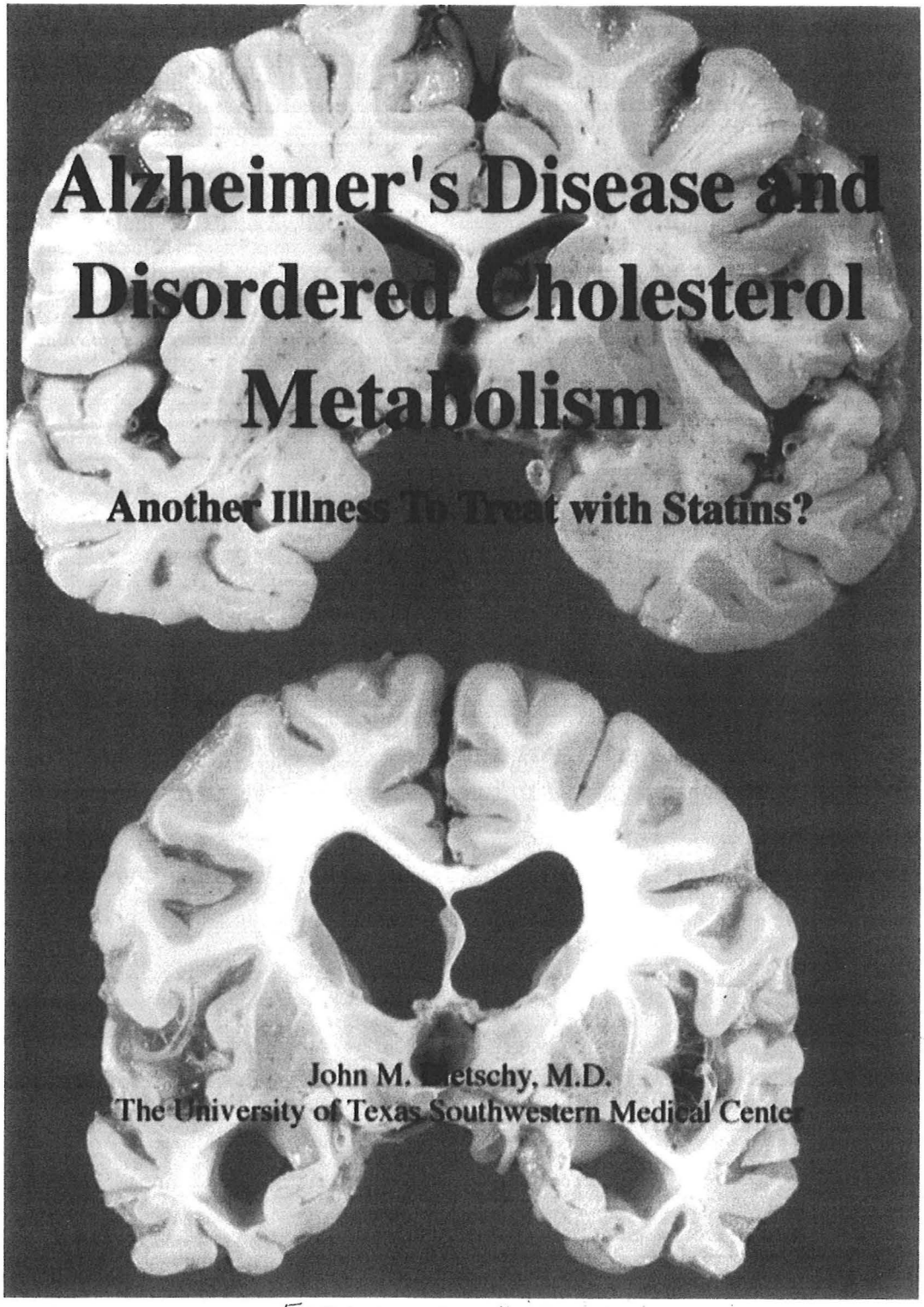
Alzheimer's Disease and Disordered Cholesterol Metabolism
Another Illness To Treat with Statins?
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Introduction

Alzheimer's disease is a common, neurodegenerative disorder that is characterized by progressive loss of higher intellectual functions. There is seldom, if ever, focal neurological defects detectable. In general, there are two clinical syndromes associated with this disorder. Uncommonly, the disease may begin before the age of 60. Such early onset Alzheimer's is commonly associated with mutations that affect the rate of amyloid-β production. The much more common syndrome, late onset Alzheimer's disease, typically begins after the age of 60 and is not associated with an identifiable mutation that affects the rate of amyloid-β production, although a minority of these patients do carry an allele for apolipoprotein E 4 (apoE 4). Over the past five years, there has been considerable progress in understanding the formation of amyloid-β from its precursor, amyloid precursor protein (APP), and the roles of three different proteases in this process. Many investigators currently believe that the Alzheimer's syndrome is caused by a defect in the processing of amyloid precursor protein brought about by either mutations or by some unrecognized environmental factor. This defect leads to the precipitation of amyloid-β, along with apo E, in synapses in specific regions of the brain. Ultimately this disorder is manifested by the development of neuritic plaques and intracellular neurofibrillary tangles that are the pathological hallmarks of this disease. Patients affected with this disease live many years and often require custodial care that makes this syndrome the third most expensive medical disorder in the United States.

While it is clear that mutations in the amyloid precursor protein or in presenilin 1 and 2 can lead to overproduction of amyloid-β and the development of early onset Alzheimer's disease, there have also been a series of reports in the older literature suggesting that there is a relationship between the plasma cholesterol concentration and the development of Alzheimer's disease. Feeding rabbits a high cholesterol diet, for example, is associated with an increase in plaque formation. In other studies, there is an association between a high plasma cholesterol concentration and an increased incidence of Alzheimer's disease. For the most part, these observations have been ignored since there has been no experimental connection made between circulating plasma cholesterol levels and the neuropathology of Alzheimer's disease. However, a recent abstract has been presented that suggests that lowering the plasma cholesterol level by the administration of HMG CoA reductase inhibitors, i.e., statins, markedly lowers the incidence of Alzheimer's disease (Fig. 1). In a retrospective analysis of approximately 50,000 patients in three Veterans Adminstration hospitals, Wolozin and his colleagues reported that "the rate of Alzheimer's disease for patients taking lovastatin or pravastatin is reduced by 60-73% compared to a total patient population or compared to patients taking other medications typically used in the treatment of cardiovascular disease or

