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UPTAKE AND TRANSPORT OF FATTY ACIDS INTO THE BRAIN AND THE ROLE OF THE BLOOD BRAIN BARRIER SYSTEM

by

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List of Abbreviations

BBB	=	Blood Brain Barrier
% RCA	=	Relative Carboxyl Activity $= \frac{\text{Radioactivity in} - \text{COOH}}{\text{Radioactivity in Total FA}} \times 100$
FA	=	Fatty Acid
PUFA	=	Polyunsaturated Fatty Acids
EFA	=	Essential Fatty Acid
PC	=	Phosphatidyl Choline
PE	=	Phosphatidyl Ethanolamine
PS	=	Phosphatidyl Serine
CEREB	=	Cerebroside
Sph	=	Sphingomyelin
SphL	=	Sphingolipids
PL	=	Phospholipids
% Incorporation	=	$\frac{\text{Radioactivity per g wet wt. of tissue}}{\mu\text{c Radioactivity administered per g body wt}} \times 100$
$18{:}2\omega 6$	=	Denotes octadecadienoic acid with the 1st double bond at the 6th carbon starting from methyl end

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I. Introduction

The restriction posed by the central nervous system on the uptake of intravenously administered dyes has been known since the early work by Ehrlich (1882). Goldmann (1909) showed that after an intravenous injection of trypan blue, the brain was not stained, although the choroid plexus and the meninges were. Later, in another experiment, Goldman (1913) injected the same dye into the CSF and was surprised to find that the whole brain was heavily stained. Since these publications, the term "Blood Brain Barrier" (BBB)* emerged and has persisted in the literature on this subject. Although the concept originated with dyes, it soon was widened to include many compounds, ions, metabolites, non-metabolites etc. A similar barrier has been proposed for the retina (Blotevogel 1924) but has not received as much attention.

In this communication it is not our intention to present a critical review of the arguments, either pro or con, on this controversial subject since many such reviews have appeared in the literature in recent years. The following list, although not complete, includes some very comprehensive reviews: (Friedmann, 1942; Bakay, 1956; Dobbing, 1961; Edstrom, 1964; Davson, 1967; Lajtha and Ford, 1968; Oldendorf, 1971b; Dhopeshwarkar and Bawa, 1971). Our intention is to discuss the uptake and transport of fatty acids into the brain and to examine the role of the BBB system as it applies to this phenomenon.

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*See list of abbreviations

II. BBB and Lipids

A. EARLY WORK USING NON-RADIOACTIVE MARKERS

Soon after Schoenheimer and Rittenberg (1936) pioneered the use of the stable isotope, deuterium, to study the dynamics of body lipids, Waelsch and co-workers (1940; 1941; 1955) using heavy water as a tracer, found that fatty acids of the growing as well as adult brain were synthesized in the brain itself and that there was no necessity to assume that the BBB was penetrable by fatty acids or cholesterol even in growing animals. McConnell and Sinclair (1937) used a non-radioactive but easily identifiable fatty acid, elaidic acid, in the diet of pregnant rats and their offspring and found that when they had reached maturity, their brain phospholipids had only one fourth as high a proportion of elaidic acid as the liver phospholipids. Bloch et al. (1943) found that when deuterium-labeled cholesterol was fed to adult dogs, all tissues except the brain and the spinal cord incorporated labeled cholesterol from the circulation. Thus, these early studies strongly indicated the restriction posed by the BBB in limiting penetration of lipids into the brain.

B. STUDIES WITH RADIOACTIVE MARKERS

Soon after ^{14}C labeled fatty acids and precursors were available for biochemical research, Sperry et al. (1953) found that a short chain fatty acid such as 1-^{14}C octanoate was readily incorporated into the brain lipids. Similarly, Laurell (1959) found that a small amount of radioactivity was incorporated into the brain lipids of rats given an intrajugular injection of 1-^{14}C palmitate.

Carroll (1962), using a series of carboxy-labeled fatty acids, came to the conclusion that only a small amount of radioactivity was incorporated

into brain total lipids. However, when the same tracer was administered to young weanling rats (12-13 days old), there was greater uptake of radioactivity in the brain as compared to adult (2-3 month old) rats.

Such studies of brain uptake with isotopically labeled compounds, although useful, have been difficult to interpret because of factors such as different metabolic rates, exchange without net accumulation, etc. Moreover, it is necessary to bear in mind that the mere presence of radioactivity in brain fatty acids following ingestion cannot be taken as a proof of passage from the blood into the brain, since the radioactivity in the brain fatty acids could be the result of synthesis from acetate derived by oxidation of the administered fatty acid.

C. EFFECT OF AGE

Increased uptake of radioactivity by the brain of 8-13 day old rats as compared to adult rats, 2-6 months old, was noted by many workers (Fries *et al.*, 1940; Bakay, 1953) and thus, much of the work done during the last decade has been performed by administering tracers to baby rats. For example, Fulco and Mead (1961) injected 1-^{14}C acetate into 13 day old rats and determined the biosynthetic pathway of lignoceric, cerebronic and nervonic acids. Kishimoto *et al.* (1965a) injected labeled acetate into 7 day old rats to study lipid changes and turnover in the developing rat brain.

Whether age has some definite relationship to the development of the BBB, and in turn, myelination or whether the differential uptake is simply a reflection of metabolic requirement and turnover associated with growth, is still a debatable question. Dobbing (1968), who favors the latter view, has discussed the subject exhaustively.

D. ROUTES OF ENTRY

When a radioactive tracer is injected intracranially to study biosynthesis of nervonic acid (Kishimoto and Radin 1963), or to study half life and turnover of 1-^{14}C palmitate (Sun and Horrocks, 1969; 1971), the route of entry can be described as "bypassing the BBB." On the other hand, perfusion via a carotid artery (Sperry _et al._, 1953) using the Geiger-Magnes brain perfusion technique, although not bypassing the BBB, undoubtedly presents a rather large localized dose to the brain, thereby introducing another unknown variable. The same argument applies to the single intracarotid injection used by Oldendorf (1971) and in our own studies (Dhopeshwarkar _et al._, 1971c). However, in these studies the mass injected was negligible.

Another variation is introduced by the concept of "sink-action" of the CSF proposed by Davson _et al._ (1961). These authors point out that if the tracer concentration in the CSF is low, it could act as a drain or sink, continuously draining the tissue of the tracer that the tissue originally acquired by way of the blood. If the tracer is introduced into the CSF, the same phenomenon occurs with the only difference, that now, it is the sink-action of the blood instead of the CSF. To circumvent this factor, Levin and Kleeman (1970) have described a technique for introducing the tracer simultaneously into the blood and the CSF.

That the route of entry is an important factor in determining and comparing the uptake of compounds by the brain and for the evaluation of the concept of the BBB, is discussed by Levin and Scicli (1969) and by Levin and Kleeman (1970).

Oldendorf (1970), by injecting a mixture of a test substance labeled with

^{14}C and ^{3}H-water into the carotid artery followed by decapitation within the short interval of 15 seconds, was able to compare the uptake of many compounds. This technique uses only the ratio of ^{14}C to ^{3}H in the brain relative to the ratio of ^{14}C to ^{3}H in the original mixture and thereby eliminates both elaborate experimental procedure and complex calculations.

