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# Lipid Metabolism of Neurons and Neuroglia

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# LIPID METABOLISM OF NEURONS AND NEUROGLIA

By

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# Introduction

## A. Background

For almost one hundred years, it has been known that the mammalian nervous system is composed of a variety of cell types, each of which sends out complex branching processes that intertwine with each other to form a mesh-like pattern of nervous tissue (for a discussion see Peters, et al, 1970; Cragg, 1968). Thus any section of nervous tissue, even if it is from a well defined anatomical region is composed of a heterogeneous mixture of cells and their processes. There are two major types of cells, the neurons which carry the actual nervous impulse, and the neuroglia, of which there are two major kinds, the astrocytes, whose function is largely unknown, and the oligodendrocytes, the cells presumably responsible for the formation and maintenance of the myelin sheath (Bunge, 1968, 1970). Estimates have been advanced concerning the number of glial cells in the brain and the figure varies from brain region to brain region and between histologists (Brizzee, et al, 1964, Jacobson, 1970).

Any compositional or metabolic experiments on nervous tissue will result in data that reflects the net average of all the cells and cell processes in the tissue sample. Methods have been developed to isolate the various functional units from nervous tissue such as cell soma (see below) and even parts

of cells such as myelin (Autilio, et al, 1964), axons (DeVries, et al, 1972) and synaptosomes (Whittaker, 1964, deRobertis, et al, 1961). (For discussion of these various methods see Appel, et al, 1972, Whittaker, 1969, deRobertis and Arnaiz, 1968).

This work was undertaken for the purpose of elucidating the roles of neurons, astrocytes and oligodendrocytes in the overall pattern of lipid metabolism of the brain. Lipids were chosen because of their importance in membranes (Nicholson and Singer, 1971, Bretschler, 1973) and membranes are particularly essential to the functioning of the nervous system; as this is where the actual conduction of information takes place. Brain contains more lipid than any other organ. Over half of the dry weight of the brain is lipid. On a total weight basis, the brain is 11% lipid while skeletal muscle contains only 5% lipid. (McIlwain and Bachelard, 1971). Most of this brain lipid is in the white matter which contains 55% lipid to 39% protein (Norton, 1972) on a dry weight basis, but even grey matter has a higher lipid to protein ratio than skeletal muscle, 0.8 as compared to 0.26 (McIlwain and Bachelard, 1971). Thus even omitting myelin which has a lipid to protein ratio of from 2.3 to 3 (Norton, 1972), lipids play a large role in the molecular structure of nervous tissue.

B. Brain Cell Preparations

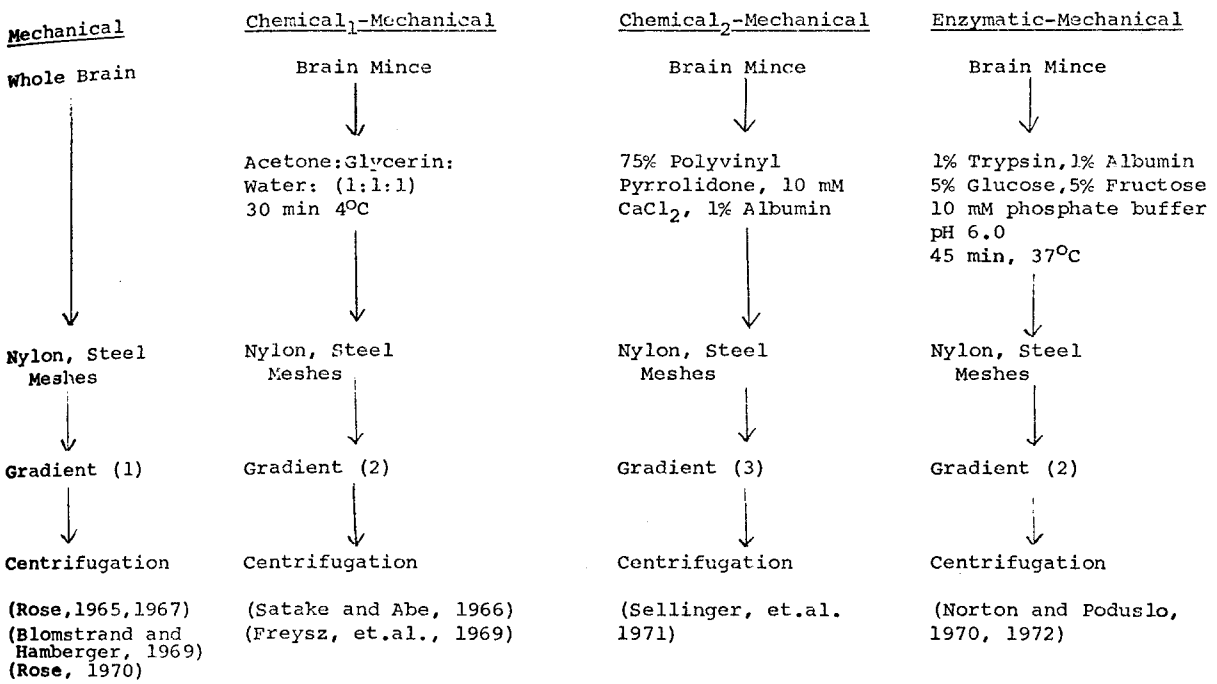
Four basic methods have been published for the isolation

of brain cell types (Figure 1). They are the mechanical, the chemical-mechanical (of which there are two) and the enzymatic-mechanical. They differ primarily in the pretreatment of the tissue (or lack of pretreatment) before disruption of the tissue into a cell suspension by sieving. There are also differences in the media employed for preparing the suspension, the material used for the gradients (Ficoll or Sucrose), the density of the gradients and the speed and time of centrifugation.

Saul Korey and his associates (1958a, 1958b and 1959) developed a method to produce a neuroglial fraction from white matter by two basic steps (a) disrupting minced tissue into a cell suspension by forcing it through meshes of small pore size and (b) separating the cell types on a discontinuous density gradient. These two basic steps are employed in all of the currently available cell separation methods. Stephen Rose (1965, 1967, Rose and Singh, 1970) developed a method to obtain both neuronal perikarya and neuroglia from whole rat brain utilizing a media of 10% Ficoll, 100mM KCl, 10mM K-phosphate, pH 7.4 to disrupt the tissue in, and a "Ficoll" density gradient for centrifugation. Ficoll is a high molecular weight (400,000) polymer of sucrose which offers the advantage of high density, low osmolarity gradients. Rose's method was altered by Blomstrand and Hamberger (1969) who modified the density

# Figure 1

SUMMARY OF AVAILABLE METHODS FOR THE PREPARATION OF BRAIN CELL FRACTIONS



gradient and placed the cell suspension in the center of the gradient so that the neurons sedimented down and the astrocytes floated up. In later experiments, Blomstrand and Hamberger (1970) found that the morphology and yield of cells is improved if the tissue is first incubated at 37° prior to sieving. Presumably during the preincubation endogenous proteases are released and some intercellular bonds are weakened making the tissue easier to sieve.

Satake and Abe (1966) introduced the step of pretreatment of the brain mince before disruption of the tissue into a suspension by placing the minced tissue into cold glycerol:acetone:water (1:1:1) on ice for thirty minutes. The treated tissue was then washed free of the acetone-glycerol-water and a cell suspension prepared by sieving. In this way Satake and Abe (1966) were able to obtain a neuronal preparation. Freysz, et al (1968) modified the Satake and Abe procedure to produce both neuronal soma and neuroglia by adding a second density gradient centrifugation to purify the glial fraction obtained from the first step. The major disadvantage with this method is that the cells obtained are not viable and also membrane lipids may be extracted by the glycerin-acetone-water.

