagrammatically in **Fig. 6**, the oligodendrocyte is an extraordinary cell that synthesizes vast sheets of plasma membrane that extend outward from the cell body. These sheets are surrounded by narrow loops or channels of persistent cytosol. Each of these sheets first wraps around a section of adjacent axon ~1 mm in length, between nodes of Ranvier. Thus, within the CNS, each oligodendrocyte may contribute plasma membranes to 10–15 different neurons, whereas in the PNS, each Schwann cell interacts with only a single neuron (102). The second step in the formation of this myelin coat involves the removal of most of the aqueous phase surrounding the tightly wrapped plasma membranes. This process involves specific proteins (e.g., myelin basic protein) that bring together in tight apposition the inner leaflets of the bilayers and, similarly, pull together the outer leaflets of adjacent plasma membranes (103). In this manner, nearly all of the aqueous electrolyte solution in both the extracellular and cytosolic compartments is "squeezed" out of the mature myelin and its capacitance is correspondingly reduced. Although more primitive forms of poorly packed myelin are found in lower phyla like Annelida and Crustacea, true compact myelin is seen only in Gnathostome vertebrates (104). Thus, the evolutionary adaptation of the cholesterol-rich plasma membrane to form compact myelin made it possible to "wire" the complex brain with numerous relatively small-diameter, low-capacitance axons that manifested very high conduction velocities.

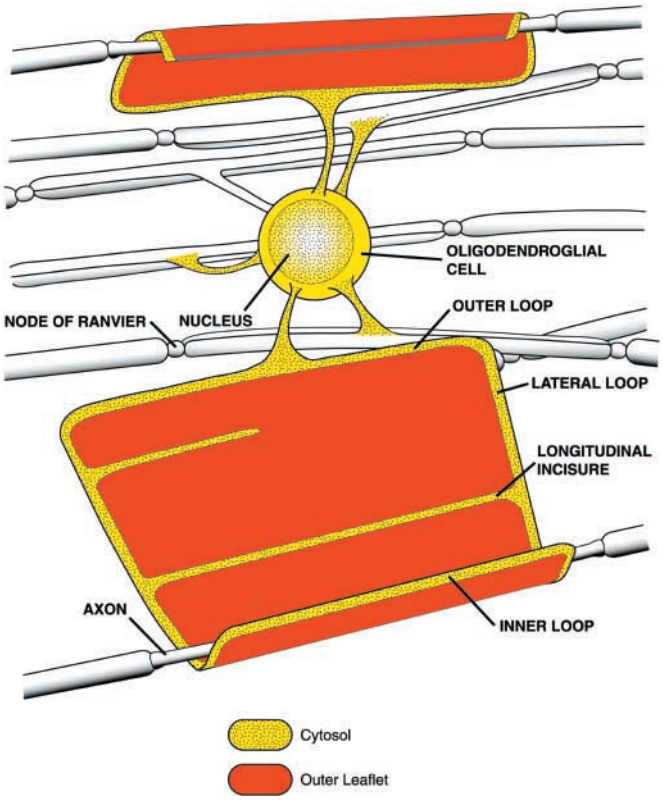

Fig. 6. Formation of myelin sheets by the oligodendrocyte. This diagram illustrates how a single oligodendroglial cell participates in the formation of sheets of cholesterol-rich plasma membranes that wrap around the axons of multiple neurons as compact myelin. The channels running around the edges of these sheets represent regions of persistent cytosol in which the inner leaflets have not come into tight apposition. This figure has been modified and redrawn, with permission of the publisher, from ref. (102).

As a result of this compaction process, the chemistry of myelinated portions of the CNS differs significantly from that found in other organs such as the liver. For example, water accounts for ~73% of the weight of the liver but only ~40% of isolated myelin (105, 106). The concentration of unesterified cholesterol equals only ~3 mg/g in the hepatocyte (Fig. 3) but may reach 40 mg/g in the spinal cord. Although this sterol accounts for a similar proportion (17–22%) of the total lipids found in plasma membranes of both the liver and the CNS, membranes in the brain contain relatively more glycolipids (28% vs. 7%) and less sphingomyelin (8% vs. 19%) than do plasma membranes of other organs like the liver (107). The fact that the cholesterol molecule exists in this relatively anhydrous, hydrophobic environment raises the issue of how such sterol is replaced during the normal turnover of cholesterol that takes place in all such plasma membranes.

The problem of brain size

Although the evolution of myelin largely solved the problem of action potential conduction velocity, this insulatory layer on axons did add additional volume to the CNS, particularly in the large brains of primates, where there was massive expansion of the neocortex. Over a very large range of brain sizes, the volume of the neocortex increased from only 16% of the volume of the whole brain in small animals like Soricomorpha to 74% in Hominoidea. In contrast, the relative volume of the cerebellum remained constant at 13% of whole brain volume, regardless of the absolute size of the brain (108, 109). As the size of the neocortex progressively evolved, so also did the volume occupied by myelinated nerve fibers. The quantitative nature of this relationship is shown in **Fig. 7**, where the volume of white matter is plotted against the volume of gray matter in 59 different species (110). As is apparent, there is a remarkably tight relationship between these two variables in all animals from the pigmy shrew to the elephant. These data support the view that as the neocortex increased in size and complexity, there was necessarily a disproportionate increase in the volume of brain devoted to "wiring." Thus, in Fig. 7, the volume of the white matter approximately increases as the 4/3 power of the volume of the gray matter (99, 110).

As cholesterol is an essential component of myelin in white matter, not surprisingly, the size of the sterol pool in the CNS also increases disproportionately as brain size in-

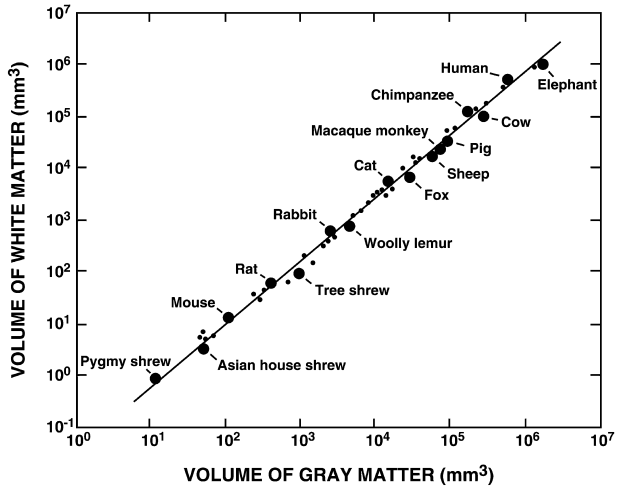

Fig. 7. Relationship between the volume of the white matter and the volume of the gray matter in 59 species. Although each data point represents a different species, only 16 of these are identified. The slope of this logarithmic relationship is such that the volume of white matter increases disproportionately to the volume of the gray matter as brain size increases. This relationship gives an apparent power law with an exponent of ~4/3. This figure has been modified and redrawn, with permission of the publisher, from ref. (110).