III. BBB and Fatty Acids

A. NATURE OF THE BRAIN FATTY ACIDS

In addition to the usual saturated and unsaturated fatty acids such as palmitic, stearic and oleic acids, the brain contains a rather large amount of polyunsaturated fatty acids with four, five and six double bonds (Biran and Bartley 1961; Johnston _et al_., 1961; Kishimoto _et al_., 1965b; Miyamoto _et al_., 1967; Rouser and Yamamoto 1969; O'Brien _et al_., 1964; Dhopeshwarkar _et al_., 1969a). Another class of fatty acids found largely in the brain is that of the α-hydroxy long chain fatty acids (Klenk 1928; Kishimoto and Radin 1959). The formation of the long chain α-hydroxy fatty acids and the odd chain fatty acids by α-oxidation was first described by Mead and Levis (1962; 1963). Finally, the occurrence of very long chain fatty acids, up to 35 carbon atoms, has been reported by Pakkala _et al_. (1966).

B. SYNTHESIS OF FATTY ACIDS IN THE BRAIN

That the brain is capable of synthesizing fatty acids as efficiently as other organs, such as liver, was demonstrated by Brady (1960). The major fatty acid synthesized by the brain synthetase was palmitic acid. Recently, a brain "microsomal" chain elongation system responsible for stearate and longer chain saturated fatty acids as well as the chain elongation-desaturation system has been characterized by Aeberhard and Menkes (1968). A brain

mitochondrial system, which elongates 16:0 as well as 22:0 and 22:1, has been recently described by Boone and Wakil (1970). As mentioned above, the α-oxidation system producing hydroxy and odd chain fatty acids was first demonstrated *in vivo* by Mead and Levis (1963) and later demonstrated in the brain "microsomal" system *in vitro* (Levis and Mead 1964; McDonald and Mead 1968; Davies *et al.*, 1966). In a recent report, Mead and Hare (1971) have implicated ascorbic acid as a cofactor in the α-oxidation system.

The brain has the necessary enzymes for synthesis (either by *de novo* or chain elongation-desaturation processes) of the required fatty acids, thus reducing the demand on transport of fatty acids synthesized elsewhere or ingested in the diet. Obviously, the essential fatty acids 18:2ω6 and 18:3ω3, which cannot be synthesized by the mammalian systems, must come from the diet and be transported into the brain by the circulating blood. Both of these fatty acids are found in trace amounts in the rat brain but their polyunsaturated products occur abundantly.

C. NUTRITIONAL STUDIES

Several years before the actual nutritional studies designed to ascertain whether dietary fatty acids penetrated the BBB and were taken up by the adult brain were undertaken, Klenk (1955) had postulated that 20:4ω6 and 22:6ω3, occurring in rather large amounts in the brain can be formed only from dietary sources of 18:2ω6 and 18:3ω3. Thus, at least these essential fatty acids must pass from the blood into the brain through the BBB.

Following this prediction, Mohrhauer and Holman (1963) found that increasing amounts of dietary linoleate and arachidonate fed to weanling rats led to increased deposition of arachidonate and docosapentaenoate, 22:5ω6, in the brain. Similarly, dietary linolenate gave rise to brain polyunsaturated

fatty acids of the ω3 family. Rathbone (1965), starting with animals that were a little older (8-12 weeks instead of weanling), came to similar conclusions.

Thus it was clearly established that dietary fatty acids could influence the composition of brain lipids. This is possible only if we assume that at least some fatty acids pass into the brain by way of circulating blood.

D. UPTAKE OF LABELED FATTY ACID PRECURSORS

The uptake of fatty acid precursor by the brain has, for the most part, centered around uptake and incorporation of radioactivity from 1-^{14}C acetate. Since earlier experiments failed to show any appreciable uptake of long chain fatty acids by the adult brain (Laurell 1959; Carroll, 1962) fatty acid precursors such as 1-^{14}C acetate were used extensively to study uptake and incorporation into brain fatty acids. Thus, 1-^{14}C acetate was injected into rats to study uptake and incorporation of radioactivity in brain fatty acids (Waelsch et al., 1941; Van Bruggen et al., 1953). Much metabolic information on the biosynthesis and transformation of brain fatty acids was obtained from the radioactivity distribution pattern obtained after administration of 1-^{14}C acetate (Fulco and Mead 1961; Kishimoto and Radin 1966). More recently, uniformly labeled glucose has been used as a fatty acid precursor by Smith (1968) in the study of myelin turnover in the adult rat. Although use of ^{14}C labeled glucose resulted in 4-6 times higher uptake of radioactivity in the brain than did 1-^{14}C acetate (Smith, 1967), the non-lipid fractions of the brain also were labeled to an appreciable extent. The main advantage of using a labeled precursor such as glucose, is that the tracer permeates freely into the brain and thus overcomes the influence of

the BBB. However, the use of labeled glucose does not permit the facile prediction of metabolic pathways of fatty acids in a manner that labeled acetate can provide. This point is discussed in detail in Sec. III., E., 1., a, b, and c.

E. UPTAKE OF LONG CHAIN FATTY ACIDS

1. Non-Essential, Palmitic and Oleic Acid

Although Miyamoto *et al*. (1967) found ready incorporation of 1-^{14}C linoleic as well as linolenic acid into the embryonic chick brain, the route of entry was via the yolk sac, by-passing the BBB. On the other hand, Gatt (1963) found practically no radioactivity in the brains of rats after intravenous administration of 1-^{14}C lignoceric acid (24:0).

With such varied and often conflicting data reported in the literature, we decided to reexamine the uptake of saturated and unsaturated as well as essential fatty acids. The main object was to determine whether long chain fatty acids, administered either by mouth or injected into the blood stream, would be taken up by the brain directly as intact molecules rather than after oxidative degradation to acetate elsewhere in the body. In order to determine the direct uptake of a carboxy-labeled fatty acid and distinguish it from the uptake of acetate derived by degradation of the administered tracer and resynthesis, we resorted to comparing the labeling pattern of fatty acids isolated from the brain after administration of labeled fatty acid (Dhopeshwarkar *et al*., 1969b; 1970; 1971a; 1971b) with that obtained after administration of 1-^{14}C acetate (Dhopeshwarkar *et al*., 1969a). The label distribution was obtained by decarboxylating the fatty acids isolated from the brain using the Schmidt decarboxylation procedure as described by Brady *et al*.

(1960). The activity in the carboxyl carbon compared to the total activity of the intact fatty acid gave the per cent relative carboxyl activity (%RCA).

The following three possible pathways can be detected by this procedure:

a. De novo Synthesis from Acetate

Alternate carbon atoms are labeled; in the case of palmitic acid, for example, all odd carbon atoms (8 out of 16) will be labeled and theoretically the carboxyl carbon will have 1/8 or 12:5% of the total radioactivity of palmitic acid. Thus, by determining % RCA and comparing it with the theoretical value for that particular chain length, one can postulate whether the fatty acid was synthesized de novo from acetate.

b. Synthesis by Chain Elongation

(1) One molecule of radioactive acetate adds to a preexisting non-radioactive precursor; this will result in 100% RCA.