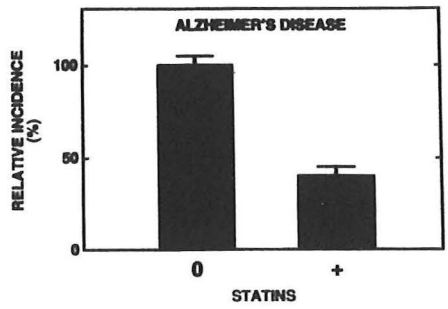

Fig. 1

hypertension." These observations, combined with a series of new experimental results reported during the last year, raise the possibility that there is, in fact, a connection between the circulating plasma cholesterol concentration and the rate of development of Alzheimer's disease. This review explores some of these new observations and outlines the potential mechanisms whereby changes in the plasma cholesterol level could be translated into changes in the rate of neuritic plaque formation within the brain.

Function of Cholesterol in the Body

Cholesterol is often discussed with respect to its detrimental effects on the endothelial lining of major arteries throughout the body. There is now little doubt that high circulating levels of cholesterol in the plasma are associated with an increased incidence of atherosclerosis. However, the major function of this molecule in the body is as a component of the plasma membrane of all cells. There is virtually no cholesterol in the membrane of the nucleus or in the membranes that make up the endoplasmic reticulum. There are, however, significant amounts of cholesterol in the golgi apparatus, but most cholesterol in the whole cell is found in the plasma membrane. This plasma membrane in a typical cell consists of cholesterol, sphingomyelin, phosphatidylcholine and lesser amounts of glycolipids. These various surface-active lipids make up the structure of the plasma membrane and provide the cell with the means to limit the movement of molecules into and out of the cell and to maintain an electrical gradient between the inside and outside of the cell. In addition, cholesterol and sphingomyelin form the structural basis for microdomains within this membrane, e.g., caveolae, that contain many nutrient receptors that are essential for the viability of the cell. Cells cannot function normally unless they have a carefully balanced, continuous supply of cholesterol to replace the sterol that is being turned-over in the plasma membrane. For example, in the Smith-Lemli-Opitz syndrome, children inherit an enzyme defect such that they cannot catalyze

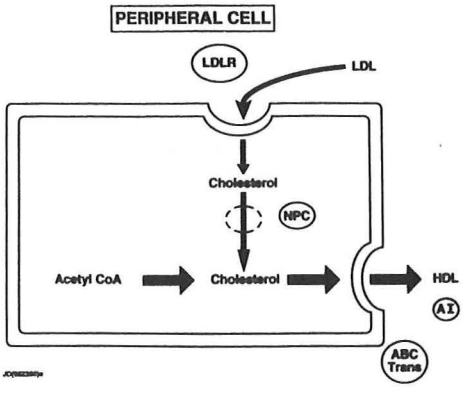

Fig. 2

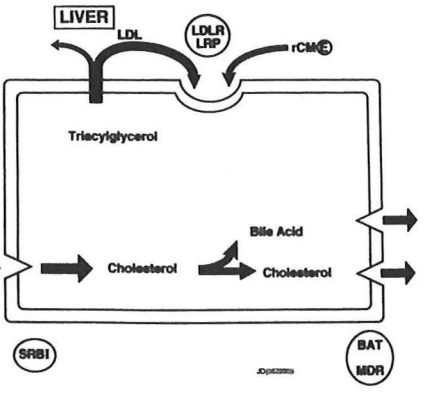

Fig. 3

the final biosynthetic step in the formation of cholesterol, i.e., the conversion of 7-dehydrocholesterol to cholesterol. As a result, cell membranes in these children have reduced amounts of cholesterol and there is accumulation of the precursor, 7-dehydrocholesterol. These children have multiple congenital anomalies, including cleft palate, congenital heart disease, genitourinary abnormalities and marked mental retardation. This syndrome vividly illustrates the critical role of cholesterol in supporting the normal function of all cells in the body including, in particular, those in the central nervous system. Because of this critical function, newly synthesized cholesterol must be constantly supplied to the cell membranes while the sterol that is replaced must be continuously excreted from the body so as not to accumulate in any particular compartment.

Flow of Cholesterol through the Body

Quantitatively, the greatest flow of cholesterol involves the movement of newly synthesized sterol from the endoplasmic reticulum to the plasma membrane of every cell in the body, the secretion of this cholesterol into the plasma, the uptake of this cholesterol by the liver and, ultimately, the excretion of this sterol into the gastrointestinal tract (Figs. 2, 3). Every tissue in the body, including the central nervous system, continuously synthesizes cholesterol from acetyl CoA . This newly synthesized sterol moves from the endoplasmic reticulum to the plasma membrane where it is inserted into structures such as caveolae and the bulk phase of the membrane. Cholesterol is also continuously shed from the plasma membrane into the pericellular fluid at a rate that is equal to the rate of *de novo* synthesis. This "shedding" process is apparently facilitated by the newly described ATB-binding cassette transporter 1 (ABC1) transporter. In the pericellular fluid, this unesterified cholesterol becomes associated with apo AI and is eventually esterified to cholesteryl ester within the structure of the HDL particle. After circulating through the plasma to the liver, this cholesteryl ester is taken up into the hepatocyte by a second transporter

protein, scavenger receptor class B type 1 (SR-BI). Ultimately, this sterol is secreted across the canalicular membrane into the bile and excreted in the feces. Before excretion, about half of this sterol is converted to bile acids which are secreted into the bile under the influence of a bile acid transporter, BAT. The unmetabolized, remaining cholesterol is secreted into the bile by a process that indirectly utilizes the multiple drug resistance (MDR) transporter. These bile acids and unesterified cholesterol ultimately appear in the feces as acidic and neutral steroids. In the adult human, the amount of cholesterol that passes through this pathway daily is approximately 1000 mg.