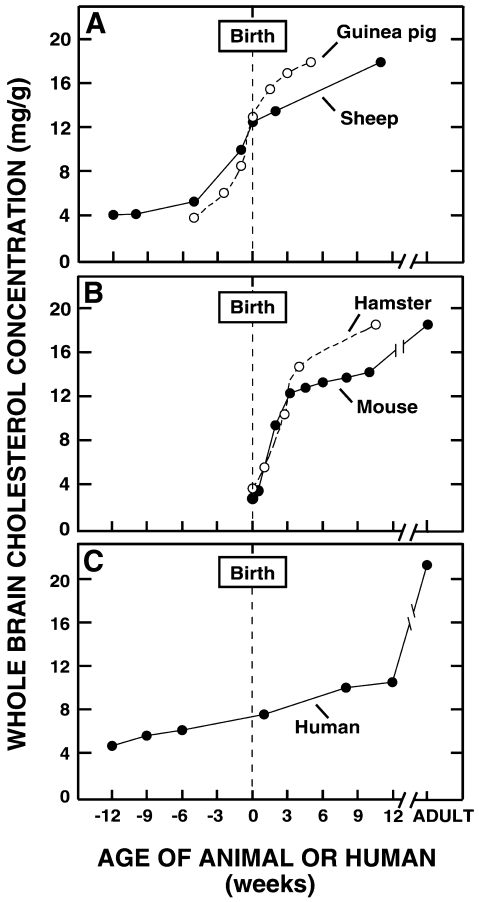

Fig. 8. Time course for myelination of the CNS in five different species. The degree of myelination in this diagram is approximated by the mean concentration of cholesterol in the whole brain, expressed as milligrams of sterol per gram wet weight. Although the gestation period is different for each of these species, the time scale has been kept constant from 12 weeks before birth to 12 weeks after birth. These data were calculated from a number of different sources, both published and unpublished (53, 111, 114, 140).

creases. The CNS of the adult mouse and human, for example, weighs ~0.5 and 1,400 g, respectively. Although the total body cholesterol pool in these two species is essentially the same, ~2,150 and ~2,200 mg/kg, respectively, the proportion of this pool residing in the CNS is significantly greater in the human (490 mg/kg, 23% of the total body pool) than in the mouse (330 mg/kg, 15% of the total body pool) (53, 59). Thus, because of the disproportionate increase in the volume of white matter required to support the greatly expanded neocortex in the human, there is a correspondingly disproportionate fraction of the whole-body pool of unesterified cholesterol in the CNS.

The problem of the female pelvis

Although solving the problem of nerve conduction velocity, this disproportionate increase in brain size created another challenge that ultimately dictated when brain growth and myelination could take place during fetal and neonatal development. Obviously, the cross-sectional area of the female pelvis limited the degree of development of the CNS before birth by limiting the size of the head that could pass through the birth canal. Various species have dealt with this problem in essentially two ways.

In **Fig. 8**, the degree of myelination at different times during perinatal development is approximated by the mean concentration of cholesterol in the whole brain. Ungulates, represented in Fig. 8A by the sheep, as a group begin myelinating the brain during late fetal development. Although these hooved animals are not noted for their intellectual capacity, this early myelination allows the newborn to be immediately mobile so that it can be led away from the birthing site before attracting the attention of local predators. The guinea pig represents another species that myelinates the CNS in the few weeks before birth so that the newborn pup is also quite mature and fully mobile (111). In contrast, many species, represented by the mouse and hamster in Fig. 8B, have essentially no myelination in the brain and are nearly helpless at birth. Thus, although the concentration of cholesterol in the brain of the sheep is ~12 mg/g at birth, it is only 3.5 mg/g in mice and hamsters. This value is similar to the concentration of sterol found in most other organs at birth in these species. However, in the 3 weeks after birth, cholesterol rapidly accumulates in the CNS. The human, also born essentially helpless, follows a similar pattern of myelination, although a number of years, rather than weeks, are required for the average concentration of cholesterol in the brain to increase from ~6 mg/g at birth to 23 mg/g found in the young adult (Fig. 8C).

From the experimental point of view, these data suggest two different models that are useful in studying choles-

terol metabolism in the developing brain. The vascular supply to the sheep fetus can be cannulated at any point during the last half of gestation, and the rate of sterol transport and synthesis in the CNS can be measured in vivo at times even before the closure of the blood-brain barrier (112, 113). In addition, the CNS of the sheep fetus is so large that it is possible to dissect out many small subregions of the brain (113). In contrast, myelination occurs rapidly in the newborn mouse over a 3 week period, so that changing transport and synthesis rates can be observed over a very brief period of time. In addition, many mice are available with genetic deletions of specific transporters or apolipoproteins that make it possible to explore the role of each of these proteins in cholesterol metabolism in the CNS (53).

The problem of excess energy expenditure

A final problem with the evolution of the CNS is explaining how the vertebrate, in general, and the encephalized primate, in particular, could afford the massive expenditure of energy required to support a large brain. In the newborn human, for example, ~60% of basal energy expenditure goes to support ion transport, neurotransmitter synthesis, and other metabolic processes in the CNS (99). In the adult, the organ-specific basal metabolic rate of the brain (230 kcal/day/kg) is nine times higher than the average basal metabolic rate (25 kcal/day/kg) for the whole body (60). The gastrointestinal tract has a similarly high rate of organ-specific basal metabolic energy expenditure (250 kcal/day/kg).

For nearly all animals, the rate of basal energy expenditure increases as the 3/4 power of body weight (58). Surprisingly, humans follow this same relationship despite their disproportionately large CNS (60). An explanation for this apparent paradox recently has been presented as the "expensive-tissue hypothesis." This hypothesis suggests that the massive expansion of the neocortex in humans came at the expense of the gastrointestinal tract. The left column in **Fig. 9** shows the weights of various organs that would be expected in a 65 kg human based upon projections from the relative weights of these same organs in various nonhuman primates. Such a hypothetical human should have a relatively small brain but a very large gastrointestinal tract to cope with foods of poor nutritional value and digestibility. However, the situation in actual humans, shown in the right column, reveals that the brain is more than three times larger and, importantly, the intestine is only half as large as projected from these calculations. Thus, it has been suggested that primitive humans with enlarging brains became more efficient hunter/gatherers and so gained greater access to more nutritious and easily digestible foods (e.g., fruits, nuts, and meat) and so no longer needed the large gastrointestinal tract. Humans had, in effect, conserved energy by greatly reducing the size of the intestine and then shifted this basal energy expenditure to support a much larger brain (60). Whether or not this hypothesis is correct, the CNS still has a very high basal metabolic rate, so that another paradox remains. Why is the rate of cholesterol turnover in the membranes of the brain apparently much slower than the rate of turnover in the plasma membranes of other organs? This question remains to be answered.

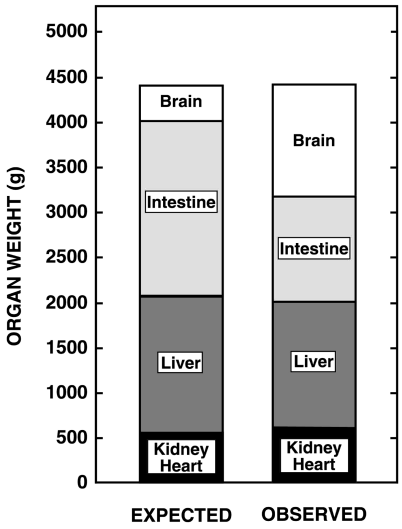

Fig. 9. The size of the brain in the human as predicted from other higher primates and as actually observed. At left are shown the weights of the various organs that would be predicted for a 65 kg human based upon extrapolation from observations in a number of other higher primates. At right are the actual weights of these same organs in the human. Although the relative size of the liver, kidneys, and heart is approximately the same as predicted from other primates, the weight of the brain has expanded greatly at the expense of the weight of the whole small and large intestine. This figure has been modified and redrawn, with permission of the publisher, from ref. (60).

BRAIN GROWTH AND CHOLESTEROL POOLS IN THE CNS

The transport and biosynthetic processes that bring about cholesterol accumulation in the CNS must account for the rates of growth and the size of the sterol pools found in the brain at different ages of development. The weight of the brain and the sterol content at different ages are shown for the mouse and human in **Fig. 10**. Between 1 and 26 weeks of age, the weight of the brain of the mouse increases from ~0.28 to 0.52 g (16.3 g/kg body weight), and the content of cholesterol in the whole brain increases from 1.5 to 10.6 mg (330 mg/kg). As is also apparent in Fig. 10B, the rate of increase in this sterol pool is greatest during the first 3 weeks after birth, after which the rate of accumulation markedly decreases as the animal matures.