(2) A radioactive acetate adds to a radioactive precursor, which may have been formed by de novo synthesis. In this case the % RCA will vary considerably depending on the relative radioactivity of the precursor and the acetate. This in turn, will depend upon the time interval between dose and sacrifice (Kishimoto and Radin, 1966).

c. Direct Uptake

Obviously, when a fatty acid is taken up directly, it will have a % RCA that is very close to that of the starting material.

Since the possibility that radioactivity will be largely confined to the carboxyl carbon can theoretically be realized both in the case of direct uptake and in chain elongation, it is advisable to determine the label distribution at two or more intervals between dose and sacrifice, thus eliminating a coincidental RCA characteristic of direct uptake when it could as well be

by chain elongation. Similarly the variable % RCA value in the chain elongation process can theoretically (by coincidence) be very close to the *de novo* synthesis value. Again, by choosing two or more intervals, this coincidence can be avoided (Kishimoto and Radin, 1966).

This reasoning has been extensively used by Mead (1961) and Klenk (1961) to establish biosynthetic pathways.

The first experiments (Dhopeshwarkar *et al.*, 1969a) were carried out on a group of weanling 13-day-old rats, and for comparison a group of adult rats approximately 90 days old was included. 1-^{14}C sodium acetate (5 μc/g body wt) was injected intraperitoneally and the animals were killed after 4 hours. The results are shown in Table I.

TABLE I

From these data it can be observed that the total lipid content per g of brain had almost doubled in the adult animals as compared to weanling rats, which agrees with earlier work by Wells and Dittmer (1967). No such profound change was found in the liver total lipids. The radioactivity in the brain was higher than that in the liver in young animals, whereas the opposite was true in the case of adult animals. In the earlier literature, this reduced uptake by adult animals was linked to the development of the BBB. However, as early as 1955, Folch Pi suggested caution in such interpretations, emphasizing that during the weanling period the brain is building new tissue at a high rate thus giving apparent high uptakes, which could be mistakenly interpreted as related to the BBB. This point has been exhaustively discussed by Dobbing (1968). After separation of cholesterol and traces of neutral lipids on a SiO_2 column, the polar lipid fraction (PL + SphL) was subjected to methanolysis and the methyl esters were separated on a SiO_2-$AgNO_3$ column (Stein and Slawson 1968) into classes according to degree of

unsaturation. Further separation was performed by preparative GLC and the fatty acids were obtained by alkali hydrolysis of the methyl esters. The fatty acids were then decarboxylated to obtain % RCA data. Table II shows the specific activities and % RCA of the various brain fatty acids.

TABLE II

Palmitic acid, the major product of the extramitochondrial fatty acid synthetase in the brain (Brady 1960), had the maximum specific activity both in weanling and adult rats. The % RCA was very close to the theoretical value of 12.5% for de novo synthesis. The polyunsaturated fatty acids, 20:4 and 22:6, had a very high % RCA. Thus, this would be the pattern of labeling if any 1-^{14}C-labeled fatty acid administered to the animals was first degraded to radioactive acetate which would be used for resynthesis of 16:0 or to chain elongate 18:2 and 18:3 in the brain itself.

With this basic information, we proceeded to determine the uptake of two fatty acids, palmitic and oleic, that are present in substantial amounts in the brain. (Dhopeshwarkar and Mead, 1969b; 1970.) We restricted our work to adult animals to reduce the influence of rapid growth and consequent high rate of lipid synthesis in the brain during the myelinating period. Carboxy-labeled palmitic acid was dissolved in corn oil and fed by mouth to 3-month-old rats, whereas 1-^{14}C oleic acid was complexed with bovine fatty acid poor albumin and injected into the tail vein. Animals were sacrificed 4 and 24 hours after administration of the dose. Table III shows the uptake of radioactivity in the blood, brain and liver total lipids.

TABLE III

It is important to note that the radioactivity of the blood in all cases dropped considerably between the 4 and 24 hour periods, but that the radioactivity in brain lipids did not show a comparable decrease. The radioactivity in brain total lipids cannot be attributed to a small amount of

blood that is trapped in any dissected brain tissue. It may also be pointed out that previously the actual amount of blood left in the brain was determined using ^{51}Cr-labeled erythrocytes. It was found that it amounted to 0.1 to 1.5% of the fresh weight of the brain or 1.2 ml of blood per 100 g brain (Davson and Spaziani 1959; Lajtha et al., 1957; Bito et al., 1966; Kabara 1967). Thus a maximum amount of blood (equivalent to 6 μl of plasma) trapped in the brain could contribute 363 counts out of 13,287 cpm/g fresh weight of the brain, in the 4 hour experiment. Contribution of radioactivity by the blood to the brain in the 24 hour experiment is only 0.35% of the total brain radioactivity per g and hence negligible. Evidence that the radioactivity in the brain was associated with all polar lipid components including myelin lipids is shown in Table X (and discussed on p 21) giving conclusive proof of true uptake and incoporation.

The uptake of radioactivity by the brain does not seem to be different whether the route of entry was oral feeding of palmitic acid or intravenous injection of albumin-bound oleic acid.

The radioactivity distribution in fatty acids isolated from the brain polar lipid fraction 4 and 24 hours after a tracer dose of 1-^{14}C palmitate and oleate was compared with data obtained from a tracer dose of 1-^{14}C acetate.

Comparing the specific activities of brain fatty acids, one can see from the data presented in Table IV that even 24 hours after administration of 1-^{14}C palmitic acid, the brain palmitic acid was still the most active component. However, this was not the case in the oleic acid experiment. The brain oleic acid, which was the most active component 4 hours after injection of the tracer, had lost its activity at 24 hours, but 20:1 had gained

←TABLE IV

in radioactivity making it the most active component. This suggests a precursor product relationship, 18:1 ⟶ 20:1. Furthermore, the experiment suggests direct uptake of both palmitic and oleic acids. The proof that this is actually the case comes from the label distribution data.

When 1-^{14}C palmitic acid was administered, irrespective of the interval between dose and sacrifice, the brain palmitate had most of the radioactivity in the carboxyl carbon, showing direct uptake of the tracer. If the fed palmitate had been extensively degraded to acetate, which was then taken up by the brain, the labeling pattern would have been similar to that obtained with 1-^{14}C acetate as tracer. That this was not the case, can be concluded from distinct differences in the label distribution (% RCA). By the same token, it was concluded that oleic acid injected intravenously was taken up by the brain directly without extensive oxidation to acetate and resynthesis in the brain.

The gain in radioactivity by eicosenoic acid (20:1) and its low % RCA shows a definite precursor product relationship. C_3 of eicosenoic acid corresponds to C_1 of oleic acid, which had high radioactivity. On the other hand, the acetate derived by oxidative degradation of the tracer would be low in radioactivity, contributing only a small amount to C_1 of eicosenoic acid resulting in a low % RCA. This agrees with the general conclusion of Fulco and Mead (1961) and Kishimoto and Radin (1963) that oleic acid is the precursor of eicosenoic acid and nervonic acid (24:1). However, Kishimoto and Radin found that very little oleate entered the cells that make sphingolipids and that much was degraded to acetate. These differences between their study and ours (Dhopeshwarkar and Mead 1970) might be due to variation in age and route of entry of the tracer.