There is a second pathway, the function of which is primarily to move triacylglycerol, but which is also capable of facilitating the uptake of small amounts of cholesterol into the body from the diet. On the typical American diet, approximately 100-200 mg of dietary sterol is absorbed each day. This cholesterol, along with nearly 100 g of dietary triacylglycerol is incorporated into the chylomicron particle that contains both apoE and apoB 48. After delivery of most of the triacylglycerol to extrahepatic organs, the remnant of the chylomicron particle is taken up into the liver cell using primarily the LDL receptor (LDLR). The liver contains a second lipoprotein receptor, LDLR related protein (LRP), that can also clear the chylomicron remnant. Any triacylglycerol that accumulates in the liver and small amounts of cholesterol are incorporated into the VLDL particle that is synthesized in the hepatocyte. After secretion into the plasma, most of the triacylglycerol in this particle is also cleared into extrahepatic organs leaving behind a small remnant particle, LDL, that contains cholesteryl ester and apoB 100. About 80% of this lipoprotein fraction is cleared directly back into the liver while the remaining 20% is distributed to all of the extrahepatic organs in the body. This LDL particle can be transported into the liver utilizing the LDLR, but not LRP. This situation arises because the two hepatic receptors have different binding characteristics. The LDLR can bind lipoprotein fractions that contain either apoE or apoB 100. In contrast, LRP binds only apoE. Thus, if a individual lacks LDLR, the chylomicron remnants will be cleared normally (using LRP) while LDL will accumulate in the plasma. The amount of cholesterol that is contributed to the body pool of plasma membrane sterol from the diet is small (100-200 mg) compared to the amount of cholesterol that is synthesized in each organ (1000 mg).

There are three features of this system that should be emphasized and that may be important for cholesterol metabolism in the central nervous system and the development of Alzheimer's disease. First, while LDLR and LRP are important for the metabolism of cholesterol in the liver and other organs, the brain contains not only these two lipoprotein receptors, but at least four other members of the LDL receptor family including a VLDL receptor, an apoE 2 receptor, and gp 330. Second, while the liver is the major source for apoE synthesis in the body, the brain is the second most important source for the synthesis of this apolipoprotein. Third, the apoE

present in human populations is found in three different isoforms that result from specific amino acid substitutions in the protein chain. These include individuals who carry the apoE 3 gene (70-80% of the population), those who carry the apoE 4 gene (10-15% of the population), and those who carry the apoE 2 gene (5-10% of the population). ApoE 2 binds less tightly to the LDLR so that LDL can compete more favorably for uptake into the liver. In contrast, chylomicrons carrying apoE 4 bind more tightly to the LDLR and so more vigorously compete with LDL for uptake into the liver. As a consequence of these differences in binding characteristics, individuals who carry an apoE 4 allele usually have a somewhat higher concentration of LDL in the plasma while those who carry an apoE 2 allele have a lower concentration. ApoE 4, therefore, is considered a risk factor for the development of atherosclerosis and, as will be outlined shortly, is a significant risk factor for the development of Alzheimer's disease. These differences in the binding characteristics of the isoforms of apoE may be critically important to understanding the pathogenesis of neuritic plaque formation and the destruction of synaptic function.

Was There Evolutionary Pressure To Control the Plasma Cholesterol Concentration and the Type of ApoE in Human Populations?

Current data suggest that there was no evolutionary selection to maintain plasma cholesterol levels at very low concentrations. This was almost certainly true because over the last 500,000 years of human evolution, the average life expectancy was of the order of 30-40 years. Only very recently, in the early 20th century, did life expectancy in the United States and Europe begin to increase beyond 50 years. During this same period of time, cooking oils became very abundant and cheap, and triacylglycerol came to account for 25-50% of the caloric intake in Western countries. Intake of this large amount of triacylglycerol, coupled with small amounts of dietary cholesterol, have resulted in a progressive increase in the plasma total cholesterol concentration which, in turn, has been associated with a near epidemic of coronary artery disease during the mid-part of the 20th century. In contrast to most of the rest of the world, where the average total cholesterol concentration is of the order of 150 mg/dl, in the United States this average value approaches 230-250 mg/dl. It should be emphasized that this increase in concentration is a normal response to the intake of the high triacylglycerol diets typical of the American population. As a consequence of these very high levels of circulating chylomicrons, VLDL and LDL particles, virtually all Americans have significant atherosclerosis. New data to be published in the summer, obtained by using intra-arterial ultrasound, will show that 85% of the American population over the age of 50 has significant coronary artery atheromas (Fig. 4). This is true even in individuals who have a normal coronary artery arteriogram since these atheroma do not impinge on the arterial lumen until late in their

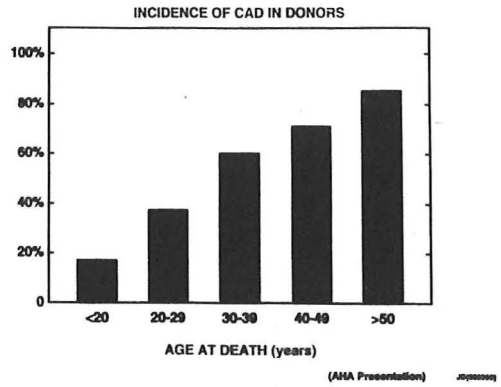

Fig. 4

development. Thus, as a consequence of the high fat intake in the American population, the majority of Americans have very high plasma cholesterol levels, a very high incidence of coronary artery atherosclerosis, and a very high incidence of acute myocardial infarction. Five major studies over the last 10 years have unequivocally demonstrated that reduction in these plasma cholesterol levels by treatment with statins dramatically lowers the incidence of these coronary events. Thus, the failure of evolutionary pressure to control the plasma cholesterol level coupled with the massive increase in triacylglycerol intake in modern humans has led to significant hypercholesterolemia in virtually all Americans. This has brought about a major epidemic of coronary artery disease and, possibly, may play a role in neurodegeneration such as seen in Alzheimer's disease.

While there seems to have been little evolutionary pressure to suppress plasma cholesterol levels, there may have been significant evolutionary selection in terms of the apoE phenotype seen in different populations. While the apoE 3 phenotype is most common, data now suggests that the ancestral allele was apoE 4. This allele is commonly found in higher primates and in some aboriginal human populations in Papua New Guinea. However, during evolution of other major ethnic groups, the apoE 4 allele apparently was progressively replaced with the apoE 3 allele in Africans (62%), Finns (70%) and other European groups (76-81%). Many Asian ethnic groups have still higher incidences of the apoE 3 allele (83-86%). If this shift from apoE 4 to apoE 3 came as a result of evolutionary pressure, it seems likely that this pressure did not arise from an attempt to regulate the plasma cholesterol level, but, rather, from an attempt to protect the brain from trauma. Individuals who carry the apoE 4 allele have a much poorer prognosis for recovery after closed-head injuries than do individuals who carry the apoE 3 allele since apoE 3 may play a central role in cholesterol metabolism and amyloid-β protein turnover in the brain.