The weight of the brain in the human increases from ~360 g in the newborn to ~1,400 g (21.5 g/kg) in the adult (Fig. 10C), during which time the content of cholesterol increases from ~2,700 to ~32,200 mg (490 mg/kg) (114). Thus, the size of the pool of cholesterol in the brain per kilogram of body weight in the mouse varies greatly between 1 week old (290 mg/kg) and 3 week old

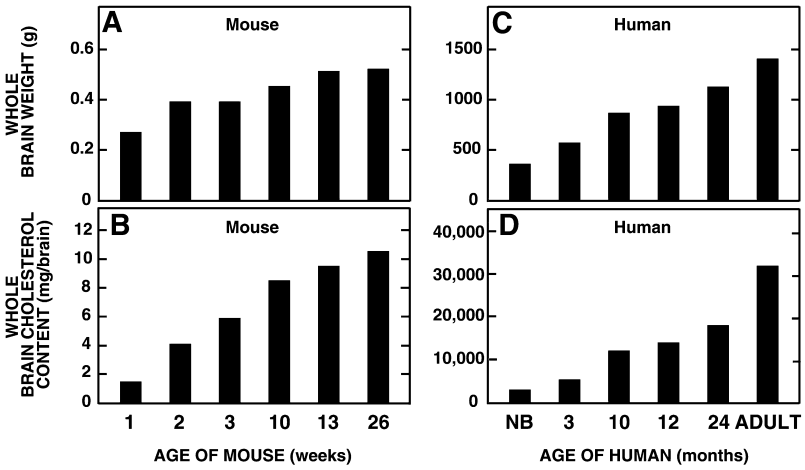

Fig. 10. The weight and cholesterol content of the whole brain at different ages in mouse and human. A and B show these values for mouse, and C and D illustrate these values in human. NB, newborn. These numbers were calculated from refs. (53, 114).

(670 mg/kg) pups but becomes constant in the adult animal at 330 mg/kg, so that in the 26 week old mouse this pool represents only 15% of the whole-body pool. In the human, however, this pool decreases slightly from 770 mg/kg in the newborn infant to 490 mg/kg in the adult, in which it occupies ~23% of the whole-body sterol pool.

In the adult CNS, this pool of unesterified cholesterol must be distributed among at least three different compartments. These include the cholesterol present in the membranes of myelin and the sterol present in the plasma membranes of neurons and glial cells. Although data are very limited, it is possible to approximate the amount of cholesterol present in each of these compartments. In the rat and mouse, it is reported that 70% and 80% of brain cholesterol, respectively, is in myelin (49, 106, 115). This would suggest that in the adult mouse the pool of cholesterol in myelin equals ~260 mg/kg, whereas the remaining 70 mg/kg presumably resides in the membranes of cellular elements. Approximately 10% of these cells are probably neurons and the remainder are different types of glial cells and vascular elements. If these figures are appropriate for the mouse, the pool of cholesterol in neurons must equal only ~7 mg/kg, whereas the remaining 63 mg/kg resides predominantly in the glial cell compartment (59). The metabolism and turnover of cholesterol in each of these three major cellular compartments is almost certainly very different.

CHOLESTEROL MOVEMENT INTO AND OUT OF THE CNS

With these pools of cholesterol identified in the CNS, it should be possible to quantify the pathways for sterol acquisition and excretion that account for the size of each of these pools and their rates of turnover. Furthermore, because these pools of unesterified cholesterol change rapidly at different ages of development (Figs. 8, 10), the magnitude of these acquisition and excretion pathways should also change markedly over the life of the animal. In theory, the cholesterol required for cellular proliferation and myelin synthesis might come from either the rapidly fluctuating levels of plasma lipoproteins (Fig. 5) or the synthesis in the different types of cells within the CNS.

Characterization of the blood-brain barrier

Any cholesterol that enters or leaves the CNS must do so by crossing the blood-brain barrier that, anatomically, is made up primarily of the unique endothelial cells found in the capillary network within the brain and spinal cord. The inner or basal membranes of these cells are also intimately associated with the foot processes of adjacent astrocytes (14). In general, in different regions of the body, capillaries have different permeability characteristics that are determined by whether or not they manifest fenestrations, tightly adherent junctional complexes, or transcellular vesicular movement (116). Capillaries of the brain apparently have essentially no fenestrae and show little bulk phase vesicular transport. Furthermore, adjacent endothelial cells are tightly adherent, so that there is also little or no paracellular molecular diffusion (117, 118). Thus, the endothelial cells of these capillaries form a very high resistance barrier where the movement of molecules, including cholesterol, between the plasma and the CNS must largely take place across the two parallel plasma membranes covering each surface of the capillary endothelial cell (14).

As with other plasma membranes, these membranes of the endothelial cells are freely permeable to water and, in addition, contain a number of different transport proteins, e.g., glucose and amino acid transporters and P-glycoprotein (ABCB1) (14, 119). In addition, these bilayers are permeable to a number of more hydrophobic molecules, including sterols, that diffuse across the blood-brain barrier down existing activity gradients (120). The maximal rate of such movement is dictated by the product

of the maximal activity that a particular molecule can achieve in the blood or extracellular fluid of the brain and its passive permeability coefficient (120–122). Importantly, when a sterol like cholesterol is hydroxylated, there is an increase in the maximal aqueous activity that can be achieved but a reduction in the passive permeability coefficient. However, the increase in solubility is proportionately greater than the reduction in the permeability coefficient, so that the net effect of hydroxylation is to greatly increase the maximal rate of passive diffusion of the molecule across the blood-brain barrier.

From these considerations, it is very unlikely that cholesterol carried in lipoproteins can reach the CNS either through fenestrations in the capillary membranes or through paracellular diffusion. However, three other pathways are at least theoretically available to promote net cholesterol movement in either direction across the blood-brain barrier. First, it is possible that the plasma membranes of the endothelial cells contain functional lipoprotein transporters such as the LDLR or SR-BI or transporters directed at unesterified cholesterol like ABCA1. Second, although vesicular transport appears to be minimal in the brain, it is still possible that a small amount of bulk-phase endocytic transcellular movement could take place in the endothelial cells. Third, it is also possible that unesterified cholesterol or, more likely, hydroxylated cholesterol could diffuse passively across the blood-brain barrier down existing activity gradients.

Cholesterol movement from the plasma into the CNS

There is now a variety of data from both in vitro and in vivo studies that can be used to identify which, if any, of these transport pathways function to promote net cholesterol uptake into the CNS. Certainly, the observation that the suckling animal routinely develops high levels of circulating lipoproteins (Fig. 5) during the time when major sterol accretion in the brain is taking place (Fig. 8) raises the possibility that lipoproteins might cross the blood-brain barrier. Consistent with this possibility, cells of the CNS are known to express the liver X receptor-β (LXRβ) (and, to a lesser degree, LXRα) nuclear receptor that can regulate transporters such as ABCA1 (16, 17, 123). Furthermore, the endothelial cells making up the blood-brain barrier express mRNAs for LDLR, SR-BI, and ABCA1 (15, 124). Under in vitro conditions, these proteins have been shown to promote the movement of cholesterol across these endothelial cells, suggesting that these transporters might be involved in either the net uptake of LDL-C into the CNS or the net excretion of sterol from the brain (15, 124).

Four other groups of studies, however, have examined this question of whether there is net cholesterol uptake into the CNS under in vivo conditions. First, the clearance of lipoprotein cholesterol into the brain has been measured in sheep, rabbit, and mouse using ^{125}I-labeled homologous LDL. In both the adult mouse and rabbit, net clearance of these particles into the CNS was undetectable (<0.5 μl/h/g) under circumstances in which the liver and adrenal gland in these same species took up LDL at rates varying from ~100 to ~1,000 μl/h/g (125, 126). Furthermore, this very low clearance rate of <0.5 μl/h/g was the same when the LDL particle was methylated to block interaction with the LDLR and in animals genetically lacking the LDLR (125, 126). Similar studies were also undertaken during the fetal and suckling periods in the sheep, as shown in **Fig. 11**. Between 90 days before birth and 18 days after birth, the concentration of cholesterol in all regions of the CNS increased dramatically, reaching ~7 mg/g in the frontal and parietal cortex and ~40 mg/g in the spinal cord. However, despite these high