The differences both in the specific activities and % RCA of oleic acid as against stearic acid indicates that biohydrogenation must be at best a minor pathway, although biohydrogenation of palmitoleate (Mead and Nevenzel 1960) and elaidate (Dhopeshwarkar and Mead 1962), even in the absence of intestinal flora (Blomstrant et al., 1963), may have been demonstrated in the whole animals. It was interesting to note that the palmitic and oleic acids taken up by the brain were incorporated into all the major brain lipid components, phosphatidyl choline (PC) being the most and sphingomyelin the least radioactive component. This is in general agreement with the observations by Sun and Horrocks (1969; 1971). Cholesterol was relatively less radioactive, indicating that its precursor, acetate (itself a breakdown product of the administered labeled fatty acid) had relatively low radioactivity.

F. UPTAKE OF ESSENTIAL FATTY ACIDS

1. Linoleic and Linolenic Acid

One could argue that since the brain has most of the necessary enzymes for synthesis of fatty acids, it may not require fatty acids synthesized elsewhere in the body or from dietary sources. However, it is now a well established fact that essential fatty acids comprising 18:2ω6 and 18:3ω3, cannot be synthesized by mammalian systems; hence, they must be provided by the diet. Moreover, Bernsohn and Stephanides (1967) have discussed a possible connection between dietary deficiency of essential fatty acids and multiple sclerosis, based on an earlier hypothesis by Sinclair (1956). Although no such definite relationship has been established, the question of whether the essential fatty acids can pass from the blood into adult brain had remained cloudy. The reason for this was that much of the earlier work

that dealt with uptake of labeled essential fatty acids was done when the BBB was poorly developed (Miyamoto et al., 1967). Nutrition studies by Mohrhauer and Holman (1963) made use of young rats, although Rathbone (1965) had used slightly older rats. These studies in animals of premyelination age were not acceptable to those who believed in the concept of BBB and who argued that there was no restriction whatsoever from BBB at that early age. If this argument is taken to be valid and the assumption made that in older animals there is a restriction to the uptake of fatty acids, one would have to conclude that the animal must depend upon the supply of essential fatty acids it received during the early or premyelination period of life. Further, in these circumstances the recycling hypothesis (Sun and Horrocks 1969) would be very useful to conserve the limited supply.

We therefore used three month or older rats and administered carboxy-labeled linoleic acid to one group and 1-^{14}C-linolenic acid to another, to determine whether these essential fatty acids could be taken up by the brain as intact molecules (Dhopeshwarkar et al., 1971a; 1971b). As in earlier experiments, animals were sacrificed 4 and 24 hours after the dose. Data on uptake into the blood, brain and liver lipids are presented in Table V.

←TABLE V

Animals that were sacrificed after 24 hours received a slightly higher dose per g of body weight than animals that were sacrificed 4 hours after the dose. However, in spite of this higher dose, the radioactivity per ml of plasma had dropped considerably between the 4 and 24 hour periods. However, during this interval, radioactivity of brain total lipids had actually increased. That this increase was not due to the higher dose, is borne out by the data on per cent incorporation. In calculating this figure, the dose given per g of tissue is taken into consideration and as can be observed in

Table V, the per cent uptake of radioactivity by the brain between 4 and 24 hours after tracer administration increased almost 80%. During the same interval, there was a drop in per cent incorporation in the liver that was more pronounced with linoleate than with 1-^{14}C-linolenate feeding.

The specific activity and label distribution data after administration of linoleic and linolenic acid are given in Table Vl.

TABLE VI

Although the amount of 18:2ω6, in the brain is of the order of 1-1.5% of the total fatty acids (Rathbone 1965; Dhopeshwarkar et al., 1969), use of argentation column chromatography (Stein and Slawson 1968) followed by preparative GLC permitted the isolation of very pure 18:2 from brain total fatty acids. Since there is virtually no 18:3, we added carrier material to isolate traces of 18:3. In this manner it was possible to determine label distribution in 18:3 but not the specific activity.

As can be seen in Table VI, the 18:2 isolated from brains of rats given 1-^{14}C-linoleate, was the most active component, indicating a direct uptake. Since the mammalian system cannot make 18:2ω6 from small molecular weight precursors, the high activity of linoleic acid isolated from brain polar lipids must be derived by direct uptake from circulating blood. Further, it is known that linoleate is converted into arachidonate (Mead 1961) and the label distribution in brain arachidonate clearly shows this to be true. C_3 of arachidonate corresponds to C_1 of the administered linoleate. Thus, C_3 is expected to have high activity and the carboxyl carbon coming from an acetate would have relatively lower activity. This is borne out in the 4 hour experiment and, more dramatically, in the 24 hour experiment. One also can observe an increase in specific activity of arachidonic acid between the 4 and 24 hour periods during which time more of the tracer was taken up and

converted into arachidonate. It may be pointed out here that Steinberg et al. (1956), who were the first to show the transformation of 18:2 to 20:4 had noted that C_1 and C_3 comprised all the activity of the arachidonic acid with no activity at all in the rest of the molecule. However, they found a rather high activity in the carboxyl carbon, unlike our studies in the brain (Dhopeshwarkar et al., 1971a), which they attributed to acetate formed by oxidative degradation of the fed material and a reflection of relative pool size for acetate and linoleate at the site of elongation. This oxidative process, however, seems to be a very slow reaction in the brain resulting in a low activity in acetate and in the derived carboxyl carbon.

Linolenic acid isolated from the brain polar lipids, even 24 hours after feeding, still had most of the activity in the carboxyl carbon indicating direct uptake. The label distribution in docosahexaenoic acid (22:6ω3) is exactly what one would predict from the precursor-product relationship. The carboxyl carbon, derived from acetate formed by oxidation of the fed material, has low radioactivity as compared to the hydrocarbon chain comprising carbons 5 to 22; carbon 5 being derived from the carboxyl carbon of linolenic acid was high in radioactivity. Thus, the observation by Miyamoto et al. (1967), in which 1-^{14}C linolenic acid was injected into the yolk sac to demonstrate uptake and conversion to 22:6ω3 in the chick embryonic brain, is true in the adult rat brain as well.

Thus the rat brain does not have to depend on the supply of essential fatty acids it received during very early periods of development and growth, but can take up these essential nutrients throughout adult life. Since the brain has no mechanism for storage of fatty acids, it seems likely that after the period of active myelination, when the need for rapid lipid synthesis is

reduced, although essential fatty acids would still gain access to the brain, only a portion would be retained. This agrees with the observation by White *et al*. (1971) that the return to normal of EFA-deficient animals, after shifting to a balanced diet, is quite rapid. If the BBB had prevented or severely restricted the uptake of EFA, full grown animals that were EFA deficient would not return to normal even if dietary corrections were made.