Norton and Poduslo (1970) used a trypsin pretreatment and a salt free isolation media containing 5% glucose, 5% fructose, 1% albumin and 10 mM phosphate buffer at pH 6.0. They have

also modified their method (Poduslo and Norton, 1972a) so that by starting with calf brain white matter and using a slightly different gradient, an oligodendroglia fraction can be obtained.

Sellinger et al (1971) devised a method involving the use of polyvinylpyrrolidone in the media for preparing the cell suspension. Zonal rotors have also been utilized by several laboratories in place of the discontinuous density gradients (Flanges and Bowman, 1968, Giorgi, 1971).

Rose (1967) and Satake and Abe (1966) recovered 7% and 6-18% respectively of the $17.4 \times 10^6$ neurons per rat brain obtained by Norton and Poduslo (1971) with their method. The figure of $17.4 \times 10^6$ neurons per brain probably represents 15-20% of the total neurons. Norton and Poduslo (1971) also recovered $3.4 \times 10^6$ astrocytes from each rat brain or approximately 3.5% of this cell population. Neither Rose (1967) nor Satake and Abe (1966) reported recoveries of glial cells.

On the basis of recovered protein, the Sellinger technique yields 11% (10 day old rats) to 1.44% (18 day old rats) to 0.68% (43 day old rats) of the recovered protein in the neuronal fraction and only 0.067% to 0.042% in the glial fraction. Norton and Poduslo (1971) recovered 11% of the DNA which equals about 11% of the cells. Since some of the brain protein is non-cell soma protein, i.e. myelin, processes, synaptosomes, etc., the Sellinger yield of neurons from 10 day old rats is probably

greater than the Norton-Poduslo yield. However, the neuronal recovery at the later ages, and most important, the very low glial yield make the method less desirable if one wants to compare neurons and glia.

Johnston and Roots (1965) found that gangliosides stabilized the plasma membrane of neurons during a micro-isolation procedure and it is possible that in the Norton-Poduslo technique, the albumin serves a similar function. In the process of isolating plasma membranes from mouse fibroblasts and other cells, Warren et al,(1967) found that a variety of substances and conditions, including a low pH, stabilized the plasma membranes of the cells they were working with. Thus, it appears that the combination of albumin and low pH used in the Norton-Poduslo technique may be optimal for stabilization of the cells while allowing disruption of the tissue. These factors, plus the digestion of intercellular proteins by the trypsin pretreatment, which makes it easier to disrupt the tissue into a cell suspension without breaking the cells, probably account for the higher yield of cells by the Norton-Poduslo method as compared to the other available techniques.

For the work of this paper, the Norton-Poduslo method was used for the following reasons: 1) it is possible to obtain the three cell types, neurons, astrocytes and oligodendrocytes, from calf brain, 2) the cells are intact on the basis of electron

microscopy; subcellular organelles and the plasma membranes are well preserved (Raine, et al, 1971), 3) on the basis of phase microscopy, the results obtained by us (See Results Section) were very similar to those of Poduslo and Norton (1972b) and 4) the method offers the highest yield of both cell types, thus allowing routine chemical analysis on the fractions.

C. Lipids of Neurons and Glia.

The available isolation of techniques have been used by several authors for the purpose of studying the lipid composition of neurons and glia. Fewster and Mead (1968 a,b) used an isolation method similar to that of Rose (1967), but starting with bovine white matter to obtain a white matter glial fraction (Fewster, et al, 1967). The cells were found to be composed of 74% protein and 26% lipid. Norton and Poduslo (1972) found oligodendroglia, also isolated from white matter to be 30% lipid on a dry weight basis. Fewster and Mead (1968b) also investigated the fatty acid and fatty aldehyde composition of the glial cell glycerophosphatides. The cell fatty acids had a composition similar to that of white matter and myelin, namely few long chain polyunsaturated fatty acids (20:4, 22:4, 22:6).

Norton and Poduslo (1971) and Hamberger and Svennerholm (1971) determined the lipid composition of their neuronal and glial preparations, the former from rat brain and the latter from rabbit cerebral cortex. Both groups found approximately

50% more lipid per cell in the neuronal soma than the glial fraction, and more cholesterol and ganglioside in the glial fraction. Although Hamberger and Svennerholm (1971) found twice the amount of glial ganglioside as compared to neurons, Norton and Poduslo (1971) found almost eight times more ganglioside in the glia than in the neurons. The phospholipid composition was found to be similar in both cell fractions by both groups, with choline phosphoglycerides present in the greatest amount followed by ethanolamine phosphoglycerides, inositol and serine phosphoglycerides. Since the isolated neurons lose their processes, during the isolation procedures, while the glia retain extensive, thin highly branched processes, it is possible that the higher lipid concentration of the glial fraction reflects the higher proportion of membrane per cell. Raghavan and Kanfer (1972) confirmed the findings of Norton and Poduslo (1971) with regard to the higher lipid concentration of the glial fraction, and they also found differences in the fatty acid composition of the neuronal and glial ceramide galactoside, the latter containing more $\alpha$-hydroxy fatty acids and long chain (C>20) non-hydroxy fatty acids.

Studies of the metabolism of lipids of the cell fractions may help to clarify the functional roles of the cell types in the nervous system and also the role of lipids in the structure and function of the cells. The first approach to the study of

neuron-glial lipid metabolism was a rather ingenious method devised by Torvik and Sidman (1965). With conventional autoradiographic methods, use of general precursors such as (1-$^{14}$C) acetate or (1-$^{14}$C) serine does not permit the study of lipid metabolism, since the tracers will be incorporated into other classes of molecules such as proteins and carbohydrates. Thus, it is impossible to ascertain whether a grain is from a lipid or a protein molecule. Torvik and Sidman (1965) circumvented this difficulty by preparing two adjacent thin sections from brains of mice that had been injected intraperitoneally with a radioactive tracer and then removing the lipids from one of the sections. Both sections were then developed for autoradiography. The grains present in the non-delipidated section and absent in the delipidated section represent lipid radioactivity. They found in vivo incorporation of (1-$^{14}$C) acetate was most rapid in the neuronal soma, followed by the neuropil and slowest in white matter. The neuropil includes astrocytes, dendrites, axons and synapses. Likewise, white matter is composed of oligodendrocytes and myelinated axons. Thus, it is understandable that the white matter was the least active since the bulk of it is myelin, which has a slow turnover for most of the lipids (Davidson, 1968).

Freysz, et al (1969) injected $^{32}$P labelled inorganic phosphate intraperitoneally into adult rats and isolated neuronal

soma and glial fractions by their acetone-glycine-water pre-treatment method. From the first to about the seventh day after injection, the neuronal phospholipids had a specific activity 50% greater than that of the glia. After the ninth day after injection, the specific activity of both cell phospholipids was the same. The neuronal phospholipid specific activity reached its peak on the fourth day and the glial cell fraction on the seventh day after injection. Thus Freysz, et al, (1969) concluded that with $^{32}P$ labelled inorganic phosphate, in the adult rat, the neuronal phospholipids are turning over faster than those of the glial fraction. Within the phospholipid classes, phosphatidylcholine was found to turn over more rapidly than phosphatidylethanolamine in both cell fractions. Since different parts of the phospholipid molecule (the glycerol backbone, phosphate, fatty acids and choline or ethanolamine, etc., base) turn over at different rates (Lennarz, 1970 and McMurray and McGee, 1972), it is necessary to study the other parts of the phospholipid molecule in order to conclude that neuronal phospholipids turn over at a greater rate than glial phospholipids. Raghavan, et al (1972a) utilized the Norton-Poduslo method and studied the <u>in vitro</u> incorporation of ($^{14}C$) serine, ($^{14}C$) ethanolamine and ($^{14}C$) choline into phospholipids of neuronal soma and glial fractions of rat brain, by the $Ca^{++}$ dependent base exchange reaction. This is the reaction in which

the base moiety of the phospholipid (for example, phosphatidylcholine) is exchanged with a free base (like ethanolamine) to produce a different phospholipid and a free base (in this example, phosphatidylethanolamine and free choline). They found little or no difference between the cell fractions, probably reflecting similar levels of the base exchange enzymes in the two cell types. However, the experiments were performed with rats ranging in age from 13 to 20 days and since the rat brain is rapidly maturing during that time period, it is possible that differences would have been found if only one age was studied. Raghavan et al, (1972a) also found that (U-$^{14}$C) glucose, glycerol and acetate were incorporated equally into the lipids of the isolated cells.