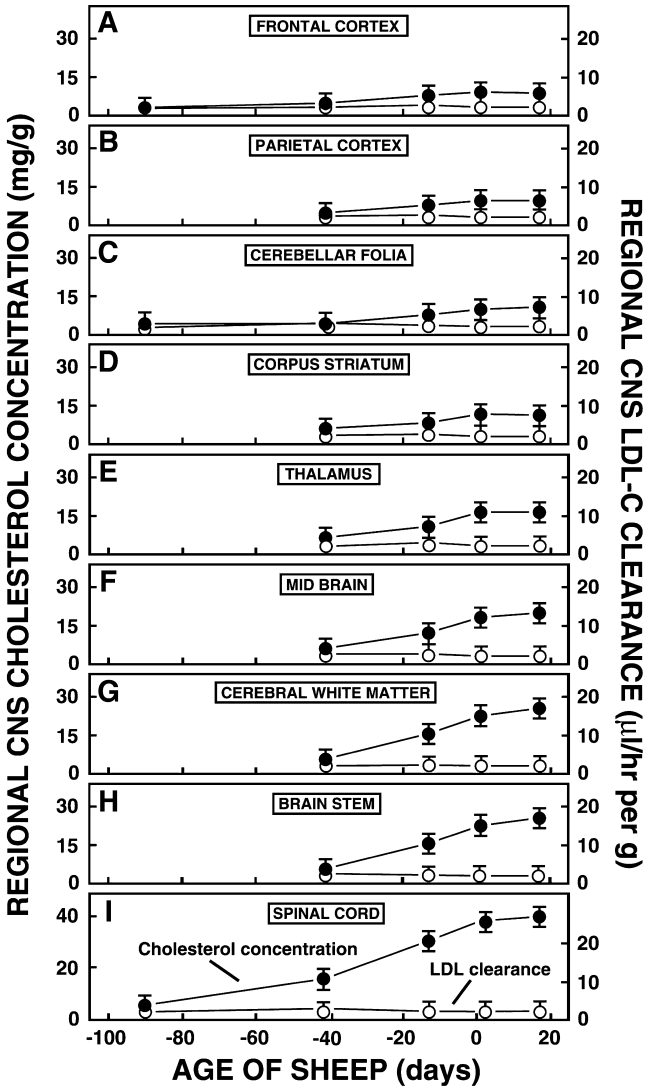

Fig. 11. TC concentration and cholesterol carried in LDL-cholesterol (LDL-C) uptake by different regions of the CNS in fetal and newborn sheep of different ages. The age range is between −90 days before birth (at day 0) to 18 days after birth. The closed circles show the concentration of cholesterol in each area at each age, and the open circles illustrate the rates of LDL-C uptake into these same regions. These latter rates are expressed as microliters of plasma cleared of LDL-C per hour per gram of tissue. In no case were these rates of LDL-C clearance into the CNS significantly different from 0. This figure has been modified and redrawn, with permission of the publisher, from ref. (113). Error bars represent ±1 SEM.

rates of sterol accretion in the brain and spinal cord, LDL clearance was still <0.5 μl/h/g, a value not different from 0. This was true in every region of the CNS and at times before the closure of the blood-brain barrier, even though there was active uptake of LDL in the liver and adrenal gland of these same animals (113). Identical results have been found in the mouse using HDL labeled with [^{14}C]cholesteryl ester. Finally, although mRNA for the LDLR is found in the brain, the level of expression does not change in the newborn mouse or rabbit when circulating hypercholesterolemia is present and maximal accretion rates of brain cholesterol are found (127, 128). Thus, these various observations provide no direct experimental evidence for the uptake of lipoprotein cholesterol across the blood-brain barrier into the CNS at any time during late fetal or postnatal development.

A second approach to this problem is to study the effect of deleting a particular transporter on the concentration and rate of synthesis of cholesterol in the target tissue. Thus, NPC1L1 and LDLR are responsible for net contributions of cholesterol to the liver and adrenal, respectively (Fig. 2). Deletion of NPC1L1 activity in the intestine or LDLR activity in the adrenal leads to a marked compensatory increase in the rate of synthesis in the target organs and, under some circumstances, a decrease in sterol concentration (36, 129). Using this same approach, the rate of synthesis and the concentration of cholesterol in the CNS have been measured in animals lacking these various, putative transporters. Loss of LDLR activity, for example, did not change the rate of synthesis or the concentration of cholesterol in the brain of the adult rabbit or mouse (126, 129). This was also true of the newborn rabbit, in which rapid cholesterol accretion was occurring in the CNS (129). In the mouse, deletion of the other two putative transporters, SR-BI and ABCA1, also did not cause a change in either the concentration of cholesterol or the rate of synthesis in the CNS (53). Thus, this second set of studies also provided no evidence that lipoproteins in the plasma were used by any transporters such as LDLR, SR-BI, or ABCA1 to make a net contribution of sterol to the CNS during the development of the young animal or in the adult.

A third set of experiments has explored the possibility that small amounts of bulk-phase endocytic transcellular movement of plasma lipoproteins take place across the endothelial cells of the blood-brain barrier. In mice fed oleic acid and cholesterol, the plasma TC was increased from 228 to 1,629 mg/dl. Even at a minimal bulk flow clearance rate of ~0.5 μl/h/g, this 7-fold increase in lipoprotein cholesterol concentration should have delivered a significant amount of cholesterol into the CNS. However, under these conditions, there also was no change in the concentration or rate of synthesis of cholesterol in any region of the CNS, including the cerebrum, cerebellum, midbrain, brain stem, and spinal cord (53). Thus, these studies again failed to demonstrate any net cholesterol movement from the plasma into the CNS.

Finally, there is a series of observations using either unlabeled or isotopically labeled sterols to examine the permeability characteristics of the blood-brain barrier. Unfortunately, such studies do not specifically measure net sterol transport into the brain because bidirectional molecular exchange could also result in the appearance of these respective sterols in the CNS. Nevertheless, the results of these experiments are useful. In one study, rats were treated with an inhibitor of cholesterol synthesis so that most organs, including the CNS, contained predominantly 7-dehydrocholesterol. When these animals were fed exogenous cholesterol, the content of this sterol in the plasma and liver promptly increased, but there was no change in the level of cholesterol in the brain (130). This result was similar to observations in both mouse and human with loss of function of the ABCG5/8 transporter that various sterols of plant origin accumulate in relatively high concentrations in the plasma and liver, but only trace quantities of these sterols could be detected in the CNS (45, 131). In another experiment, rat pups were fed artificial milk diets intragastrically between 5 and 16 days of age. When the cholesterol concentration in this artificial milk was increased 6-fold, the concentration of cholesterol in the liver also increased 6-fold, but it remained unchanged in the brain. Furthermore, the deuterated cholesterol present in the milk came to label ~70% of the sterols in the plasma and liver but virtually none of the cholesterol in the brain (132). In baboons administered [^{14}C]cholesterol intravenously, virtually all organs became heavily labeled after 70–85 days. The exception was the brain, which acquired virtually no radioactive cholesterol (133). In a similar experiment in humans, no radioactive sterol was found in the brain 6 days after the intravenous administration of [^{14}C]cholesterol. As in the baboon, this latter study indicated that the CNS was not part of the miscible pool of cholesterol that was present in nearly all other organs of the body (134).

Taken together, these many observations in different species represent a compelling body of evidence that there is no net, or even bidirectional, movement of cholesterol from plasma lipoproteins across the endothelial cells of the blood-brain barrier to the cells of the CNS. This seems to be the case in the mature animal, in which sterol turnover is very slow, as well as in the developing fetus and suckling newborn, in which the rates of sterol acquisition are greatest. These findings, therefore, raise the question of whether there can be a causal relationship between the concentration of plasma cholesterol and abnormalities such as depression, violent behavior, and dementia.