IV. Specificity of Fatty Acid Uptake

A. PREFERENTIAL UPTAKE OF LINOLENIC OVER LINOLEIC ACID

A careful look at the percent composition of fatty acids of brain total lipids shows one predominant feature. The brain fatty acids are rich in polyunsaturated fatty acids and in particular the fatty acids of the linolenic acid ($\omega 3$) family (Biran and Bartley 1961; Johnston *et al*., 1961; Miyamoto *et al*., 1967; Dhopeshwarkar *et al*., 1969; Rouser and Yamamoto 1969). In liver fatty acids, for example, there is an appreciable amount of linoleic acid, but very small amounts of linolenic acid. Thus, even if there were equal amounts of $20{:}4\omega 6$ and $22{:}6\omega 3$ in the brain and the liver, the overall composition in the liver would be made up predominantly of the $\omega 6$ family. This feature of brain fatty acids poses a question of whether there is any specific uptake of 18:3 over 18:2 in the brain. We already had the data on the uptake of various fatty acids by the brain and decided to evaluate the data with respect to this question, as shown in Table VII.

TABLE VII

Looking at the per cent incorporation data (ratio of radioactivity per g of tissue to that of radioactivity administered per g body weight x100) it does not seem to indicate any preferential uptake of linolenic acid over linoleic acid. However, if one compares the radioactivity per g of tissue in the liver to that in the brain it is found that the ratio is higher for

18:2ω6 than for 18:3ω3. This decreased ratio indicates that there is greater uptake of radioactivity from 18:3ω3 in the brain than in the liver and the opposite was true in the case of 18:2ω6. Similar conclusions can be drawn from the ratio of radioactivity in the plasma (per ml) to that in brain.

In another study, uptake of radioactivity in the brain following one hour continuous infusion of carboxy-labeled fatty acid-albumin complex via the carotid artery was studied in rabbits (Dhopeshwarkar and Levin). The results are shown in Table VIII.

← TABLE VIII

In this experiment, the grey matter comprising the first two 400 μ-slices of the dissected brain and the white matter made up of the fourth and fifth slices (Levin and Kleeman 1970) were used for lipid extraction and radioactivity determination. It was found that in all cases, the ratio of radioactivity of brain tissue per g to plasma per ml was higher when 1-^{14}C linolenic acid was infused than with linoleic acid. Thus it appears that there is a selective uptake of 18:3ω3 by the brain tissue <u>in vivo</u>. It is well to remember here, that neither of the two EFA occurs in the brain in any appreciable amounts, but their products 20:4 6 and 22:6 3 are major unsaturated fatty acids.

B. RAPID UPTAKE VIA THE INTRACAROTID ROUTE

In most of the studies cited so far, the interval between administration of tracer dose and sacrifice of the animals has been hours or days. The main reason for this was a general belief that brain lipid metabolism, at least in adult animals, is rather slow and that a shorter time interval would not be adequate to obtain all data regarding uptake, metabolic transformations and biosynthesis. Our attention was drawn to a study by Oldendorf (1970; 1971a) who had used a single carotid injection of a mixture of tritiated

water and ^{14}C-labeled test compound to determine brain uptake. The animals were decapitated in 15 seconds, just enough time for one complete pass through cerebral circulation. We used this method (Dhopeshwarkar et al., 1971c) to determine the uptake and incorporation of 1-^{14}C acetate into the brain. The data are shown in Table IX.

TABLE IX

Within this short interval of only 15 seconds after injection, there was considerable uptake of radioactivity resulting in a specific activity of brain lipids 10 times greater than that of the liver lipids. This was the first time that brain did not come out second best in uptake of radioactivity after a tracer dose to an adult animal. It was interesting to note that the radioactivity of blood plasma per ml was very low, thus eliminating the possibility of any appreciable contribution from trapped blood. Moreover the radioactivity of the brain was in the form of complex lipids.

Three conclusions can be drawn from these data. First, considerable radioactivity in the brain cholesterol must mean rapid metabolic conversion of acetate to cholesterol. Second, the label distribution of palmitic acid isolated from the purified polar lipid fraction (PL + Sph L) suggested total *de novo* synthesis from acetate. Third, all major polar lipid components were radioactive, indicating incorporation of newly synthesized fatty acids into complex lipids.

Thus most of the major lipid metabolism reactions occurred very rapidly, within 15 seconds of dose administration plus less than 50 seconds between decapitation and tissue dispersion in chloroform:methanol. This rapid uptake raises many questions, one of which is how can there be a rapid synthesis of complex molecules such as cholesterol and polar phospho- and sphingolipids and also at the same time a very long stable half life of these brain lipids?

For example, radioactivity in brain cholesterol has been shown by Davison *et al*. (1958) to be persistant up to 220 days. We have shown evidence of rapid *de novo* synthesis of cholesterol from acetate. The only possible explanation for this apparent contradiction, available at present, is that the brain cholesterol has more than one compartment; one turning over very rapidly and the other, very slowly (Kabara 1967).

The earlier findings that the BBB causes the brain to come out second best to the liver (Kishimoto and Radin 1966) is not true in all cases. For example, when the brain is presented with the tracer via a carotid injection, there is greater uptake by the brain than the liver, at least in the case of acetate; the opposite is true when tracer is injected intravenously or intraperitoneally. Whether this is true for all compounds is not known. However, the carotid injection does not by-pass the BBB and so the dramatic uptake shows that the BBB plays a minor role, if any, under these circumstances.

V. Incorporation of Radioactivity into Brain Polar Lipids

The Radioactivity from carboxy-labeled fatty acids and acetate was completely incorporated into brain complex lipids and there was no radioactive free fatty acid fraction detectable in the brain. The results are shown in Table X.

TABLE X

Examination of the 1st two columns in Table X shows quite a remarkable difference between specific activities of various components when acetate was injected intraperitoneally followed by sacrifice after 24 hours, and when it was administered by the intracarotid route and the animals were killed just 15 seconds later. In the former case PC was the most active

component, whereas, in the latter case PS had the highest specific activity. Thus, the route of entry as well as the interval between dose and sacrifice seem to influence the label distribution. From the earlier work of Borkenhagen et al. (1961), it is known that liver mitochondria can decarboxylate PS to PE. Wilson et al. (1960) had shown that brain slices could decarboxylate L serine to ethanolamine. However, Ansell and Spanner (1962) reported that decarboxylation and stepwise methylation to form PC was not a major pathway in the brain. This would explain a rather low activity of PE; another reason could be that although PS could lead to the formation of PE via decarboxylation, the PE, having a rapid turnover (Ansell and Spanner 1968), might have lost a portion of the activity, although 15 seconds seems to be too short an interval for this to happen.

That the route of entry could be the sole influence is ruled out, because 1-^{14}C palmitate irrespective of the type of administration gave rise to the highest radioactivity in the same component, PC. Wright and Green (1971) found that when 1-^{14}C palmitate was injected into the femoral vein and the liver dissected out after 1 minute, PC was the most active component. They also found that this radioactive component was predominantly in the plasma membrane fraction. The high activity in PC following intracarotid injection of 1-^{14}C palmitic acid and decapitation in 15 seconds might be similarly associated with the plasma membrane fraction. However, in our studies, PC was the most radioactive component even at longer intervals, contrary to the experiments reported by Wright and Green wherein the high activity of PC was lost to the liver triglyceride fraction. In the brain, triglycerides form a very minor component and this may be one of the reasons for the observed differences, in addition to organ specificity and

experimental conditions. Sun and Horrocks (1969) also found that PC was the most active component after an intracerebral injection in 4 month old mice. However, when the time interval was shortened to 6-10 minutes, they observed high activity in diacylglycerols (Sun and Horrocks 1971).