Goracci,et al, (1973) used the Blomstrand and Hamberger method of cell preparation with adult rabbits. They found that the $Ca^{++}$ dependent incorporation of (3-$^{14}$C) serine and (1,2-$^{14}$C) ethanolamine into phospholipids was 6-8 times greater in neuronal plasma membranes and microsomes than in the corresponding glial subcellular fractions. In a later study, the same group of workers (Bingalia et al, 1973) studied the incorporation of phosphorylcholine, phosphorylethanolamine, cytidine-5'-phosphate choline and cytidine-5'phosphate ethanolamine into phospholipids of isolated neurons and glia. Both CDP-choline and CDP-ethanolamine were incorporated into neuronal cholinephosphoglycerides

(CPG) and ethanolaminephosphoglycerides (EPG) respectively, five to nine fold more rapidly into the corresponding glial lipids. The same was true when the precursors phosphoryl-ethanolamine and phosphorylcholine were used, although these precursors were incorporated at a rate of about 1 to 2% of that of the corresponding cytidine nucleotides. The activity of the enzymes choline-phosphotransferase (E.C.2.7.8.2) and ethanolamine phosphotransferase (E.C. 2.7.8.1) were also studied. The $K_m$ for cytidine 5'-phosphate ethanolamine and diacyl glycerol, the pH optimum and requirement for divalent cations were similar in the two cell types. However, the $K_m$ for cytidine 5'-phosphate choline was lower ($2.3 \times 10^{-4}$M compared to $1 \times 10^{-3}$M) in glia than in neurons. The authors were not sure if this could be interpreted to mean that the glia possessed a different enzyme than the neurons. They concluded that the cytidine-dependent enzyme system for choline and ethanolamine phosphoglyceride synthesis was more concentrated in the neuronal fraction, as compared to glia.

The enzymes galactocerebroside galactosidase and ceramide galactosulfotransferase were found to be present at similar specific activities while ceramide glucosyltransferase, an intermediate enzyme in ganglioside synthesis was found only in neurons (Radin, et al, 1972). Jones, et al (1972) found that neurons incorporated DL (3-$^{14}$C) serine and N-acetyl (4-$^{14}$C)

neuraminic acid into gangliosides to a greater extent than the glial enriched fraction, supporting the conclusion of Radin et al, (1972) that ganglioside biosynthesis may be localized in neurons. Raghavan et al, (1972b) studied the acid hydrolase β-galactosidase, β-glucosidase, α-galactosidase, α mannosidase, N-acetyl-β-glucosaminidase, N-acetyl-β-galactosaminidase, acid phosphatase, β-glucuronidase, arylsulfatase, glucocerebrosidase and galactocerebrosidase and found all of them to be present at similar specific activities in both cell fractions.

Phospholipase $A_1$ and $A_2$ activities of the neuronal and glial fractions were studied by Woelk et al, (1973). The found different pH optima for the two enzymes in the neurons and glia and an 8-fold greater activity for phospholipase A, and 5-fold greater activity for phospholipase $A_2$ in the neurons as compared to the glia.

In conclusion then, there are differences in the lipid composition and metabolism of the neuronal and glial cell fractions, but whether or not these differences are related to the functions of the cells, is yet to be determined.

## Materials and Methods

### A. Cell Isolation Procedure.

#### 1. Neuronal Soma-Astroglia and Myelin Preparations

For the preparation of neuronal soma and astroglia, the starting material is either ten rat brains minus cerebellum (10 days old), six rat brains minus cerebellum (21 days old) or from ten to fifteen grams of calf brain grey matter. The tissue is minced finely (1 $mm^3$ pieces) with a scalpel in a watch glass on ice in a medium of 5% glucose, 5% fructose, 1% ablumin and 10 mM $KH_2PO_4$-NaOH buffer pH 6.0. (Hereafter referred to as HAP). The sugars are of AR grade from Mallincrodt Chemical Works, St. Louis, Mo. and the alubmin is Cohn fraction V from Sigma Chemical Co., St. Louis, Mo. The minced tissue is incubated at 37°C for one hour in HAP (10 ml per gram of tissue) with either 1% trypsin (rat brain) or 0.5% trypsin (calf grey matter). The trypsin is 2x crystallized from bovine pancreas, salt free, from Nutritional Biochemicals, Inc., and the trypsin solution is made fresh just prior to use and filtered. All solutions including HAP, trypsin and sucrose are readjusted to pH 6.0 as necessary.

Following the incubation, the mince is cooled to 0° on ice and a mixture of 90% calf serum, 10% phosphate buffer (10 mM pH 6.0) is added, 1 ml for every 5 ml of trypsin solution. (The calf serum is fetal, lyophilyzed from Sigma Chemical Co., 1.7

grams of the powder is added to 45 ml of $H_2O$ and 5 ml of the buffer is added). All further steps are carried out at 0-4°C. The tissue is washed free from the trypsin by centrifugation at 120 x g for five minutes, discarding the supernatant and resuspending the tissue in fresh HAP media. This is repeated twice.

The washed tissue is then forced through a 150 micron nylon mesh by stroking with a glass rod and applying a gentle vacuum under the mesh. The meshes can be held either over a Hirsh funnel or in a Millipore filter funnel (No xx20-047-20). While disrupting the tissue, 90 ml of a solution of 0.85 M sucrose in HAP is added to keep the tissue wet and prepare the suspension of cells for density gradient centrifugation. The original Norton-Poduslo method calls for preparing the crude suspension in plain HAP, but it was found that with the older rat brains the myelin sedimented to the bottom of this layer and prevented the cells from going through, thus reducing the yield. Suspending the disrupted tissue in 0.85 M sucrose allows the myelin to float to the top and not hinder the sedimentation of the cells. This is the only significant departure from the original Norton-Poduslo method. Following disruption of the tissue, the suspension is filtered through a stainless steel mesh of 74 micron pore size. This is repeated from three to five times and monitored by phase microscopy to ensure that disruption of the tissue is complete.

Fifteen ml of the cell suspension is then layered over a gradient of 5 ml 2.00 M sucrose, 5 ml of 1.55 M sucrose, 5 ml of 1.35 M sucrose and 8 ml of 0.9 M sucrose in the 39 ml tubes of the IEC SB-110 or Spinco SW 27.1 rotor, both of which hold six such tubes. The sucrose solutions are made up in HAP. These gradients are then centrifuged for ten minutes at 3300 x g (5000 rpm).

The neuronal soma layer on the 2.00 M sucrose and require no further purification. The astrocytes layer on the 1.35 M sucrose. Both cell types are removed with a syringe and a 16 gauge needle. The crude astrocytes from the first gradient are diluted slowly to 90 ml with HAP. Twenty-two ml of this is then layered on a gradient of 5 ml 1.40 M sucrose and 10 ml 0.9 M sucrose; four such tubes are used. This second gradient is centrifuged for twenty minutes at 3300 x g. The purified astrocytes layer over the 1.40 M sucrose. Both cell types are collected by diluting the suspensions from the sucrose gradients slowly to 160 ml with HAP and centrifuging at 630 x g for 20 min. The cells collect in the pellet.