Cholesterol synthesis in the CNS

If there is no movement of lipoprotein cholesterol from the plasma into the CNS under circumstances in which massive expansion of the pools of sterol in the brain and spinal cord is taking place, then de novo synthesis must account for all cholesterol accretion and turnover in these tissues. However, these rates of synthesis are grossly underestimated when substrates such as [^{14}C]acetate, [^{14}C]octanoate, or [^{14}C]glucose are used to make the measurements either in vitro or in vivo. This large error comes

from the failure of these substrates to readily penetrate the blood-brain barrier and, equally important, from uncertainties about the specific activity of the precursor pool of [^{14}C]acetyl-CoA generated from these substrates and used for cholesterol biosynthesis (135, 136). One of the only valid measures of the absolute rate of sterol synthesis in vivo in the CNS, or in any other organ, comes from quantitating the rates of incorporation of either the ^{2}H or ^{3}H atom from [^{2}H]water or [^{3}H]water, respectively, into the cholesterol molecule. The conversion of these incorporation rates into rates expressing the absolute amount of cholesterol synthesized, in turn, requires knowledge of both the specific activity of cell water in the CNS, relative to the specific activity of water in the plasma, and the number of H atoms from water that are incorporated into each cholesterol molecule. Both of these critical values have been experimentally determined in several species (137–139).

Utilizing this technique, absolute rates of cholesterol synthesis have been measured in at least four species at different ages of development. In the mouse, rat, hamster, and sheep, for example, various regions of the CNS manifest higher rates of synthesis than are found in most other organs of these young animals during perinatal development (51, 53, 62, 115, 140). These rates, however, decrease dramatically as the size and level of myelination of the CNS reach constant values in the mature animal (51, 53, 115). Furthermore, as shown in **Fig. 12**, within the major regions of the CNS, the rate of sterol synthesis correlates closely with both the rate of cholesterol accretion and the ultimate concentration of cholesterol found in each of these regions. Thus, in the mouse, the highest rates of synthesis (Fig. 12A–E) seen in any region are found during the first 4 weeks after birth, when the highest rates of accumulation (Fig. 12F–J) are observed. Similarly, the highest rates of synthesis are also seen in those regions like the brain stem and spinal cord (Fig. 12D, E) that ultimately achieve the highest concentrations of sterol (Fig. 12I, J). Overall, careful quantitative measurements in both the rat and mouse indicate that the absolute rates of cholesterol synthesis during this early phase of brain development are essentially identical to the observed rates of cholesterol accumulation (51, 53, 115). In the mouse at 3 weeks of age, for example, these rates of synthesis and accumulation both equal ~0.27 mg/day (53).

Importantly, these rates of sterol synthesis in young animals also correlate closely with the rates of synthesis of myelin basic protein and cerebroside, two other important components of compact myelin (115). This finding im-

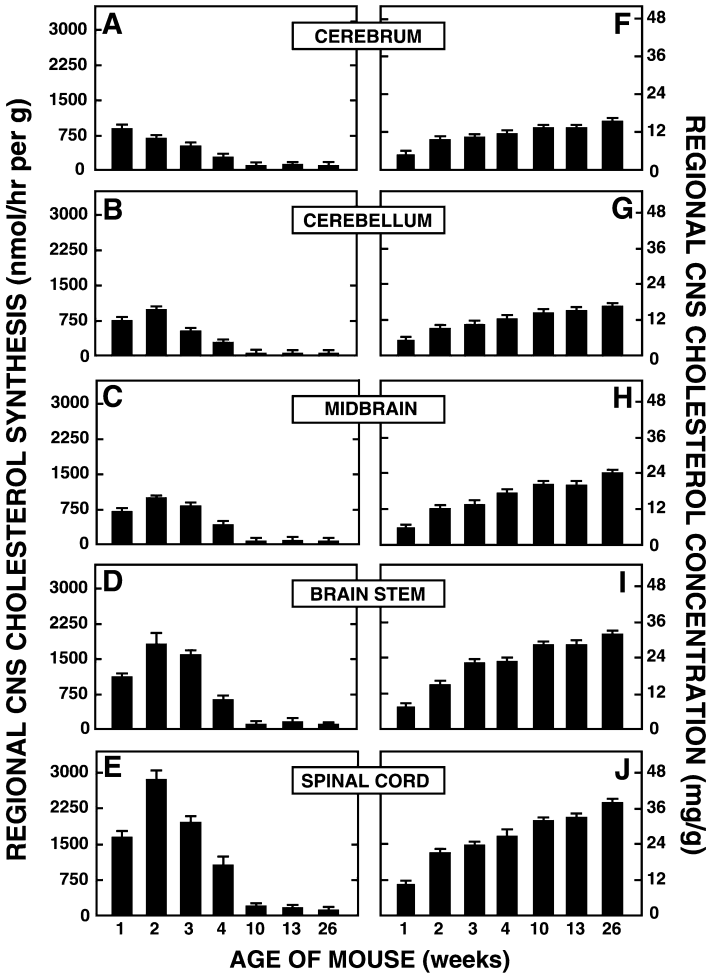

Fig. 12. Rate of cholesterol synthesis and concentration of sterol in different regions of the CNS of the mouse at different times ranging from 1 to 26 weeks of age. A–E show the rate of synthesis measured in vivo and expressed as nanomoles of [^{3}H]water incorporated into sterols per hour per gram of tissue. F–J show the concentration of cholesterol in each of these same regions. This figure has been modified and redrawn, with permission of the publisher, from ref. (53). Error bars represent ±1 SEM.

plies that most of this early sterol synthesis is occurring in oligodendrocytes and is largely associated with myelin production. As an aside, similar studies have been carried out in Schwann cells and in the regenerating sciatic nerve in the PNS. Again, it was found that essentially all cholesterol required for myelin formation in these cells came from endogenous synthesis and not from exogenous, lipoprotein cholesterol (141–143).

Thus, although there is no evidence that lipoprotein cholesterol is used by the CNS, these various reports establish that cholesterol is synthesized locally in all regions of the brain and spinal cord at rates that appear to equal exactly the observed rates of sterol accumulation in these same regions. Early during brain development, most of this synthesis probably occurs in oligodendrocytes and is associated with myelin production. Presumably, only a small fraction of this synthesis occurs in association with glial cell and neuron proliferation. As the CNS matures, however, myelin production decreases to very low levels and the pool of cholesterol in the CNS reaches a constant value. Under these circumstances, the rate of cholesterol synthesis declines to a much lower, but easily measurable, level that probably more closely reflects synthesis in the glial cell and neuron compartments (24). In the 26 week old mouse, for example, with a cholesterol pool of 330 mg/kg, synthesis declines 87% to only 0.035 mg/day. However, at this age, even this lower rate of synthesis is still 3-fold higher than the observed rate of sterol accretion (0.012 mg/day). Thus, as the animal approaches maturity, mechanisms must be activated to promote the net excretion of cholesterol from the CNS into the plasma (53, 59).

Cholesterol movement from the CNS to the plasma

The first hint of the nature of these excretory pathways came from early observations that the CNS contained enzymes capable of hydroxylating and further metabolizing cholesterol (144, 145). Such sterol hydroxylases are expressed in many organs, as shown for the mouse in **Fig. 13** [reviewed in refs. (146, 147)]. The first of these to be described was the enzyme cholesterol 7α-hydroxylase (CYP7A1) that initiates bile acid synthesis and is expressed only in the liver [reviewed in ref. (41)]. Later, a second enzyme capable of hydroxylating the sterol molecule in the 27 position (CYP27A1) was described and found to be expressed in a number of tissues outside of the liver (Fig. 13). This sterol 27-hydroxylase is thought to play a role in facilitating the movement of at least a portion of the cholesterol synthesized in various peripheral organs to the liver for excretion (146).

Although early studies suggested that there was sterol 27-hydroxylase activity in the brain, subsequent experiments did not support this conclusion, and in the mouse, there is little expression of the mRNA for this enzyme in the CNS (Fig. 13) (144, 148). Instead, it was demonstrated in humans that the major hydroxylated sterol excreted from the CNS was 24(*S*)-hydroxycholesterol (148). As shown in Fig. 13, the mRNA for the enzyme responsible for the synthesis of this oxysterol, cholesterol 24-hydroxylase

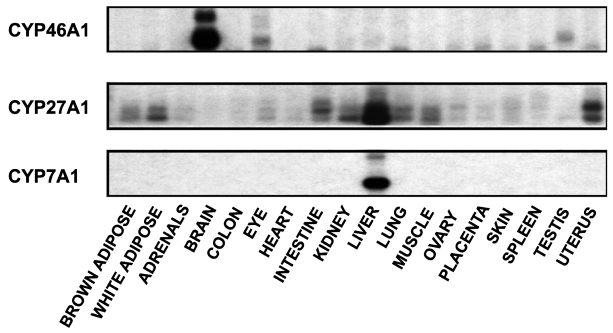

Fig. 13. Expression of the sterol hydroxylase mRNAs for CYP46A1, CYP27A1, and CYP7A1 in various tissues of the mouse. This figure has been modified and redrawn, with permission of the publisher, from ref. (59).