The high radioactivity of PC was observed in all experiments except when 1-^{14}C linolenic acid was administered. In this experiment the radioactivity distribution was very unusual in that the cerebroside fraction (which was rather poorly labeled in all other cases, except with 18:2) appears to have an increased proportion of the radioactivity, resulting in specific activities of the cerebroside fraction almost equal to that of lecithin. Unfortunately, there was not enough material to characterize this fraction and identify the most active component of the complex cerebroside mixture. This highly active cerebroside gave two spots on TLC similar to the authentic standard. The lower spot, presumably containing hydroxy fatty acids (Hooghwinkel et al., 1964; Svennerholm and Svennerholm 1963), was the one that had most radioactivity. Further characterization and confirmation is being pursued in our laboratory.

VI. Fatty Acid Transport into the Brain

PREFERRED FORM OF FATTY ACID TRANSPORT

Although it had been conclusively demonstrated that long chain fatty acids both saturated and unsaturated, including essential fatty acids, are readily taken up by the adult rat brain *in vivo*, a question remained as to the preferred form in which fatty acids are transported into the brain.

It was already known from the early work by Fredrickson and Gordon (1958) that although free fatty acids (FFA) make up only 1 to 5 per cent of plasma lipids, they have a very rapid turnover that can account for most of the net

lipid transport in the blood. The myocardium of man and dog, for example, is known to extract free fatty acids from the circulating plasma (Gordon 1957; Rothlin and Bing 1961). The circulating fatty acids are associated with plasma albumin (Gordon 1958) forming the fatty acid albumin complex. These complexes are formed immediately after FFA release into the bloodstream and are transported to the sites of utilization where the fatty acids are transferred from binding sites on the albumin to sites on the cell surface. There has been considerable discussion as to whether the brain should be included among "all other tissues" in which fatty acids are utilized. For example, Bragdon and Gordon (1958) found that when ^{14}C labeled FFA or chylomicra were administered intravenously, virtually all the tissues of the rat became labeled except those of the brain. This was then attributed to the existence of the BBB. The other reason for considering brain to be different from other tissues, was the fact that lipid metabolism in the brain was considered to be rather slow and formed a closed system,by which the brain used the products of degradation for resynthesis; thus, the need for transporting such compounds as preformed fatty acids was, for all practical purposes, unnecessary. Recent results reported by Spitzer and Wolf (1971) indicate that FFA supplied by CSF may serve as oxidizable substrates for the brain, we decided to examine the actual form in which fatty acids were transported into the brain (Dhopeshwarkar et al., 1972).

Three main possibilities were examined. The fatty acids could be transported into the brain as free fatty acids, as components of triglycerides or as components of phospholipids. A group of rats was given an intravenous dose of 1-^{14}C palmitic acid-albumin complex and killed after one hour. In these rats, the triglycerides of the circulating plasma had 78% of the total

lipid radioactivity, which is in agreement with the observations reported by Laurell (1959). Another group of animals was subjected to functional hepatectomy involving removal of the entire intestinal tract in addition to isolating the liver from the circulating blood. 1-^{14}C palmitate was injected intravenously and the animals killed after 1 hour. In these rats the FFA of the circulating plasma had 78% of the total activity. In another group of animals 1-^{14}C palmitate was injected intracarotidly and the animals were killed within 15 seconds. We reasoned that since the time interval was very short, the circulating plasma lipids would have a very radioactive FFA fraction; experimentally it turned out to contain 90% of the total radioactivity. Finally, the last group of animals was given an intravenous injection of 1-^{14}C dipalmitoyl lecithin (sonicated in physiological saline) and killed after 1 hour (Dhopeshwarkar and Subramanian.) In these rats, phospholipids contained 62% of the total plasma lipid radioactivity.

When the radioactivity in the brain was examined, it was found that maximum activity appeared in the brain when the circulating plasma FFA were high in radioactivity and not when either the plasma triglyceride or phospholipid were the most radioactive components. This indicates that free fatty acids are the preferred form of transport into the brain (Table XI). This does not agree with the observations by Holzl and Franck (1969), who proposed lecithin as a vehicle for fatty acid transport. No comparison of uptake with free fatty acid was made in their study, which was aimed to show that the BBB did not restrict the passage of intravenously administered lecithin into the brain.

TABLE XI

VII BBB and Malnutrition

A. EFA DEFICIENCY

Various workers have investigated the effects of EFA deficiency on the structure of cell membranes giving evidence that the skin of rats deficient in EFA is more permeable to water than is that of normal rats (Basnayake and Sinclair 1956). It has been reported that EFA deficiency resulted in fragility of erythrocytes and capillaries (Kramar and Levine 1953). Applying these observations to the brain, De Pury and Collins (1963) have noted that EFA deficiency caused alteration in the cytoplasmic membrane structure leading to increased uptake of ^{32}P into brain phosphatidic acid. Gerstl et al. (1965) have proposed that Pelizaeus-Merzbacher Disease (familial centrolobar sclerosis) is caused by a genetically controlled deficiency of arachidonic acid. EFA deficiency had been implicated in multiple sclerosis (Sinclair 1956; Baker et al., 1963; Thompson 1966; Bernsohn and Stephanides 1967). Whether changes in myelin metabolism are primary or secondary to metabolic lesions occurring elsewhere is unknown (Smith, 1967); thus, whether EFA deficiency is an etiological factor is still a speculation at best. Galli et al. (1970) stressed the importance of nutritional factors during the very early period before and immediately after birth and found that EFA deficiency initiated in rats prior to birth and continued for one year produced profound changes in brain weight and lipid composition. White et al. (1971) however, found that animals that were EFA deficient even during the brain's most actively growing period, were able to recover completely on restoration to the control diet.

From this discussion, it seems that EFA deficiency could have some effect on the permeability of brain capillaries affecting the BBB. No direct

evidence has yet been presented.

B. OTHER DEFICIENCIES

Thiamine deficiency has been implicated in its effect on the proper functioning of the BBB by Warnock and Burkhalter (1968). When the labeling pattern of brain glutamic acid was studied after injecting 2-^{14}C pyruvate, it was found that in thiamine deficiency, pyruvate entered the brain because of malfunctioning of the BBB. In normal well fed animals, pyruvate did not permeate from the blood into the brain. This effect of thiamine deficiency may not be restricted to pyruvate. Whether this is the cause of the polyneuritis observed in thiamine deficiency in pigeons is also still speculative. More work along these lines will clarify some of the unknown parameters of BBB.

Damage to BBB by toxicants, such as mercury, has been studied by Steinwall (1959) and Steinwall and Snyder (1969).