Cholesterol Metabolism in the Central Nervous System

Compared to the other organs in the body, there has been relatively little research done on the characteristics of cholesterol movement across the central nervous system or among the various types of cells within the brain and spinal cord. The brain is different from all other organs in the body in that it has two different pools of unesterified cholesterol. In most organs, as outlined above, the unesterified cholesterol is primarily found in the plasma membrane of the various cell types. In such organs, the concentration of cholesterol is relatively low and usually in the range of 2-5 mg/g wet weight of tissue. These concentrations are much higher, however, in various regions of the central nervous system. In areas of the brain in which there is a great deal of gray matter, such as the cerebrum and cerebellum, cholesterol concentrations typically are in the range of 8-12 mg/g. In more highly myelinated regions such as the mid brain and spinal cord, these concentrations can reach nearly 40 mg/g. These differences exist, of course, because unesterified cholesterol not only makes up a major portion of the plasma membrane of nerve cells and glial cells, but, in addition, cholesterol is a major component of myelin.

Myelination is a relatively recent evolutionary development. Myelin is not found in any invertebrate or jawless vertebrate. For example, modern day hagfish and lampreys do not have myelination within their central nervous system. Myelin first appeared in animals with an articulated jaw. Apparently, the evolution of effective predators critically depended upon a more efficient central nervous system that could rapidly integrate sensory input with effective output of neural signals to muscle groups. The production of myelin is simply a specialized use of a plasma membrane-like structure to insulate the axon of the nerve cell. The specialized oligodendrocyte synthesizes redundant plasma membrane which is wrapped round and round the axon. After condensation of the cytosol, this plasma membrane forms a multi-layered, insulating coat that increases the rate of impulse conduction down the axon and reduces the energy expenditure necessary to maintain this signal. The composition of the plasma membrane making up myelin is similar to the composition of plasma membranes in other organs. There are some important differences, however. In myelin there tends to be relatively less sphingomyelin and a greater amount of glycolipids (such as galactocerebroside, various gangliosides, etc.) so that the membrane is decorated with various carbohydrate moieties. Currently, the function of these complex gangliosides is uncertain. Selective knockout of the biochemical pathways producing these various gangliosides does not result in a significant phenotype.

It is clear that the evolution of myelination occurred at a time when the development of larger predators demanded a central nervous system that could rapidly respond to sensory input signals. However, the need to myelinate a larger and larger central nervous system created two major

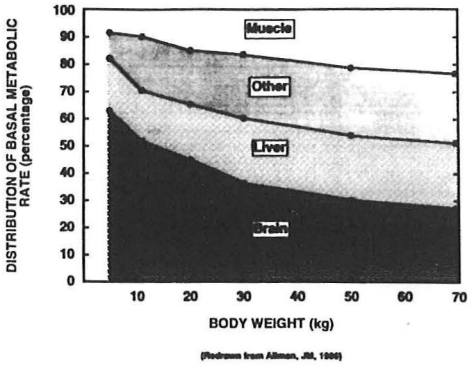

Fig. 5

problems. The first was that myelination disproportionately increased the size of the brain. As more and more neurons were added to the brain, and the volume of the neocortical gray matter increased from 1 ml to 1000 ml, the volume of the neocortical white matter increased by a factor of 4/3. Potentially, this disproportionate increase in volume with the development of increasing brain complexity could have limited overall brain size. This problem apparently was solved in two ways. In ungulate animals, myelination of the brain occurs largely before birth so that the newborn is very active and can flee predators within minutes after birth. In contrast, animals, including primates and humans, which can be nurtured for months or years largely myelinate the brain after birth. This provides the opportunity for greater brain growth and complexity until, at least, mature brain size is reached and the bones of the skull fuse.

A second problem, peculiar to very large brains, is the enormous amount of energy expenditure necessary to maintain electrical activity. This problem was solved, in part, by myelination which significantly reduces the amount of energy needed to maintain nerve conduction. However, the marked increase in brain size that occurred between the higher primates and humans required additional adaptations. Compared to the great apes, humans have a brain that is three times larger, but a gastrointestinal tract that is only half the size. It has recently been postulated that as homo sapiens evolved, the bigger brain allowed early humans to more effectively forage and seek food supplies that were more concentrated with respect to triacylglycerol and protein. This, in turn, allowed for the evolution of a smaller, and more energy efficient, gastrointestinal tract. As this evolution continued, and humans became effective predators of other animals, sufficient energy input could be obtained to allow continued brain development. Even in modern humans, however, two thirds of the basal energy expenditure in the newborn is devoted to supporting the brain (Fig. 5). Even in older humans, fully one third of the energy expended in the basal state goes to support the brain. This adaptation, however, has come at a price. Modern humans, with loss of nearly half the size of their

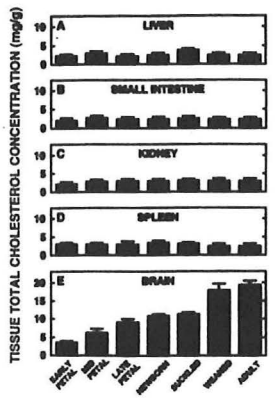

Fig. 6

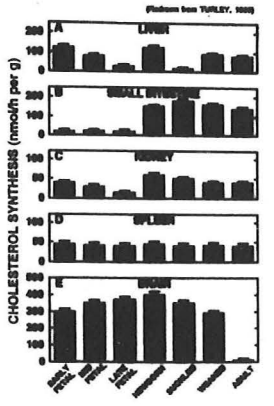

Fig. 7

gastrointestinal tracts, can no longer survive on a diet of leaves and other plant parts typical of the diets of the great apes such as gorillas.