(CYP46A1), is expressed essentially only in the brain, and relatively high concentrations of the product of this reaction are also found in the CNS but not in most other tissues (148, 149). Furthermore, although both 24(*S*)- and 27-hydroxycholesterol are found in the cerebrospinal fluid of humans, the 24(*S*)-hydroxycholesterol apparently comes directly from cells of the CNS, whereas the 27-hydroxycholesterol diffuses in from the plasma and, ultimately, from its sites of synthesis in other extrahepatic organs (150). Both of these molecules eventually reach the liver, are converted to bile acids, and are excreted from the body (151). These latter findings illustrate the relative ease with which these hydroxylated sterol molecules cross the membranes of the blood-brain barrier.

The specificity of this 24-hydroxylation reaction in promoting net cholesterol movement out of the brain was further supported when knockout mice lacking CYP7A1, CYP27A1, and CYP46A1 became available (152–154). If one or more of these enzymes was essential for net cholesterol excretion from the CNS, then deletion of this (these) pathway(s) should result in the suppression of cholesterol synthesis and, possibly, the accumulation of sterol in the brain. In fact, in all three knockout mice, the concentration and pool of cholesterol in the CNS was unchanged compared with control animals, but synthesis was suppressed by ~35% in the *Cyp46a1*$^{-/-}$ mice but not in animals lacking 7α- or 27-hydroxylase activity (59). This suppression of synthesis was greatest in those areas of the brain with relatively more gray matter, like the cerebrum and cerebellum, and was much less in the heavily myelinated regions, like the brain stem and spinal cord (59). Furthermore, the concentration of 24(*S*)-hydroxycholesterol in the brain and plasma declined to very low levels in the *Cyp46a1*$^{-/-}$ animals (149, 153). Thus, the CNS of the mouse apparently responded appropriately to the loss of this excretory pathway by suppressing endogenous synthesis by an amount exactly equal to the mass of cholesterol that would normally be excreted as 24(*S*)-hydroxycholesterol. As an aside, there was no change in cholesterol synthesis in any other organ of the *Cyp46a1*$^{-/-}$ mouse, whereas there were significant changes in sterol balance in some of these tissues in the *Cyp7a1*$^{-/-}$ and *Cyp27a1*$^{-/-}$ animals (149, 155).

Clearly, these various studies established that the formation and excretion of 24(*S*)-hydroxycholesterol is a major pathway for removing cholesterol from the CNS. Three additional studies in mice, rats, and humans have quantified the magnitude and relative importance of this particular excretory pathway. In the rat exposed in vivo to ^{18}O, the rate of 24(*S*)-hydroxycholesterol formation in the brain was approximately half the rate of de novo cholesterol synthesis taking place at the same time (156). Furthermore, this ^{18}O-labeled 24(*S*)-hydroxycholesterol apparently passed across the blood-brain barrier into the plasma without further modification. Using the *Cyp46a1*$^{-/-}$ mouse to make similar measurements, the rate of 24(*S*)-hydroxycholesterol excretion from the CNS (0.9 mg/day/kg) was also found to be approximately half the rate of cholesterol synthesis (2.2 mg/day/kg) taking place in the brain of 7 week old animals (59). Because part of this newly synthesized cholesterol was still being sequestered within the CNS in mice of this age (0.8 mg/day/kg), net sterol excretion equaled 1.4 mg/day/kg. Thus, ~0.9 mg/day/kg (64%) of this sterol excretion occurred as 24(*S*)-hydroxycholesterol, whereas 0.5 mg/day/kg (36%) occurred as cholesterol or some other unidentified metabolite (59). It should be noted that this flux of sterol from the CNS to the liver (1.4 mg/day/kg) is very small compared with the rate of excretion of sterol taking place from all of the other extrahepatic tissues (~100 mg/day/kg) (43).

The net contribution of 24(*S*)-hydroxycholesterol by the CNS to the blood also has been measured in 12 humans, 20–35 years of age, and was found to equal 6.4 mg/day (0.091 mg/day/kg) (157). If, as in the mouse, this contribution represented ~64% of the total excretion of sterol from the brain, then in the human the net flux of cholesterol out of the CNS must equal ~0.14 mg/day/kg. Given that the total pool of cholesterol in the CNS of the human is 490 mg/kg, this rate of excretion would represent a turnover rate of 0.03% of the pool each day (compared with a whole-body turnover of 0.7% per day). Similarly, in the mouse, in which the pool of cholesterol in the CNS is 330 mg/kg and the rate of excretion is 1.4 mg/day/kg, ~0.4% of the pool is turned over each day (compared with a whole body turnover of 8% per day). Thus, in both the mouse and human, the overall rate of cholesterol turnover in the CNS is only ~1/20th the rate of turnover found in the whole animal or human.

However, there is evidence of considerable variation in these turnover rates in the different compartments within the CNS. For example, as shown in **Fig. 14** for the mouse, it was reported that the cholesterol 24-hydroxylase was not fully expressed until the animal reached ~4 weeks of age. This was at a time when cholesterol synthesis associated with myelin formation was rapidly declining and when the rate of sterol accretion was decreasing below the rate of synthesis (Fig. 12) (153). Furthermore, studies using in situ mRNA hybridization and immunohistochemistry revealed that CYP46A1 was expressed exclusively in neurons of the mouse brain, particularly in a subset of large, metabolically active neurons such as pyramidal cells of the cortex and Purkinje cells of the cerebellum (153). Thus, if the rate of 24(*S*)-hydroxycholesterol excretion from the CNS of the mouse (0.9 mg/day/kg) represents turnover of cholesterol in the plasma membranes in a small subset of metabolically active neurons, and if the pool of cholesterol in this subset of cells is ~4 mg/kg or less, then the rate of turnover of this pool of membrane cholesterol must exceed 20% per day. Such a high turnover rate would be consistent with the very high basal metabolic rates probably found in these same neurons. If, on the other hand, the remaining CYP46A1-independent component of sterol excretion (0.5 mg/day/kg) reflects primarily the turnover of cholesterol in the pool of sterol in the membranes of glial cells (63 mg/kg), then this turnover rate would equal ~0.8% per day, a rate that is much lower than that found in the large neurons but that is similar to turnover in some other cells of the body. However, it is uncertain to what extent turnover in the much larger pool of myelin cholesterol (260 mg/kg) might influence this calculation. In any event, although overall cholesterol turnover in the CNS of the mouse is low (0.4% per day), these data suggest that cholesterol metabolism is very different in the various compartments, with exceedingly high rates of turnover in some types of neurons, much lower rates of turnover in glial cells, and exceedingly low rates of turnover in myelin (49, 158).

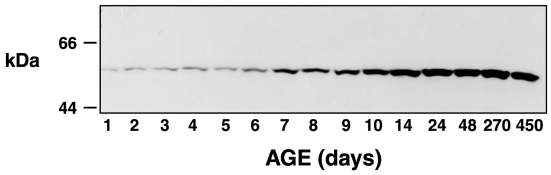

Fig. 14. Expression of CYP46A1 protein in the brain of mice of different ages. This figure has been modified and redrawn, with permission of the publisher, from ref. (153).

Thus, the facts that histochemically CYP46A1 is expressed in neurons, that both the mRNA and protein for this enzyme are found in greatest abundance in those areas of the CNS with the greatest volumes of gray matter, and that cholesterol synthesis in the *Cyp46a1*$^{-/-}$ mouse is suppressed to the greatest degree in areas like the cerebrum and cerebellum (but not the spinal cord) all strongly support the hypothesis that the excretion of 24(*S*)-hydroxycholesterol reflects cholesterol turnover in neurons and not in glial cells or myelin (59). The origin of the CYP46A1-independent component of sterol excretion from the CNS is less clear but may represent cholesterol metabolism and turnover in glial cells and, possibly, myelin.