VIII. Discussion of the Concept of the BBB as it Applies to Lipids

Considering all the evidence for direct uptake of fatty acids, lecithin and cholesterol one can generalize on several points. The restricted uptake of radioactivity from labeled lipids by the adult brain, as compared to the immature brain, may very well be explained on the basis of metabolic requirements and turnover rates (Dobbing 1968). We have conclusively shown uptake of labeled fatty acids by the adult brain *in vivo* without appreciable degradation to acetate elsewhere, followed by resynthesis in the brain. The magnitude of uptake in the adult seems to depend on the route of entry and the form in which the test compound is presented to the brain. For example, when the tracer was administered via a carotid injection, very rapid uptake

and incorporation into complex lipids was observed; in fact, the uptake by the brain was greater than by the liver. If the BBB had a profound influence on the uptake of lipids (fatty acids), the brain uptake would not be widely different when tracer was injected intravenously, intracarotidly or intraperitoneally. However, our data show that either when the liver is isolated from blood circulation or when the tracer is injected intracarotidly (thereby giving the brain the first chance to pick up the label) fatty acids were taken up readily by the brain. Our own studies, as well as those by Holzl and Frank (1969), have shown the uptake of the intact lecithin molecule. A small but definite uptake of ^{14}C cholesterol by adult animals has been shown by Kabara (1967). From all these findings, we return to the hypothesis proposed by Davson (1955) that, in general, lipid-soluble compounds are not restricted by the BBB and enter the brain much more freely than do water-soluble polar compounds. Thus, the term "Blood Brain Relationship" suggested by Dobbing (1968) is definitely more appropriate than the commonly used "Blood Brain Barrier" in describing lipid uptake by the brain.

IX. Summary

Long chain fatty acids - palmitic, oleic and the essential fatty acids (linoleic and linolenic) were taken up by the adult brain and incorporated into complex lipids including the so called "myelin lipids"- cerebrosides and sphingomyelin. The labeling pattern of fatty acids isolated from the brain showed that the resynthesis of fatty acids from acetate, itself derived by oxidative degradation of the administered carboxy-labeled fatty acid, was only a minor metabolic reaction, thereby proving direct uptake of the intact molecule. There is some indication that linolenic acid is taken up in slightly higher rate than linoleic acid by the brain, when the ratios of

radioactivity in the brain and liver are compared. This preferential uptake may result in higher concentration of the $\omega 3$ family fatty acids commonly found in the brain.

When labeled acetate or palmitate was injected into the carotid artery followed by decapitation within 15 seconds, there was greater uptake of radioactivity in the brain than in the liver. Brain palmitate as well as cholesterol was synthesized *de novo* from intracarotid injected acetate within 15 seconds, and the polar lipids were all labeled. This indicated a very rapid lipid metabolism *in vivo* in the adult brain contrary to earlier views.

Since the uptake of radioactivity in the brain was highest in animals whose plasma lipids contained a highly radioactive FFA component (hepatectomized and carotid-injected groups) as compared to animals whose plasma lipids contained either highly radioactive triglycerides (control group) or phospholipids (^{14}C lecithin injected group), it was concluded that FFA are the preferred form of transport into the brain.

The BBB did not influence the uptake of these lipid soluble compounds and so the lower incorporation of radioactivity into adult, as compared to young brain, can be explained on the basis of metabolic requirement and turnover. Actually if the brain is presented with a tracer via carotid injection, this age-dependent difference can be overcome. In the absence of any barrier effect, we strongly support the use of the term "Blood Brain Relationship" in relation to lipid metabolism in the brain.

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TABLE I

Incorporation of Sodium 1-^{14}C Acetate into Brain and Liver Lipids of Weanling (13 days old) and Adult Rats (3 months old), Four Hours after I. P. Injection

	Rats			Total Lipids			% Incorporation
	No.	Body wt. (av) g	Dose given µC/g body wt.	Wt. per g wet wt. of tissue (mg/g)	Sp. Act. cpm/mg	Radioact. per g wet wt of tissue	Radioact. per g tissue / Radioact. Administ. * X 100
BRAIN LIPIDS							
Weanling	10	30	5	51.8	27,752	1.44×10^6	12.9
Adult	6	310	5	107.5	4,744	5.1×10^5	4.6
LIVER LIPIDS							
Weanling	10	30	5	43.2	7,972	3.4×10^5	3.1
Adult	6	310	5	43.9	50,621	2.22×10^6	20.0

* µC/g body wt.

TABLE II

Incorporation of Sodium 1-^{14}C Acetate into Brain Fatty Acids of

Weanling and Adult Rats

Fatty Acid	% Composition of Total Methyl Esters by GLC		Specific Activity cpm/mg		Radioactivity in the Carboxyl Carbon (% of total)	
	Weanling	Adult	Weanling	Adult	Weanling	Adult
16:0	17.1	13.2	79,395	20,480	12.5	13.5
18:0	14.7	15.8	40,586	7,133	20.7	25.6
18:1	14.1	18.4	37,324	1,879	28.1	30.4
20:4	18.0	14.8	3,223	815	87.8	84.1
22:6	21.3	21.1	775	318	89.6	70.2

TABLE III

Incorporation of Radioactivity from 1-^{14}C Palmitic and 1-^{14}C Oleic Acid into Blood, Brain and Liver Total Lipids

Fatty Acid Administered	Route of Entry	Blood Total Lipids: Interval between Dose and Sacrifice Hrs	Blood Total Lipids: Sp. Act. cpm/mg	Blood Total Lipids: Plasma Radioact. cpm/ml	Brain Total Lipids: Sp. Act. cpm/mg	Brain Total Lipids: Total Radioact. cpm/g wet wt.	% Incorporation (Radioact./g wet Tissue / Radioact. Administered * X 100): Brain	% Incorporation: Liver
1-^{14}C Palmitic Acid	Oral Feeding as Oil Emulsion	4	21,688	60,509	125	13,287	2.39	----
		24	2,510	7,003	111	11,799	2.12	39.0
1-^{14}C Oleic Acid	I.V. Injection as Albumin Complex	4	4941	13,794	124	13,181	2.37	69.1
	Oral Feeding	24	1703	4,751	80	8,504	1.53	12.5

* 0.25 μc/g. body wt.