Cholesterol Metabolism in the Brain

Even though the brain represents the largest collection of cholesterol in the body, only recently have the sources of this sterol and the turnover characteristics been described. During the development of the mammalian fetus, the concentration of cholesterol in nearly all of the organs outside of the central nervous system are the same as in the adult animal (Figs 6, 7). For example, cholesterol concentrations in the intestine, kidney and other organs are approximately 2.5-4.0 mg/g in the developing fetus, the newborn animal and the adult. This concentration of cholesterol, which is primarily located in the plasma membrane of the various cells, is remarkably constant throughout life. Only in the cells of the liver does the concentration of cholesterol fluctuate with the amount of cholesterol eaten in the diet. In contrast, in the primate, the concentration of cholesterol in the developing brain of the fetus remains low until birth. Over the next few years, as brain development and myelination proceed, these concentrations progressively increase until mature brain size is achieved. Within the central nervous system there are regional differences in the concentration in cholesterol. In the mature brain, for example, these concentrations are approximately 10 mg/g in areas of the brain that are predominately gray matter while those areas of the brain that have myelinated axon tracts can achieve concentrations of sterol that reach 40 mg/g. Direct measurements have failed to reveal any transfer of cholesterol from the plasma to the central nervous system during the time that this brain development is taking place. Other studies, however, suggest that the rate of cholesterol synthesis in the neurons and glial cells is sufficient to fully account for the large mass of unesterified cholesterol that is found in the mature brain. Furthermore, there are also regional differences in rates of cholesterol synthesis during

active myelination. The lowest rates are seen in the gray matter while much higher rates of synthesis are found in those areas of the brain that have large amounts of myelin. Once mature brain size has been reached, rates of synthesis drop to much lower levels. Nevertheless, in every species in which rates of cholesterol synthesis have been measured in the mature brain low, but significant, rates of synthesis have been reported. Thus, among neurophysiologists, the concept has evolved that the tissues of the central nervous system are isolated by the blood brain barrier from the pool of cholesterol circulating in lipoproteins in the blood. It has been calculated that all, or nearly all, of the cholesterol required for brain growth and myelination is derived from *de novo* synthesis within the central nervous system compartment. Once the brain has achieved its mature size, the rate of synthesis is markedly reduced, but this rate never drops to zero. Given the fact that the pool of cholesterol in the brain does not undergo further change throughout the life of the mature animal, this continued, low rate of synthesis implies that there must be some turnover of cholesterol within the central nervous system on a continuing basis.

Cholesterol Turnover in the Whole Human and across the Central Nervous System

While it has been possible for a number of years to measure net cholesterol turnover across the whole animal or human, there are virtually no data in which measurements of turnover of cholesterol across the central nervous system have been reported. Because of its critical role in the plasma membrane, the rate of cholesterol turnover in any animal is generally related to the basal metabolic rate in that animal. Humans that utilize about one kcal of energy per hour per kg body weight will turn over about 12 mg of cholesterol per day per kg. The turnover of cholesterol in smaller animals like the rabbit, rat and mouse equals 55, 100 and 160 mg/d per kg, respectively, because of the much higher basal metabolic rates in these smaller animals.

Unfortunately, until very recently, there have been virtually no measurements of the rate of cholesterol movement across the central nervous system. Clearly, the mature brain continues to make cholesterol at a low rate, but it has been difficult to demonstrate that the brain also takes up cholesterol from the circulating plasma. However, the mechanism for secretion of cholesterol out of the central nervous system apparently has been identified. The brain uniquely expresses an enzyme that is capable of adding a hydroxyl group to the 24 position of cholesterol to produce 24-hydroxy-cholesterol. This enzyme is expressed only in the central nervous system, and it has recently been shown that the brain makes a net contribution of 24-hydroxy-cholesterol to the plasma. This is a very important observation since it raises the possibility that by measuring the rate of 24-hydroxy-cholesterol movement through the plasma space, one can

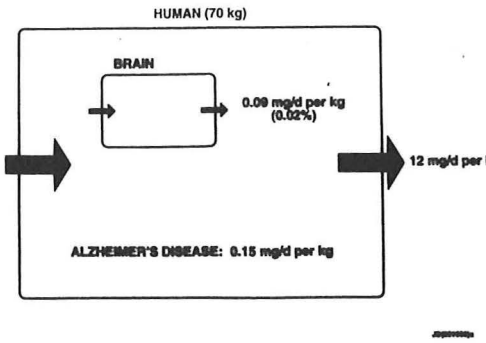

Fig. 8

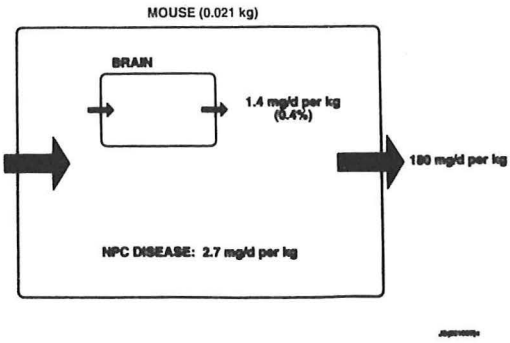

Fig. 9

indirectly measure the rate of net cholesterol turnover in the central nervous system.

By using this method in humans and more direct methods in experimental animals, it has now become possible to actually quantitate the rate of movement of cholesterol across the central nervous system (Figs. 8, 9). In general, in the three species in which such measurements are available, the rate of cholesterol turnover in the whole human or whole animal is of the order of 120 times the rate of turnover in the brain. In a 70 kg human, for example, approximately 12 mg of cholesterol are utilized each day per kg body weight by all of the organs of the body. Only 0.09 mg/d per kg are turned over in the brain, however. Thus, only 0.02% of the pool of sterol in the brain is replaced each day. These values are essentially twice as high in a smaller animal like a 23 kg baboon and are much higher in a very small mouse. Not only has it now become possible to actually quantitate the flow of cholesterol across the central nervous system, but there are also preliminary data suggesting that the rate of this sterol turnover may be a measure of the rate of neurodegeneration occurring within the central nervous system. For example, in humans with Alzheimer's disease, it has recently been reported that the rate of loss of cholesterol from the brain (measured as 24-hydroxy-cholesterol) is increased substantially. Similarly, in a mouse model of Niemann-Pick disease type C (NPC), the rate of cholesterol loss from the brain is nearly doubled. Presumably, this reflects the rapid death of neurons, the demyelination and the net loss of cholesterol from the brain that is manifest in this disease.