Effect of neurodegeneration on cholesterol excretion from the CNS

Currently, there are few data on how neurodegeneration and demyelination affect cholesterol excretion from the CNS. In the mouse with a mutation in NPC1, there is cholesterol accumulation throughout the body, including neurons and glial cells in the CNS (29, 159, 160). These

animals begin to exhibit neurological symptoms at ~7 weeks and die with severe neurological deficits at ~11 weeks of age. The death of neurons in these animals is fairly selective, so that by 7 weeks of age nearly 90% of the Purkinje cells in the cerebellum have disappeared, although neurons in other regions are less severely affected (161, 162). There is also histological evidence of mild demyelination in the brains of these animals. Not surprisingly, net cholesterol excretion from the CNS of these mice is increased (2.3 vs. 1.4 mg/day/kg). This increase is attributable to a 3-fold increase in the CYP46A1-independent pathway (1.8 vs. 0.5 mg/day/kg), whereas the excretion of 24(*S*)-hydroxycholesterol is apparently reduced (0.5 vs. 0.9 mg/day/kg). Presumably, this decrease in CYP46A1 activity reflects the reduction in the mass of large metabolically active neurons in the brains of these animals, whereas the increased excretion of sterol through the CYP46A1-independent pathway comes from cellular activities designed to clear the CNS of cellular debris and fragmented myelin.

Apparently, no measurements have yet been made of net 24(*S*)-hydroxycholesterol excretion from the human brain in patients with neurological disease, as has been reported in young normal subjects (157). However, in one study, the concentration of this oxysterol was shown to be slightly reduced in various regions of brains from patients with Alzheimer's disease compared with control subjects (163). As in the *Npc1*$^{-/-}$ mouse, this finding may reflect the loss of neural cell mass and, hence, a reduced rate of 24(*S*)-hydroxycholesterol synthesis.

Other studies have attempted to use the steady-state concentration of 24(*S*)-hydroxycholesterol in the plasma as an indirect measure of the rate of formation of this oxysterol by the human CNS. However, in a careful analysis of this technique, it was shown that the plasma concentration of this sterol is profoundly affected by the relative sizes of the brain and liver at different ages and by the concentration of circulating lipoproteins (164). Thus, the plasma concentration of 24(*S*)-hydroxycholesterol varies 3-fold as the plasma TC concentration is varied 3-fold. Given these observations and the likely possibility that changes in the concentration or fractional catabolic rate of LDL or HDL may influence steady-state concentrations of this oxysterol, it is difficult to prove that the small changes in the plasma concentration of 24(*S*)-hydroxycholesterol reported in Alzheimer's disease or after administration of a statin actually reflect changes in the production rate of this sterol by the CNS (165–167). Clearly, measurements of absolute rates of 24(*S*)-hydroxycholesterol output from the human CNS need to be made in patients with different types of neurological diseases.

CHOLESTEROL MOVEMENT AMONG THE CELLS WITHIN THE CNS

Although these various measurements have provided at least preliminary estimates of the rates and mechanisms of net cholesterol movement into and out of the CNS, there are currently no quantitative measurements of the rate of sterol transport among the different cell compartments within the brain and spinal cord. The observations that many lipoprotein transporters, ABC transporters, and apolipoproteins are expressed in the CNS suggest that these proteins may be involved in this crucial movement of cholesterol between cells. Although quantitative data are lacking, studies in various isolated cell systems suggest several pathways that may be involved in this process, and these are outlined in **Fig. 15** for the normal mouse (A) and for the animal with one form of neurodegeneration, Niemann-Pick type C disease (B).

Although neurons can synthesize cholesterol, four lines of evidence suggest that the growth of these nerve cells and effective synapse formation depend upon an additional supply of sterol. First, in isolated sympathetic neurons from the PNS exposed to a statin, axon growth and regeneration could be supported with lipoprotein cholesterol (168, 169). Second, in isolated retinal ganglion cells from the CNS, axon extension was stimulated using media derived from glial cells that contained apoE and cholesterol. Furthermore, this growth stimulation was abrogated by receptor-associated protein, an inhibitor of sterol uptake by LDLR-related protein (LRP) (170). Third, in animals with a mutation in NPC1, cholesterol accumulated in most neurons, suggesting that these cells normally took up large amounts of apolipoprotein-associated cholesterol through the clathrin-coated pit pathway (159, 160). Fourth, retinal ganglion cells in vitro were shown to form synapses very poorly unless coincubated with glial cells. The stimulatory factor produced by these glial cells was found to be cholesterol coupled to apoE (171, 172).

Thus, as shown in Fig. 15A, within the mature CNS cholesterol is probably synthesized in both neurons and glial cells. Apparently, active axon growth, as well as synapse formation and remodeling, require additional sterol that cannot be supplied by the distant cell body of the nerve cell. To meet this need, astrocytes synthesize and secrete apoE-associated cholesterol that is taken up by the axons using one of the LDLR family of receptors such as LRP. This process apparently is required to preserve the high concentration of cholesterol in the distal axons needed for the maintenance of synapse integrity (173). This same group of metabolically active neurons may also be the site for the rapid turnover of sterol through the formation and excretion of 24(*S*)-hydroxycholesterol. If this is the case, then the rate of excretion of this oxysterol (0.9 mg/day/kg) must equal the sum of the rates of cholesterol synthesis in both the neurons and a portion of the astrocyte population. As also shown in Fig. 15A, a second pathway must exist that promotes the normal turnover of cholesterol in the membranes of other glial cells and in myelin. Presumably, this low rate of turnover is also facilitated by apoE. However, there is little specific information on how the cholesterol molecule moves into and out of mature myelin unless the channels of persistent cytosol are involved (Fig. 6). In any event, it is not possible to identify the magnitude of the respective contributions of cholesterol from astrocytes, microglia, and oligodendro-