In those experiments where myelin was prepared, the floating layer from the 0.85 M sucrose was removed after centrifugation. Ten to twenty volumes of ice cold deionized water was added, the material was mixed well and allowed to sit on ice for 20 minutes. The myelin was then collected by centrifugation at

12,000 x g for ten minutes. The crude myelin went into the pellet.

2. Oligodendrocyte Preparation

The procedure for the preparation of oligodendroglia is similar to that for neurons and astrocytes and where there are differences they are described in the following paragraph.

The starting material is 50-60 grams of calf brain white matter. This tissue is minced finely with a scalpel and incubated in 200 ml of 0.1% trypsin in HAP at 37°C for 90 min. Following incubation, the mixture is cooled and the tissue washed free from trypsin solution by centrifugation (three times). The washed tissue is then sieved through the nylon and steel meshes. Forcing white matter through the nylon mesh is more difficult than grey matter or rat brain, the screen clogs and must be changed 2-3 times or a larger piece of mesh must be used. The suspension is made up in 280 ml of 0.9 M sucrose. Twenty-two ml of this suspension is layered over a gradient of 5 ml each of 1.55 M sucrose, 1.40 M sucrose and 0.9 M sucrose. As before, all sucrose solutions are made up in HAP. These gradients are centrifuged at 3300 x g for ten minutes. Two such runs are made to accommodate all of the cell suspension. The oligodendroglia layer on the 1.55 M sucrose, and are diluted and pelleted as before. The myelin floats on the 0.9 M sucrose and is prepared as is the rat myelin. Poduslo and Norton, (1972b) have discussed

these methods extensively.

B. Lipid Methodology

Lipids were extracted by the method of Folch et al, (1957), by placing the tissue in chloroform:methanol (2:1) and adding 0.2 volumes of 0.9% saline. All solvents were of AR grade or better (redistilled in glass). For the acetate experiments, the lower phase was washed five times with theoretical upper phase (chloroform:methanol:water, 3:48:47) to remove all free acetate. In the fatty acid experiments, the lipid extract was washed three times. Lipid classes were separated by column and thin layer chromatography. The column was 0.5 cm x 3.0 cm (Rouser et al, 1967) and made of silicic acid. The washed lipid extract was taken to dryness, dissolved in a small amount (5 ml) of chloroform and applied to the column. The neutral lipids (cholesterol, mono, di and triglycerides, free fatty acids) were eluted with 150 ml of chloroform. The glycolipids (cerebrosides and sulfatides) were eluted with 300 ml of acetone and the phospholipids with 150 ml of methanol. The unincorporated free fatty acid was found in the chloroform fraction. The final 20 ml of all three fractions contained no radioactivity. When the phospholipid fraction from a fatty acid experiment was applied to a thin layer plate (see below) and developed, the solvent front contained little or no radioactivity and the specific activity of all the phospholipids eluted from the TLC

plate was within 20% of the value obtained from the total column fraction. This indicates that the radioactivity in the phospholipid fraction was not due to free fatty acids. An aliquot of the phospholipid was taken for liquid-scintillation counting in a Packard Model 3003 liquid scintillation counter, using 5 gm/100 ml PPO and 100 mg/100 ml POPOP as a scintillation fluid. The lipid aliquot was taken to dryness directly in the scintillation vial. Counting efficiency was 85% as determined by an internal $^{14}C$ toluene standard. A second aliquot was removed for phosphorus determination by the method of Bartlett (1959).

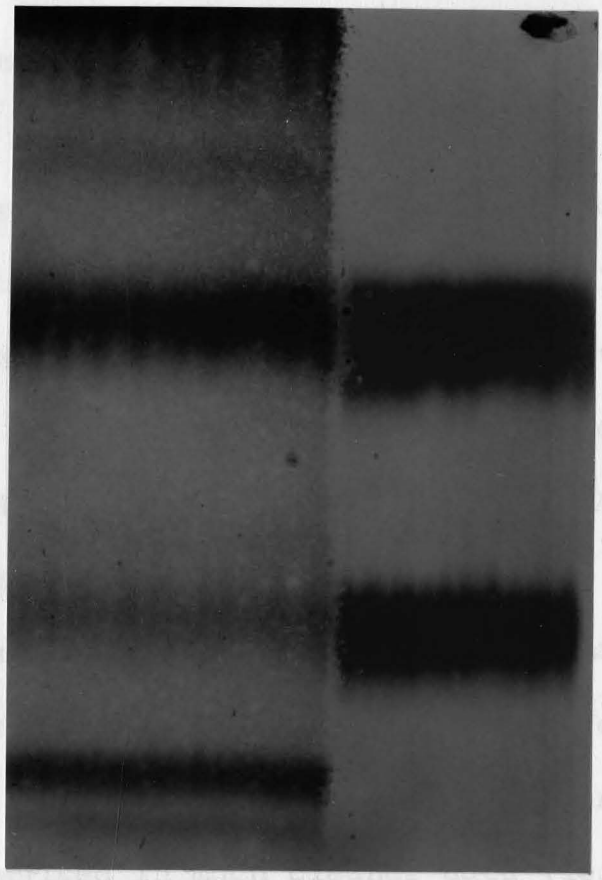
The remainder of the phospholipids was evaporated to a small volume (0.2 ml) and streaked across a Silica Gel HR (Brinkmann) thin layer plate (0.3 mm thickness, for a description of the plate preparation see Skipski, 1968) and was developed in chloroform--methanol:acetic acid-water (60:35:2:2), and then air dried for 10 minutes. In order to detect the lipid classes, 7/8 of the plate was covered with a glass plate and the exposed section was sprayed with 50% $H_2SO_4$. The glass plate was then moved to uncover an additional 1/8 of the plate (1/4 was now exposed) and the chromatogram was sprayed again, this time with ninhydrin. The plate was then put in an oven at 120°C for ten minutes and then air cooled. The result was one channel

sprayed to detect all the lipids ($H_2SO_4$) and another sprayed to detect the lipids which give color with ninhydrin such as ethanolamine and serine phosphoglycerides, with 3/4 of the plate remaining colorless. Identification of the lipid classes was checked with authentic standards (Supelco, Inc.) and the reaction with ninhydrin. In this way, ethanolamine-inositol-, serine-, choline- phosphoglycerides and sphingomyelin could be located. A sample plate is shown in Figure 2. The lipids from the unsprayed half were then scraped off and either eluted from the silica gel by the method of Skipski et al, (1964) or placed directly into a scintillation vial for counting. In those experiments where the specific activity of the phospholipid class was determined, an aliquot of the eluate was taken for liquid scintillation counting as above and one aliquot was used for phosphorus determination by the method of Bartlett, (1959). In the experiment where two dimensional TLC were used, the method was that of Rouser et al, (1967), with solvent system as shown on the page (Figure 10).