TABLE IV

Distribution of Radioactivity in Fatty Acids Isolated from Brain Polar Lipids 4 and 24 Hours after Administration of Tracer

Tracer	Interval between Dose and Sacrifice	Fatty Acid Isolated from Brain Polar Lipids (Pl. + SphL)							
		16:0		18:0		18:1		20:1	
	Hrs	Sp. Act. cpm/mg	% RCA	Sp. Act. cpm/mg	% RCA	Sp. Act. cpm/mg	% RCA	Sp. Act. cpm/mg	% RCA
1-^{14}C Sodium Acetate	4	----	13.5	----	25.6	----	30.4	----	49.8
1-^{14}C Palmitic Acid	4	2057	86.9	132	26.6	----	----	----	----
1-^{14}C Oleic Acid	4	----	----	40	53.5	1221	84.3	306	14.1
1-^{14}C Sodium Acetate	24	----	14.1	----	20.3	----	23.3	----	38.6
1-^{14}C Palmitic Acid	24	1805	85.1	243	21.6	----	----	----	----
1-^{14}C Oleic Acid	24	----	----	93	23.2	911	58.1	3145	10.1

TABLE V

Incorporation of Radioactivity from 1-^{14}C Linoleic and 1-^{14}C Linolenic Acid into Blood, Brain and Liver Total Lipids

Fatty Acid Administered	Dose Fed μc/g body wt *	Interval between Dose and Sacrifice Hrs	Blood Total Lipids Sp.Act. cpm/mg	Blood Total Lipids Plasma Radioact. cpm/ml	Brain Total Lipids Sp.Act. cpm/mg	Brain Total Lipids Total Radioact. cpm/g wet wt	% Incorporation (Radioact./g wet Tissue / Radioact. Administered* X 100) Brain	% Incorporation Liver
1-^{14}C Linoleic Acid	0.16	4	32,504	90,686	50	5315	1.5	81.4
	0.21	24	6,703	18,701	120	12,756	2.7	47.8
1-^{14}C Linolenic Acid	0.16	4	15,818	44,132	41	4358	1.2	44.7
	0.21	24	3,984	11,115	108	11,480	2.5	38.1

TABLE VI

Distribution of Radioactivity in Fatty Acids Isolated from Brain Polar Lipids 4 and 24 Hours after Administration of Tracer

Tracer	Interval	Fatty Acid Isolated from Brain Polar Lipids (Pl. + SphL)						
		18:2		18:3	20:4		22:6	
	Hours	Sp. Act cpm/mg	% RCA	% RCA	Sp. Act cpm/mg	% RCA	Sp. Act cpm/mg	% RCA
1-^{14}C Sodium Acetate	4	----	----	----	----	84.1	----	70.2
1-^{14}C Linoleic Acid	4	1439	92.0	----	99	28.1	52	68.4
1-^{14}C Linolenic Acid	4	----	----	91.0	----	----	42	23.0
1-^{14}C Sodium Acetate	24	----	----	----	----	83.3	----	81.9
1-^{14}C Linoleic Acid	24	6857	88.0	----	870	3.5	51	72.4
1-^{14}C Linolenic Acid	24	----	----	92.4	39	81.7	765	12.4

TABLE VII

Incorporation of Radioactivity from Carboxy Labeled Fatty Acids into BRAIN Lipids 4 Hours after the Dose.

1. Percent of Administered Dose Incorporated $\left(\frac{\text{Radioactivity per g Tissue}}{\text{Radioactivity Administered } *} \times 100 \right)$

2. Distribution of Radioactivity between Liver, Blood Plasma and Brain

Fatty Acid	% Composition of Total Fatty Acids by GLC (Av.)	Tracer	Amount Administered μC/g body wt. *	Brain Lipids		Ratio	
				Radioact. per g wet wt. of tissue cpm/g	% Incorporation	Radioact. per g tissue LIVER/BRAIN	Radioact. PLASMA/ml / BRAIN/g
16:0	13.2	1-^{14}C Acetate	5	5.1×10^5	4.6	4.0	----
18:0	15.8	1-^{14}C Palmitic Acid	0.25	13.3×10^3	2.4	48.0	4.5
18:1	18.4	1-^{14}C Oleic Acid	0.25	13.2×10^3	2.4	29.2	1.0
20:4	14.8	1-^{14}C Linoleic Acid	0.16	5.3×10^3	1.5	53.0	16.6
22:6	21.1	1-^{14}C Linolenic Acid	0.16	4.4×10^3	1.2	36.7	9.8

TABLE VIII

Comparison of Uptake of Radioactivity from Carboxy Labeled Fatty Acids Infused via Carotid Artery in Rabbits

Tracer Infused over a Period of 1 Hour	Blood Plasma cpm/ml	Whole Brain			Gray Matter			White Matter		
		cpm/g	% Uptake *	Ratio (cpm/g) Tissue/Plasma	cpm/g	% Uptake *	Ratio (cpm/g) Tissue/Plasma	cpm/g	% Uptake *	Ratio (cpm/g) Tissue/Plasma
10 μC 1-^{14}C Palmitate	2608	6939	0.95	2.66	11,116	1.5	4.26	7673	1.05	2.94
10 μC 1-^{14}C Linoleate	3156	676	0.09	0.21	743	0.1	0.23	547	0.07	0.17
10 μC 1-^{14}C Linolenate	1239	955	0.13	0.77	1049	0.14	0.85	903	0.12	0.73

* % Uptake = Radioact. per g Tissue X 100 / Radioact. Administered (0.33 μc / 100 g body wt.)

TABLE IX

Incorporation of Radioactivity from 1-^{14}C Acetate into Brain, Liver and Plasma Lipids 15 Seconds after Carotid Arterial Injection

	Total Lipids		
	Brain	Liver	Plasma
Sp.Act. cpm/mg	326	31	168
cpm/g tissue	35×10^3	1.2×10^3	467

Cholesterol	Polar Lipids
86 cpm/mg	294 cpm/mg

Total Fatty Acids

	Palmitic Acid	Stearic Acid
Sp.Act.	595	91
% RCA	13.4	43.1

TABLE X

Incorporation of Radioactivity from Carboxy Labeled Fatty Acids into Various Polar Lipids of the Rat Brain

Fatty Acid Administered	Route of Entry	Period between Dose and Sacrifice	Sp. Act. (cpm / mg)				
			PC	PE	PS	SPH.	CEREB.
1-^{14}C Acetate	Intraperitoneal	24 Hrs.	130	75	46	30	51
1-^{14}C Acetate	Intracarotid	15 Sec.	86	45	239	59	138
1-^{14}C Palmitic Acid	Intracarotid	15 Sec.	181	122	83	63	26
1-^{14}C Palmitic Acid	Oral Feeding	24 Hrs.	304	116	83	14	22
1-^{14}C Oleic Acid	Oral Feeding	24 Hrs.	266	156	71	42	67
1-^{14}C Linoleic Acid	Oral Feeding	24 Hrs.	179	79	79	38	71
1-^{14}C Linolenic Acid	Oral Feeding	24 Hrs.	101	84	76	23	100

TABLE XI

Uptake of Radioactivity by the Brain and Liver after Injection of 1-^{14}C Palmitic Acid and 1-^{14}C Di-Palmitoyl Lecithin

	Group	Period between Dose and Sacrifice	Blood Plasma Radioact.			Brain Lipids		Liver Lipids	
			% of TG	Total FFA	Radioact. PL	Sp.Act. cpm/mg	cpm/g wet wt.	Sp.Act. cpm/mg	cpm/g wet wt.
I	Control (I.V. Inj.)	1 Hr.	77.7	10.9	1.6	49	5242	6836	276,188
II	Hepatectomized (I.V. Inj.)	1 Hr.	10.6	77.4	11.9	314	36,688	243	9556
III	Intra Carotid Injection	15 Sec.	0.7	90.1	2.3	878	93,331	1831	73,956
IV	I.V. Inj. of 1-^{14}C Di-Palmitoyl Lecithin	1 Hr.	9.6	1.8	62.3	24	2019	8760	353,904