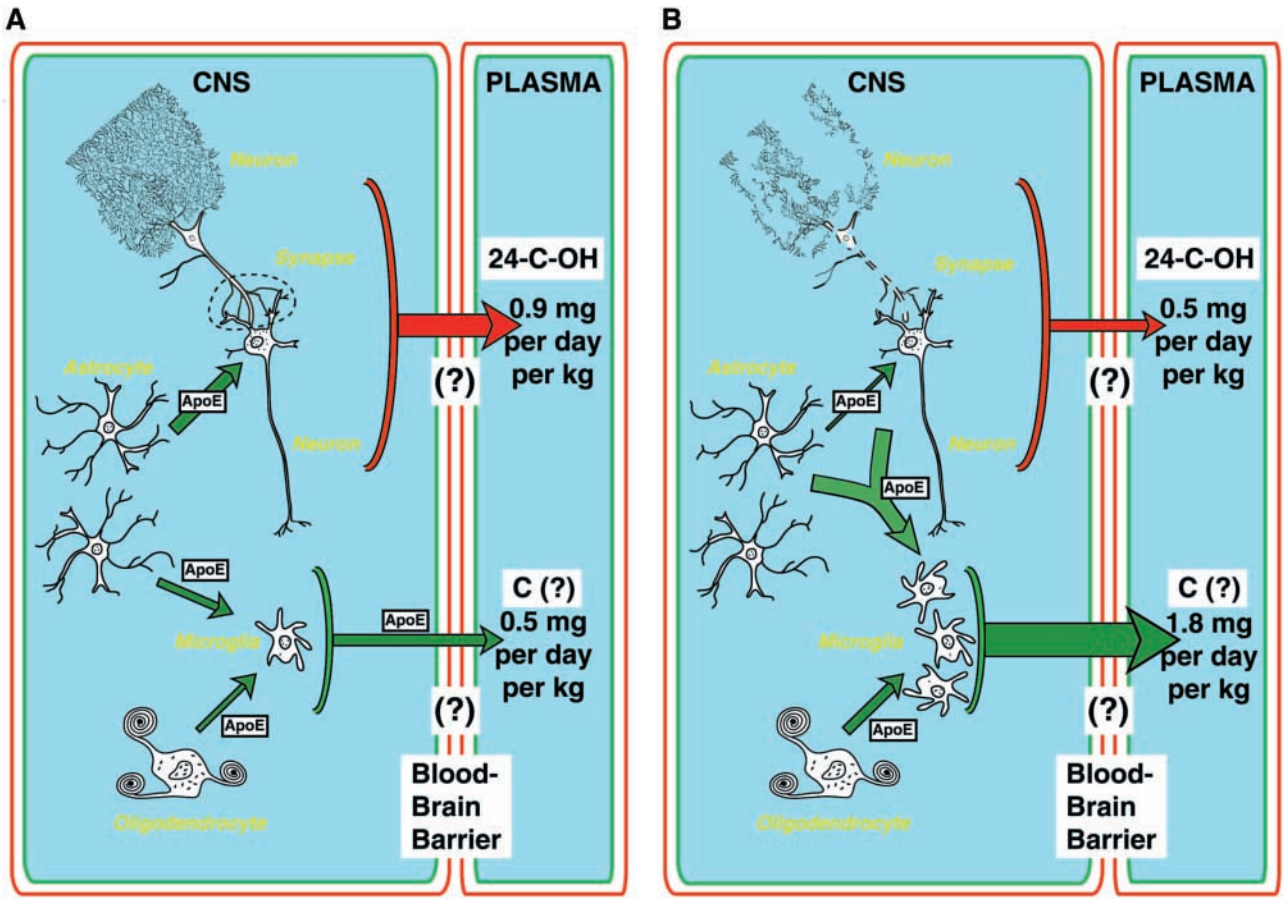

Fig. 15. Movement of cholesterol between different cellular compartments of the CNS and across the blood-brain barrier into the plasma. The numbers show the rates of movement of sterol out of the CNS as either 24-hydroxycholesterol (red) or cholesterol or some other metabolite (green) in the mouse. The pools of unesterified cholesterol vary in the compartments of neurons (7 mg/kg), glial cells (63 mg/kg), and myelin (260 mg/kg). A illustrates these values in the normal mouse, and B gives these values in mice with one form of neurodegeneration (i.e., Niemann-Pick type C disease). The question marks indicate that the mechanisms of sterol movement across the blood-brain barrier are poorly understood.

cytes to the net output of 0.5 mg/day/kg seen in this CYP46A1-independent pathway. Currently, there also are no data on what specific transport mechanism may be involved in the movement of this sterol from the CNS across the blood-brain barrier into the plasma.

Figure 15B illustrates the changes that occur in these pathways during one form of neurodegeneration, Niemann-Pick type C disease. In the 7 week old mouse with this syndrome, there is the loss of many large neurons. Presumably, the decrease in the output of 24(*S*)-hydroxycholesterol reflects this reduction in neural cell mass and the reduced rate of cholesterol turnover through the astrocyte/neuron pathway. However, overall, there must be an increased loss of cholesterol from the CNS in this syndrome because of ongoing neurodegeneration and demyelination. Presumably, both microglia and apoE are involved in this process of enhanced cholesterol excretion. Microglia, unlike macroglial cells, are derived from bone marrow monocyte precursors (174, 175). During the development of the CNS, these microglial cells have been shown to engulf and destroy Purkinje cells undergoing programmed cell death (176). It is reasonable to assume, therefore, that these cells play a similar role during neurodegeneration by engulfing these same large neurons that are spontaneously dying because of the mutation in NPC1 and that are expressing members of the caspase family. The brains of these mice also exhibit increased mRNA levels for apoE but not for other proteins such as ABCA1, LDLR, SR-BI, sterol-regulatory element binding protein-1c (SREBP-1c), or SREBP-2 (59). Additional support for the role of apoE in this process comes from the observation that clearance of the products of degenerating neurons from the injured brain is retarded in the *Apoe*$^{-/-}$ mouse (177). Thus, as summarized in Fig. 15B, in the face of active neuron death and demyelination there is a reduced flow of cholesterol out of the CNS through the 24(*S*)-hydroxycholesterol pathway. In contrast, however, there is a much increased load of sterol coming from the plasma membranes of dying neurons and degenerating myelin that must be removed. Apparently, increased synthesis of apoE and activation of microglia play a major role in this enhanced excretory process.

Importantly, there is virtually no information on the possible roles of other cholesterol transporters in facilitating sterol movement out of the CNS. mRNA levels can be detected in the brain for a variety of proteins, including LXRα, LXRβ, ABCA1, ABCG1, the SREBPs, and many others (16, 17). Furthermore, many of these mRNA levels can be significantly altered either by deleting LXR expression or by administering the experimental animal an agonist of the LXR nuclear receptors (16, 17, 178–180). However, it is not clear how, or if, these changes in mRNA levels reflect changes in any of the specific sterol fluxes outlined in Fig. 15. For example, would administration of an LXR agonist enhance the flow of cholesterol through either the CYP46A1-dependent or -independent pathway? Such measurements have not yet been made. Would the accumulation of excess cholesterol in the brain caused by neurodegeneration and demyelination lead to increased sterol excretion through the activation of LXR target genes? Unfortunately, in the only published study of this type only the mRNA level of apoE was increased (59). Clearly, further investigations are required to establish which, if any, of the many potential cholesterol transporters is (are) involved in the net movement of sterol out of the CNS and whether altering any of these pathways might affect the biochemistry or function of the brain.

CONCLUSIONS

Although quantitative data from experimental animals and humans remain very limited, sufficient information is now available to describe the broad outlines of cholesterol metabolism in the CNS. During perinatal development, when the plasma cholesterol level is fluctuating widely, there are very high rates of synthesis in all regions of the brain and spinal cord that primarily represent the construction of vast sheets of plasma membranes and compact myelin by oligodendrocytes. As the CNS matures and the pool of cholesterol in the brain becomes constant, the rate of synthesis markedly declines and more closely reflects normal sterol turnover in the plasma membranes of neurons and glial cells and in myelin. At this time, mechanisms for sterol excretion from the CNS are activated. These mechanisms include the 24-hydroxylation of cholesterol by a subset of neurons with very high metabolic rates and another, poorly defined excretory pathway that may predominantly reflect the turnover of cholesterol in the membranes of glial cells and myelin. Movement through each of these pathways likely involves apoE, but it is not clear at this time if any other sterol transporters, such as ABCA1, are rate limiting to these excretory pathways.

This scenario, if correct, suggests two important clinical conclusions. First, there is no evidence that the concentration of cholesterol in the plasma directly influences cholesterol metabolism in the CNS. This is fortunate because currently in excess of 15 million individuals in the United States are being treated with pharmaceutical agents to decrease their LDL-C levels below 100 mg/dl to reduce the incidence of coronary artery disease (181–185). Second, despite this conclusion, the possibility that pharmaceutical agents such as HMG-CoA reductase inhibitors could decrease the incidence of dementia remains viable. When given orally, all currently available statins partially inhibit cholesterol synthesis in the CNS. If, for example, this inhibition slowed cholesterol movement through the astrocyte/neuron/24(*S*)-hydroxycholesterol pathway, it could affect the rate of processing of amyloid precursor protein (186, 187). Whether such inhibition does affect the processing of such proteins in vivo or some other aspect of neuron function remains to be proven.

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ERRATA

In the article "Substrate reduction reduces gangliosides in postnatal cerebrum-brainstem and cerebellum in GM1 gangliosidosis mice" by Kasperzyk et al., published in the April 2004 issue of the *Journal of Lipid Research* (Volume **46**, pages 744–751), the affiliations should read as follows:

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In the article "Prediction of PPAR-α ligand-mediated physiological changes using gene expression profiles" by Frederiksen et al., published in the March 2004 issue of the *Journal of Lipid Research* (Volume **45**, pages 592–601), the digital object identifier (DOI) should read: DOI 10.1194/jlr.M300239-JLR200.

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