Can this Net Movement of Cholesterol across the Central Nervous System Be Manipulated by Diet and Drugs?

If there is a relationship between the plasma cholesterol concentration and the development of Alzheimer's lesions, then this net flow of cholesterol across the central nervous system should be manipulable by either changes

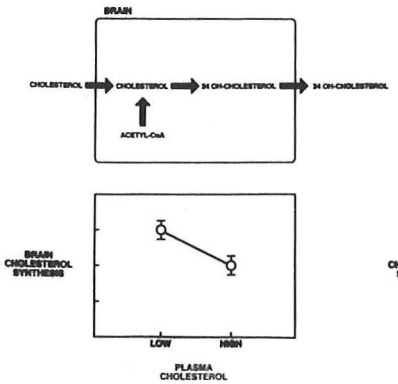

Fig. 10

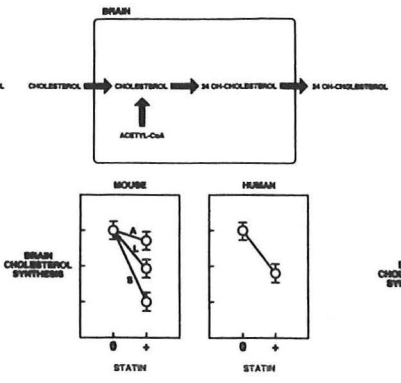

Fig. 11

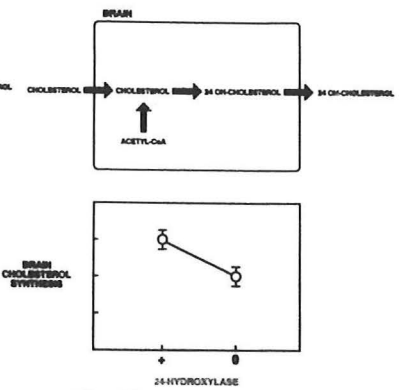

Fig. 12

in the diet or by the administration of pharmaceutical agents that alter cholesterol synthesis or secretion. There are virtually no data available in the literature to indicate that such manipulations are possible. However, there are now a series of preliminary observations which suggest that cholesterol metabolism in the brain is responsive to external forces. First, under normal conditions of cholesterol feeding in adult animals, there is usually no alteration in the rate of cholesterol synthesis in the brain. However, if the young animal is made hypercholesterolemic at a time when active brain growth and myelination are occurring, then it is possible to partially inhibit the rate of cholesterol synthesis in various parts of the brain (Fig. 10). It is not known what transport process is responsible for the movement of cholesterol from the plasma space into the central nervous system. One set of *in vitro* studies has suggested that the LDLR is present on endothelial cells lining brain capillaries and this transporter is capable of translocating cholesterol carried in LDL into the brain. However, direct measurements of LDL uptake into the brain in intact animals has failed to reveal such a process. Nevertheless, these observations that cholesterol synthesis in the brain can be inhibited by raising the plasma cholesterol level imply some transport process exists for net transfer of sterol from the blood into the brain. Second, there is now little doubt that statins can penetrate the central nervous system and inhibit cholesterol synthesis in the brain. For example, when single oral doses of atorvastatin, lovastatin, or simvastatin are administered to an experimental animal, there is immediate, significant suppression of cholesterol synthesis in the brain (Fig. 11). Furthermore, an abstract has recently been published suggesting that the same effect is observed in humans. In these studies, it was reported that statins significantly reduced the plasma level of 24-hydroxy-cholesterol which stro-hydroxy-cholesterol has been reduced by the administration of these statins. Third, in animals that lack the 24-hydroxylase, there is also inhibition of cholesterol synthesis in the brain (Fig. 12). These data imply that when the excretion of cholesterol from the brain is reduced by elimination of the 24-hydroxylase, the cells of the brain must necessarily reduce their rate of cholesterol synthesis or else excessive amounts of

cholesterol will accumulate. Taken together, these three sets of observations unequivocally show that there is active, net turnover of cholesterol across the central nervous system and, further, that this turnover can be manipulated by altering the plasma cholesterol concentration, by the administration of drugs that inhibit cholesterol synthesis in the brain, and by changing the rate of net excretion of sterol across the blood brain barrier. These observations, therefore, raise the possibility of altering the incidence of Alzheimer's disease if, in fact, net cholesterol balance across the central nervous system influences the formation of the neuritic plaque.

The Internal Recycling of Cholesterol in the Central Nervous System

While these latter observations unequivocally prove that there is net movement of cholesterol across the central nervous system, there is also compelling data that there is also internal recycling of cholesterol among the different cell types present in the brain. There are several lines of *in vitro* data suggesting that during nerve cell growth or regeneration there is uptake of cholesterol that depends upon the presence of apoE. After nerve damage, for example, there is increased expression of lipoprotein receptors, and nerve repair will proceed if there is a source of cholesterol and apoE 3 in the media. This repair process is defective, however, if apoE 4 is present. Presumably, the cholesterol in the environment becomes associated with apoE 3 and this particle, in turn, is taken up by lipoprotein receptors on the nerve cell membrane. This particle presumably is then processed through the lysosome, and the unesterified cholesterol is transferred by NPC protein to the cytosol where it can be utilized for new membrane synthesis and nerve growth. In the intact brain, it is nearly impossible to quantitate the rate of this internal recycling between the neurons and various support glia. One way in which to estimate this recycling, however, is to interrupt the internal processing of lipoprotein cholesterol by mutating the NPC protein. In this condition, the neurons will take up the apoE/cholesterol complexes but they will be trapped within the lysosomal compartment. When this is done, even in the 1-day-old animal, there is significant cholesterol accumulation in the brain, and the individual nerve cell bodies show accumulation of vesicles that stain positively for cholesterol. Thus, this type of experiment strongly suggests that in addition to net cholesterol movement across the central nervous system, there is also quantitatively important internal recycling of cholesterol between nerve cells and the support glia that may be critically important for nerve repair and nerve remodeling.