Fatty acids were analyzed by gas-liquid chromatography. The phospholipid fraction from the column was taken to dryness in a glass tube, fitted with a teflon lined screw cap. Methyl esters of the fatty acids were produced by the addition of two ml of 14% $BF_3$ in methanol (Supelco, Inc.), the air flushed out with nitrogen and the tube sealed. The sealed tube was then

Figure 2

Thin-layer Chromatogram of Rat Brain Phospholipids



Ten mg of rat brain phospholipids were streaked across a silica gel HR plate and the plate developed in $CHCl_3$-$CH_3OH$-HAc-$H_2O$ (60:35:2:2). The right side was sprayed with ninhydrin (0.2% n-butanol) and the left with 50% $H_2SO_4$. The lipids are 1. sphingomyelin, 2. choline phosphoglycerides (CPG), 3. serine phosphoglycerides (SPG), 4. inositol phosphoglycerides (IPG) and 5. ethanolamine phosphoglycerides (EPG).

placed in a boiling water bath for five minutes. Following methylation, the tube was cooled on ice and an equal volume of NaCl saturated water added. Five ml of redistilled pentane was added and the mixture shaken. The pentane was removed and a second five ml added, the tube shaken and the pentane removed. This was then repeated a third time. The procedure, when applied to a standard amount of radioactive (1-$^{14}$C) linoleic acid, gave a recovery of 95% of the radioactivity in the pentane fraction, and of this 90% could be recovered off of the GLC. (See below). The fatty acid methyl esters (FAME) were then purified by evaporating the pentane almost to dryness and applying the FAME to a small silicic acid column made in a Pasteur pipette plugged with glass wool. The methyl esters were then eluted with 4% petroleum ether in pentane (5 ml). This purification step resulted in much cleaner data (less "tailing" of the radioactivity) from the GLC. Typically, a yellow unidentified residue was left on the silicic acid column. The purified fatty acid methyl esters were analyzed on either a Varian 2100 with a flame-ionization detector attached to a Varian 1200 digital integrator to determine peak areas and percent distribution of the phospholipid fatty acids or a Varian 90-P with a thermal conductivity detector. The latter was attached to a Packard 850 automatic fraction collector and the fatty acids were collected

in Dow 550 coated terphenyl cartridges. After collection, the terphenyl was placed in scintillation fluid for counting. The results were expressed as percent of total fatty acid radioactivity. For both instruments the column material was 10% diethyleneglycol succinate with gas chrom Q as the solid support. The column temperature was a constant 190°C with the detector and injector at 250°C. The carrier gas was Argon for the Varian 2100 and helium for the Varian 90-P. The fatty acid peaks were identified by the relative retention time of the peak compared to stearic acid. Authentic standards (Supelco, Inc.) were run in order to obtain the relative retention time of each fatty acid. A typical gas chromatograph is in Figure 3.

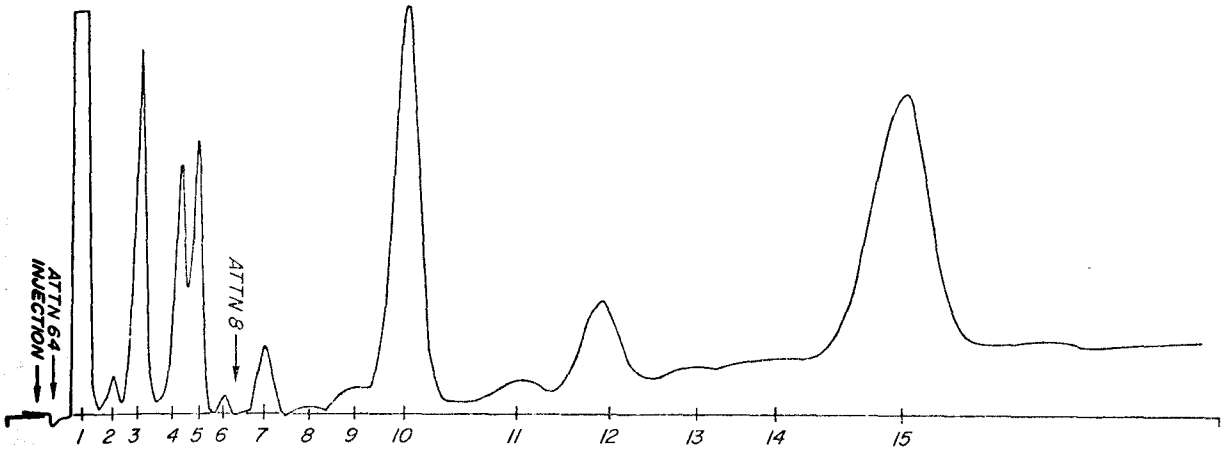
Decarboxylation of the fatty acids was accomplished by the method of Goldfine and Block (1961).

C. Incubations and Injections

Isolated cells and slices were incubated in a Krebs-Ringer medium composed of 123 mM NaCl, 6 mM KCl, 1.5 mM $MgSO_4$, 12 mM glucose and 20 mM phosphate buffer pH 7.4. Slices were incubated in this medium alone with the precursor. Cell incubations (except those in synthetic tissue culture media) were performed in the same media with 1% albumin added. The albumin was added because it was found that it helped to prevent the cells from breaking up during the incubation. The changing of the cell media from the EAP of isolation to the Krebs-Ringer plus albumin of incubation is a

Figure 3

GAS CHROMATOGRAPH OF WHOLE RAT BRAIN PHOSPHOGLYCERIDE FATTY ACIDS




Lipids were extracted, phospholipids purified, and fatty acid methyl esters of the phospholipids prepared as described. Approximately 100 micrograms were injected into the Varian 90-P to obtain this chromatogram. The conditions for the gas chromatography are described under Materials and Methods. The helium flow rate was 30 ml/min. Initial electrometer attenuation was 64. This was changed to 8 after peak 6.

The peaks are: 1. solvent, 2. 14:0, 3. 16:0, 4. 18:0, 5. 18:1, 6. 18:2, 7. 18:3 + 20:1, 8. 20:2, 9. 20:3, 10. 20:4, 11. 20:5 + 22:3, 12. 22:4, 13. 22;5 (n-6), 14. 22:5 (n-3), 15. 22:6 (n-3).

crucial step. After pelleting the cells from the HAP by centrifugation, the HAP is poured off and the inside of the tube is cleaned with a cotton swab to remove traces of HAP. Then, keeping the cells in a compacted pellet and ice cold, the new medium is slowly poured over the pellet of cells so as not to disrupt the pellet. After 15-20 minutes on ice, the cells can be dispersed by gently swirling the tubes and the incubation can then be initiated. After a five minute preincubation, the radioactive tracer was added.

All radioactive compounds were from Amersham-Searle and had specific activities of from 55 to 60 mCi/mM. (1-$^{14}$C) sodium acetate was obtained as the dry powder and dissolved in 0.9% NaCl. Appropriate quantities of this were added directly to the incubation mixture. Typically 1 ml of saline was added to 1 mCi of (1-$^{14}$C) sodium acetate to give 1 uCi per microliter.

The fatty acids were added to the incubations as the albumin complex. The fatty acid in benzene solution, was taken to dryness and an equimolar amount of 0.01 N KOH added. From 0.1 to 0.3 ml of a 12% albumin solution in saline was added to the test tube of fatty acid and the mixture sonicated for three, thirty second periods, keeping it on ice the whole time. This procedure solubilized all of the radioactivity. Aliquots were then added to the incubation mixtures or injected intracranially into rats. In some experiments isolated cells were incubated in the

media described and the lipids then extracted and the analysis performed. In other experiments, slices from rat brain were incubated first and the cells then isolated by the Norton-Poduslo method. Following isolation, the cell lipids were extracted and analyzed.

The experiments to study the effect of trypsin on the incorporation of acetate and linolenate into EPG and CPG were performed in the following way. Fifteen (linolenate experiments) or thirty (acetate experiments), brains and livers from 10 day old rats were sliced, pooled and divided into six aliquots. Six different types of incubations were then performed. The control (A) was incubated for one hour in the incubation media described above at 37°C with 10 μCi of (1-$^{14}$C) sodium acetate. The total volume was five ml. In (B) the same type of incubations was performed but with the addition of 8 mg ATP 2 mg NADPH, 1 mg NADPH and 1 mg CoASH. For (C) and (D) the brain or liver samples were first preincubated for 45 min at 37° in HAP, then washed free from the medium as described for cell preparations and the slices then incubated as in (A) and (B) respectively. (E) and (F) were done as (C) and (D) but with 1% trypsin in the HAP.

Following incubation, the lipids were extracted in the usual way, column and thin layer chromatography performed and specific activities determined.