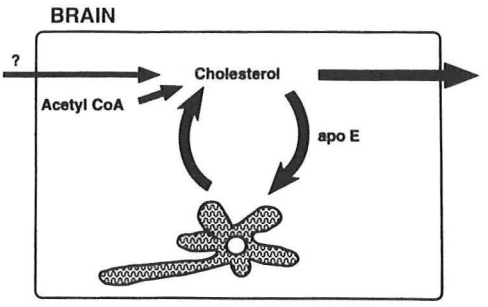

Fig. 13

Summary of Cholesterol Movement Across the Central Nervous System

These various observations concerning net cholesterol movement across the CNS and among the cells of the brain is summarized in Fig. 13. There is clearly net movement of cholesterol across the central nervous system. Net input of cholesterol comes from *de novo* synthesis and, possibly, uptake of cholesterol from the plasma. The excretion of cholesterol from the CNS presumably takes place through the formation of 24-hydroxy-cholesterol and, possibly, by other mechanisms. As implied in this diagram, there is probably also quantitatively important recycling of cholesterol among the cells of the brain, particularly after damage to nerve cells or during remodeling. This recycling process undoubtedly depends upon the presence of apoE in the brain. Several clinical observations support this conclusion. Following closed head injury, for example, the prognosis for recovery depends upon the type of apoE present in the patient. Those who carry one or more alleles for apoE 4 have a much worse prognosis than do individuals who carry apoE 2 or apoE 3. This clinical situation is also seen in professional boxers who sustain repeated trauma to the brain. Those professional fighters who carry the apoE 4 allele are much more likely to have severe disability, and very high traumatic brain injury scores, than individuals who have had the same number of fights but carry only the apoE 3 allele. Thus, taken together, the preliminary data now available strongly suggest that there is net cholesterol transfer across the central nervous system and active recycling of cholesterol among the different cell types in the brain. ApoE is probably intimately involved in this recycling process, and the apoE isoform present in the brain dictates the degree of recovery after acute brain injury. The critical question is whether these events directly or indirectly influence the rate of amyloid deposition in the brain and the severity of the Alzheimer's syndrome.

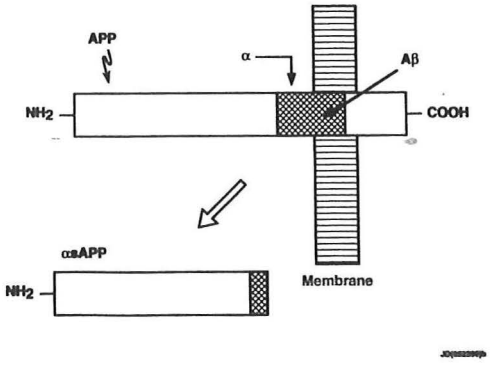

Fig. 14

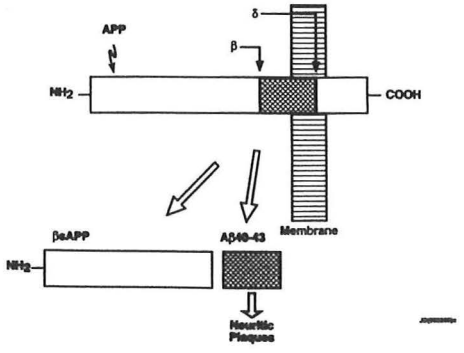

Fig. 15

Cell Biology of the Amyloid Precursor Protein Molecule

In the last few years, the identification of the molecule that gives rise to the fibrillogenic amyloid-β molecule has provided considerable insight into the pathogenesis and genetics of Alzheimer's disease. The molecule of importance is called amyloid precursor protein (APP) that is encoded by a gene on chromosome 21. This molecule is of uncertain function, is ubiquitously expressed on neurons and in the cells of a number of organs and is of variable molecular weight. Neurons commonly express a molecule that has 695 amino acid residues. Interestingly, deletion of this molecule does not result in a significant phenotype, probably because cells express similar other molecules of this type. The APP molecule has one 23-residue, hydrophobic stretch near its carboxy-terminal region. This region is thought to anchor the APP molecule in membranes such as the endoplasmic reticulum, golgi, trans-golgi network and endosomes. During the transport of the newly synthesized APP molecule from the endoplasmic reticulum to the plasma membrane, there is cleavage of the molecule at certain critical steps. On the one hand, the molecule may be cleaved at a point that is 16 amino acids into the amyloid-β region of the molecule (Fig. 14). This cleavage is carried out by a protease designated α-secretase. This cut releases the large soluble ectodomain fragment (αsAPP). This soluble fragment is apparently released into the central nervous system fluid, transported to the peripheral circulation and degraded. It should be noted that since this cut occurs 16 amino acids into the amyloid-β peptide region, generation of amyloid-β is precluded.

The APP peptide may also undergo alternative cleavages at two other sites. The APP holoprotein may be cleaved at the amino acid residue just in front of the amyloid-β region (β-secretase). This may be followed by proteolytic cleavage downstream (γ-secretase) that liberates the small amyloid-β peptide having either 40 amino acid residues or 42 amino acid residues (Fig. 15). It is not known what controls the length of this product. However, about 90% of the amyloid-β that is formed is of the 40-residue form

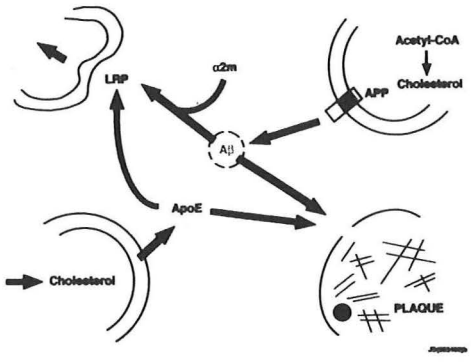

Fig. 16

(Aβ 40) while approximately 10% is Aβ 42. Aβ 40 is relatively soluble and may be normally cleared from the central nervous system. However, Aβ 42 contains two additional hydrophobic amino acid residues and is highly fibrillogenic. It may be deposited in a defuse form in the brain or, in the presence of apoE, will form very insoluble fibrils in specific regions of the brain. Amyloid-β deposits may be seen in a diffuse form without glial or neuritic reaction in areas of the brain not normally affected by Alzheimer's disease such as the cerebellum or thalamus. This material may also accumulate in the basement membrane of cerebral capillaries and arterials. Classically, this fibrillar amyloid material is deposited in the limbic system and associated cortices along with apoE, and there is recruitment of activated glial cells into the same region to form the classical plaques.