The methodology for the <u>in vivo</u> studies was as follows:

The intracranial injections were done with a Hamilton 50 ul syringe into the skull at a midline point between the eyes and ears. Twenty-three to twenty-five rats were injected for experiments where only sixteen were needed. Upon sacrifice at the appropriate time interval, those animals with excess intracranial blood at the site of injection were discarded. The brains from eight of the remaining rats were mixed with fifty grams of calf brain white matter which was used as a carrier for the preparation of oligodendrocytes in the usual way. Both oligodendrocytes (a mixture of calf and rat cells) and a myelin fraction that contained both rat and calf myelin mixed together were obtained. The specific activities of the phospholipids of both of these fractions was obtained. Another eight rat brains were used for the preparation of rat neuronal soma, astroglia and myelin. The phospholipid specific activities of these three fractions were then determined in the usual way. By dividing the specific activity of the rat myelin (S.A. rat myelin) obtained from the neuron-astrocyte preparation by the specific activity of the mixed rat plus calf myelin (S.A. rat + calf myelin) obtained from the oligodendrocyte preparation, a dilution factor (d.f.) was obtained that represents the number of times the rat myelin was diluted by the calf myelin in the mixed preparation. Thus d.f. = $\frac{\text{S.A. rat myelin}}{\text{S.A. rat + calf myelin.}}$ If, for example, the dilution factor turned out to be 30, this would

mean that the radioactive rat myelin was diluted thirty times by unlabeled calf myelin. Now, if one makes the assumption that the rat oligodendroglia are diluted by calf oligodendroglia by the same amount the rat myelin is diluted by the calf myelin, then the dilution factor can be multiplied by the specific activity of the mixed calf + rat oligodendrocyte fraction in order to calculate the specific activity of the rat oligodendrocytes. In other words, (S.A. of rat + calf oligo.) x (d.f.) = (S.A. of rat oligo.). For example, if the mixed oligodendrocyte fraction had a phospholipid specific activity of 10 and the dilution fraction was 30, then the calculated specific activity of the rat oligodendroglia would be 300. The assumption that the rat myelin is diluted by the calf myelin to the same extent that the oligodendroglia is diluted cannot be proved by these experiments, but some evidence is in its favor. Its use results in phospholipid specific activities for rat oligodendroglia <u>in vivo</u> similar to those for calf oligodendroglia <u>in vitro</u> and the rat neuron and astrocyte phospholipids from <u>in vivo</u> experiments resemble those of calf neurons and astroglia <u>in vitro</u>. Also, the dilution factor should be greater than six since less than eight grams of rat brain was mixed with 50 grams of calf white matter. Norton and Poduslo (1973) estimated that, at most, 25% of rat brain is white matter, so the theoretical value for the dilution factor is then 6.25 (50 grams calf white matter/8

gm rat brain x 4.0 (Proportion of grey/white matter in rat brain) or 25. The fraction usually came out between 29 and 32, meaning that about 1/28 to 1/32 of the cells of the mixed oligodendrocyte fractions were from the rat brain. This agrees well with the theoretical value. The use of this dilution factor is discussed in depth in the results section.

## III Results and Discussion

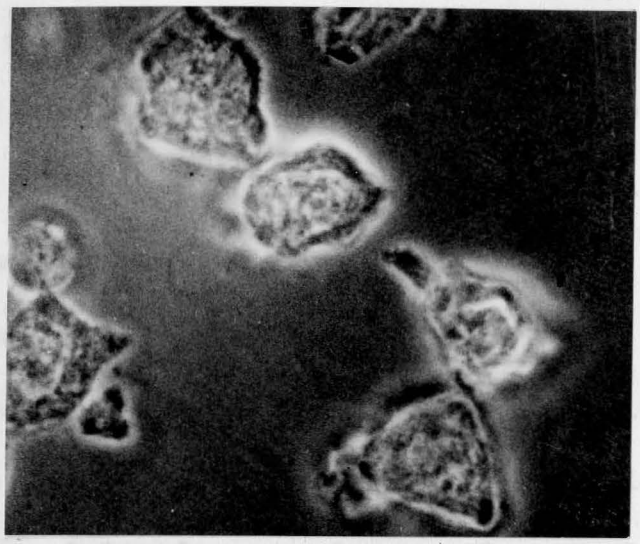
The experimental results are divided into 5 sections. The first section, A, presents photographs of the three cell types and discusses their morphology. Section B is the preliminary work done to determine the best method of studying the cell lipid metabolism; by incubating brain slices prior to cell isolation or by incubating the individual cells after isolation. This section contains data comparing the two types of incubation and also data on the effects of trypsin or lipid metabolism. The third section, C, is *in vitro* work on the incorporation of lipid precursors into lipids of neuronal soma astroglia from rat brain and the fatty acid composition of the phospholipids of the cell types. Section D concerns experiments on the incorporation of lipid precursors into lipids of neuronal soma, astroglia and oligodendroglia of calf brain *in vitro*, and Section E presents similar studies on *in vivo* incorporation of linolenic acid into the lipids of the three cell types from the rat.

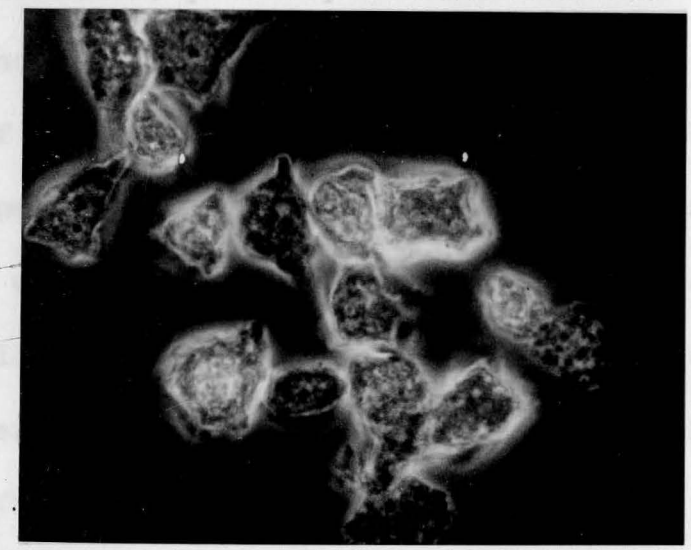
### A. Characterization of the Cellular Fractions

Under the phase microscope, both the neuronal soma and astroglia fractions are nearly identical to those obtained by Norton and Poduslo (1970). The neuronal fraction (Figure 4) is characterized by large cells with a large nucleus, a single prominent nucleolus and abundant, dense cytoplasm. Most of the cell processes are lost, but some cells retain short, thick

Figure 4

Neuronal Soma Isolated From Rat Brain
1000X
Phase Contrast





processes. The only non-neuronal contamination is an occasional free nucleus. Neurons from calf grey matter had a similar appearance (Figure 5). Following incubation of isolated cells for incorporation of radioactivity, the neurons appear more vacuolar and some of the cells break up, since subcellular particles and broken cells appear at the end of the incubation period. This degeneration of the isolated cells is greatly inhibited by the inclusion of 1% albumin and/or 5% glucose in the incubation media.