Model for the Interaction of Cholesterol, ApoE and Amyloid-β

These various observations on the role of plasma cholesterol level, apoE and amyloid-β production allow the development of a model that suggests how the rate of amyloid plaque formation, and the incidence of Alzheimer's disease, might be modified by changes in the circulating plasma cholesterol level or the administration of statins that enter the brain and partially inhibit cholesterol synthesis. As illustrated in Fig. 16, amyloid-β is produced within the brain from the metabolism of amyloid precursor protein. This molecule may become part of a neuritic plaque or, alternatively, the amyloid-β may bind to α-2-macroglobulin. This complex of α-2-macroglobulin/amyloid-β is known to be taken up by lipoprotein receptors such as LRP and degraded. Cells in the central nervous system are also known to produce apoE. ApoE may interact with amyloid-β to facilitate the formation of amyloid fibrils and so enhance the rate of formation of neuritic plaques. In addition, high concentrations of apoE may compete for the uptake of the α-2-macroglobulin/amyloid-β complex by LRP and so retard the rate of intracellular degradation. It should be noted that the mutations that lead to the overproduction of amyloid-β account for <2% of the cases of

clinical Alzheimer's disease. Any model, such as this, must account for the fact that the majority of cases of clinical Alzheimer's disease have no demonstrable mutations in the APP or presenilin molecules, and most occur in patients who carry the apoE 3 isoform of apoE.

The Role of ApoE in the Formation of Neuritic Plaques

The role of apoE in the formation of plaques is best illustrated by the recent studies utilizing a mouse model for plaque formation. In this model, the mouse has been engineered to contain the human APP gene and produces the amyloid-β peptide at a relatively constant rate. In addition, the ability of this mouse to make its own apoE has been deleted. This animal can then be used to explore the relationship between various human apoE molecules and neuritic plaque formation under conditions where the rate of synthesis of the amyloid-β peptide is constant. In this model, in the absence of apoE there is no plaque formation. There is formation and deposition of amyloid-β throughout the brain in a diffuse form, but amyloid fibrils are not produced and neuritic plaques are not found in the brains of these animals. However, if the animal is engineered to produce a small amount of apoE 3 (heterozygous animal) then a small number of plaques are found. When the gene-dose for this apoprotein is doubled (homozygous for apoE 3) many more plaques are observed. Finally, if human apoE 4 is introduced into this model, the number of plaques formed increases dramatically. Thus, under circumstances where the amount of amyloid-β protein being formed is relatively constant, the rate of plaque formation is dependent upon the amount of apoE 3 also present in the brain and whether or not there is also apoE 4.

There are now several lines of evidence that amyloid fibril formation from amyloid-β critically depends upon the presence of apoE. This apolipoprotein apparently behaves as a kind of chaperon that allows the individual molecules of amyloid-β to hydrogen bond to adjacent molecules in such a manner that identical residues on these adjacent chains are aligned directly, i.e., are in register. While apoE 3 is capable of this chaperon function, apoE 4 binds even more avidly to the amyloid-β chain and so promotes even greater plaque formation than the more common isomer. Thus, data such as these strongly suggest that the presence of apoE 3, and particularly apoE 4, are absolutely necessary for neuritic plaque formation. In the absence of apoE, plaque formation does not occur even in the face of significant amyloid-β production. This finding is also consistent with the observation that apoE is almost always co-precipitated with the amyloid-β peptide in the mature plaque.

The Role of Cholesterol in Regulating the Rate of Neuritic Plaque Formation

There are at least three observations which suggest mechanisms whereby a change in the rate of cholesterol synthesis and the rate of cholesterol entry into the central nervous system could alter this rate of apoE-dependent precipitation of amyloid-β. First, in isolated neurons it has been reported that the rate of amyloid-β production is dependent upon the status of the cholesterol pools in the cells. Depleting the membranes in these cells of cholesterol leads to a greatly reduced rate of amyloid-β production. Second, *in vivo*, it has been found that treatment with statins reduces the rate of amyloid-β production from endogenous amyloid-β precursor protein in the brain. Third, it has also been demonstrated that cholesterol feeding, and an elevation of the circulating plasma cholesterol level, is associated with an increase in the concentration of apoE in the plasma and in the brain. Presumably, this finding reflects the fact that an elevated plasma cholesterol level is associated with entry of sterol into the central nervous system and initiation of this increased rate of apoE production. Thus, an increase in the membrane content of cholesterol in the cells of the central nervous system could be associated with an increased rate of amyloid-β production and an increased rate of apoE synthesis. Together, these events should lead to increased neuritic plaque formation.

Summary

Thus, taken together, these various observations now raise the possibility that there is, in fact, a relationship between the incidence of Alzheimer's disease and the plasma cholesterol level. If the events pictured in Fig. 16 are correct, then slight expansion of the cholesterol pools in the cells of the central nervous system secondary to an increase in the circulating plasma cholesterol level would be expected to increase the rate of amyloid-β production and the rate of apoE synthesis. The increased level of apoE, particularly in the presence of increased amounts of amyloid-β, would be expected to compete with the degradation of the α-2-macroglobulin/amyloid-β complex and enhance the rate of β sheet formation from the amyloid-β. This process would be accentuated if the individual also carried an allele for apoE 4. Treatment with a statin would be expected to have two effects. These drugs clearly enter the central nervous system and inhibit cholesterol synthesis. If critical membranes within the neurons become partially sterol depleted, the rate of amyloid-β synthesis would presumably be reduced. At the same time, lowering the circulating plasma cholesterol level would reduce the entry of cholesterol into the central nervous system and, presumably, reduce the rate of synthesis of apoE. In theory, these two events should markedly reduce the rate of plaque formation.

Whether or not this series of events is correct remains to be established. These various observations, however, strongly support the possibility that circulating plasma cholesterol levels do play an important role in promoting neuritic plaque formation in the central nervous system and the evolution of Alzheimer's disease. Certainly if the retrospective study illustrated in Fig. 1 proves to be valid, these new data are so compelling that it may be timely to initiate a prospective study in which statins are tested for their ability to significantly reduce the incidence of Alzheimer's disease in both the uncommon, early onset syndrome and the much more common dementia seen in patients over the age of 60. At the very least, these data suggest that the emphasis in Alzheimer's disease should be shifted away from the cell biology of amyloid-β precursor protein to the critical role of apoE and cholesterol in the pathogenesis of this syndrome.

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