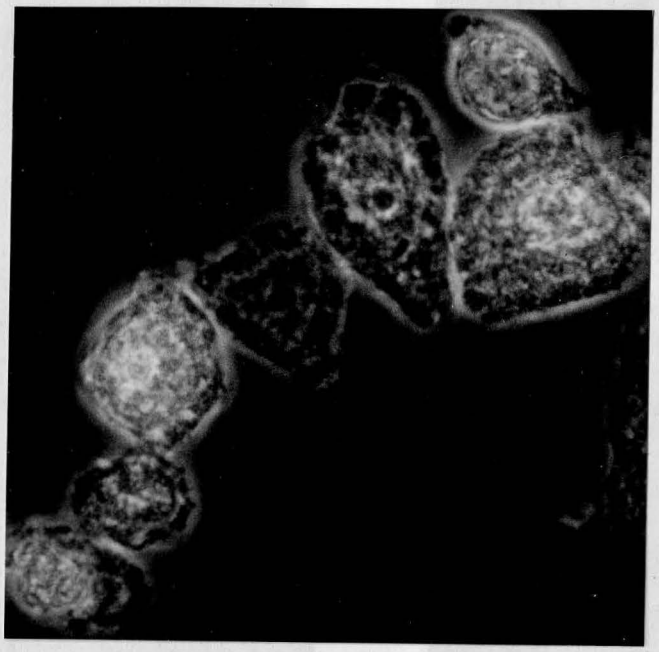
The astroglial fraction (Figure 6) contains mostly astrocytes. These cells are characterized by small cell bodies, a small nucleus and little cytoplasm. These cells retain thin highly, branched processes. Calf astrocytes were also similar in appearance. Non-glial contamination consists of some subcellular particles, probably mitochondria and broken cell membranes. Incubation of the isolated astrocytes resulted in breaking of a few of the cells and shrinking of the cell bodies.

No apparent difference in the morphological characteristics of the two cell types was observed with rats of different ages.

The oligodendroglial fraction, whether from calf brain white matter (Figure 7) or a mixture of calf white matter and rat brain (Figure 8) contains small cells with a thin rim of very dense cytoplasm. The oligodendrocytes are the smallest of the three cell types.

Figure 5

Neuronal Soma Isolated From Calf Brain
1000X
Phase Contrast



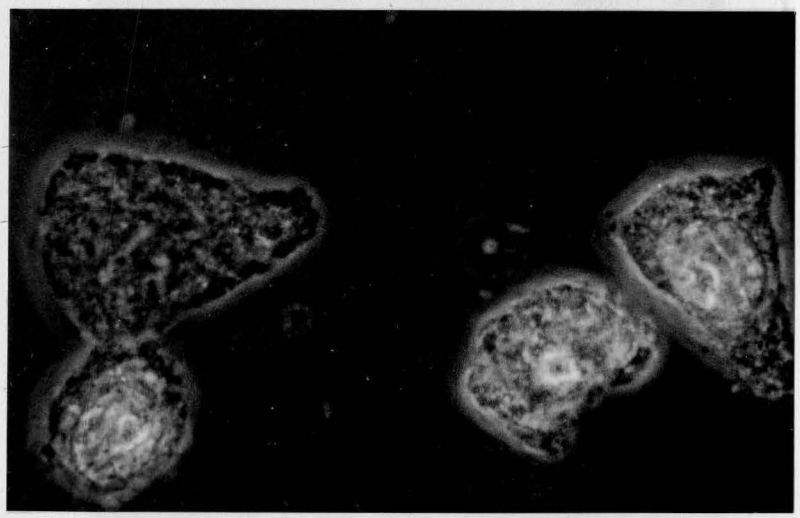
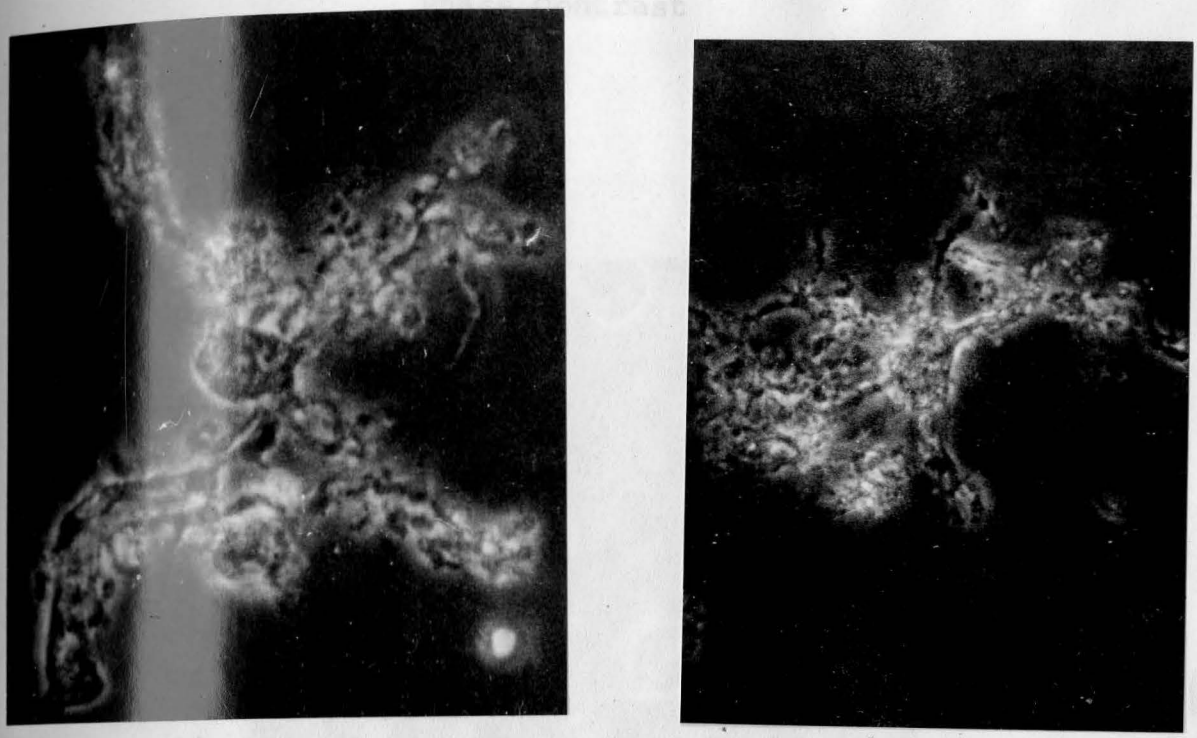


Figure 6

Astroglia Isolated From Rat Brain
2000X
Phase Contrast



1000X
Phase Contrast

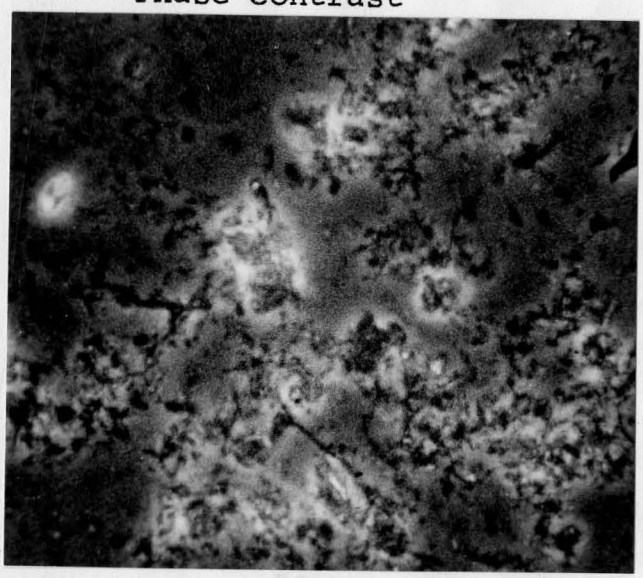


Figure 7

Oligodendrocytes Isolated From Calf Brain White Matter
1000X
Phase Contrast

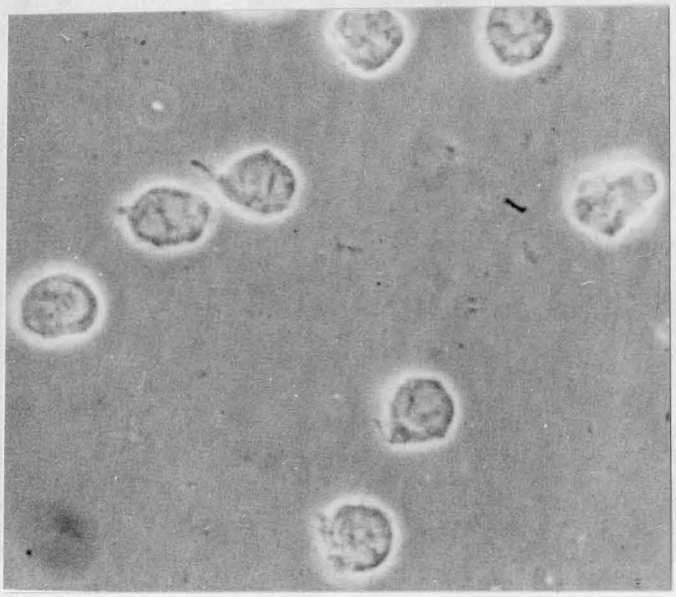
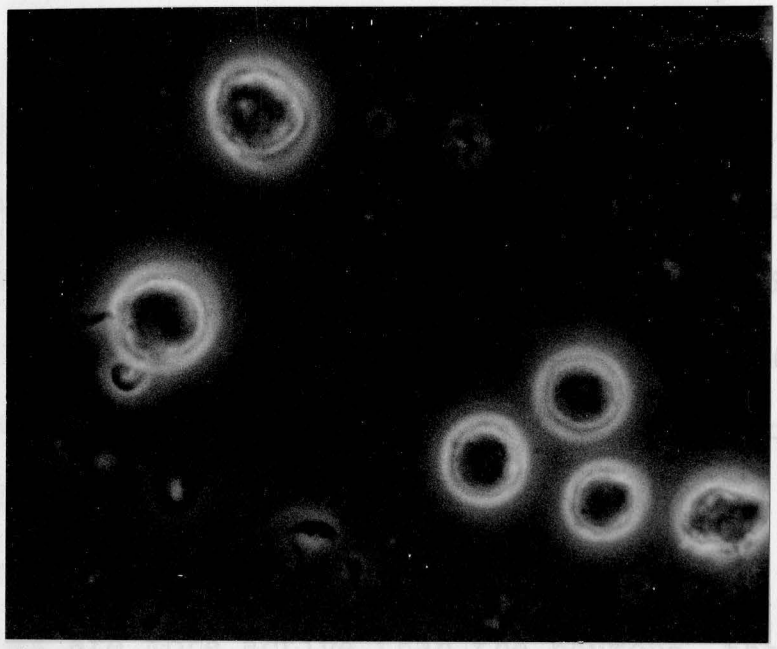


Figure 8

Oligodendrocytes Isolated from a Mixture of Calf Brain White Matter and Rat Brain
1000X
Phase Contrast



## Incubation of Cells versus Slices

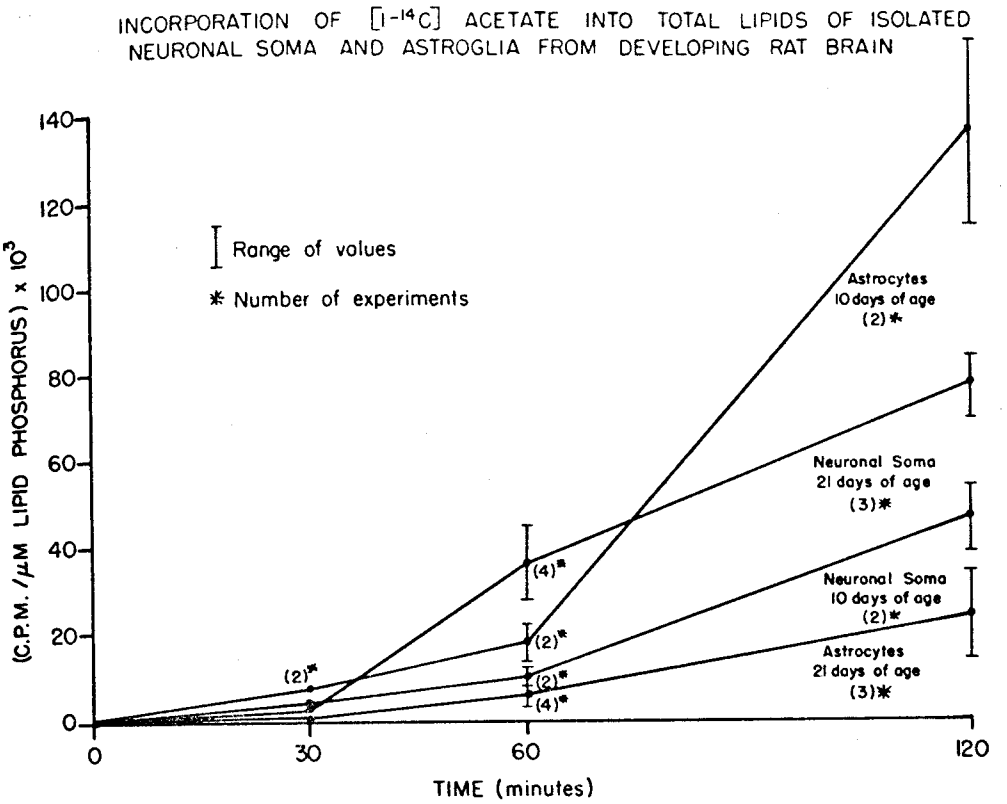
Isolated neuronal soma and astroglia from both 10 and 21 day old rats actively incorporate (1-$^{14}$C) acetate into total lipids (Figure 9). In the case of cells from 21 day old rats, the neurons are more active than the glia, incorporating 36 and 78 cpm/nM lipid phosphorus into total lipids at one and two hours of incubation while the glia contain 5.8 and 25 cpm/nM lipid phosphorus at the same time periods. Thus the isolated neurons are from 3.1 (2 hr incubation) to 6.2 (1 hr incubation) times as active as the isolated astrocytes in incorporating (1-$^{14}$C) acetate into total lipids. There is an apparent lag for the first 30 minutes of incubation.

With cells from 10 day old rats, the situation is reversed: the astrocytes are more active than the neurons, the neuronal to glial ratios being 0.36, 0.51 and 0.35 at 30, 60 and 120 minutes of incubation respectively

While the astrocytes from ten day old rats are more active than those from the 21 day old animals, at both one and two hours of incubation, the opposite is true for the neuronal soma. The older neurons are more active than those from the younger animals with respect to the incorporation of (1-$^{14}$C) acetate into total lipids.

When slices from 21 day old rat brains were incubated with

Figure 9




Isolated cells were incubated in the Krebs-Ringer medium (1 ml) described in the text with 10 uCi of (1-$^{14}$C) acetate. At the appropriate time, a sample was withdrawn and the total lipid specific activity determined.

(1-$^{14}$C) acetate and the neuronal soma and astroglial fractions subsequently isolated. the neuronal lipids contained 1.5 cpm/nM lipid phosphorus, while the glial lipids have a specific activity of 0.6 cpm/nM lipid phosphorus (Table 1). With slices from 10 day old rats, the astrocyte lipids had a higher specific activity than those from the neuronal fractions, 7.9 and 5.2 cpm/nM lipid phosphorus respectively. The neuronal/glial lipid specific activity ratios obtained from slice incubations were similar to those obtained when isolated cells are incubated with (1-$^{14}$C) acetate, namely 2.5 and .66 at 21 and 10 days of age respectively. The fact that the isolated cells incorporated a greater amount of acetate than the slice incubated cells was probably due to the greater permeability of the isolated cells. Also in the slice incubations, the substrate has to penetrate the slice. This rate of incorporation into the isolated cells reflects the relative degree of metabolic integrity of the isolated cells, since no pyridine nucleotides or ATP was added to the incubation media.

The pattern of incorporation of (1-$^{14}$C) acetate into the lipid classes of cells incubated after isolation is shown in Figure 10. Relative sterol synthesis increased in both neuronal soma and astrocytes from 10 to 21 days of age as did the synthesis of choline phosphoglycerides (CPG) to a small ex-

Table 1

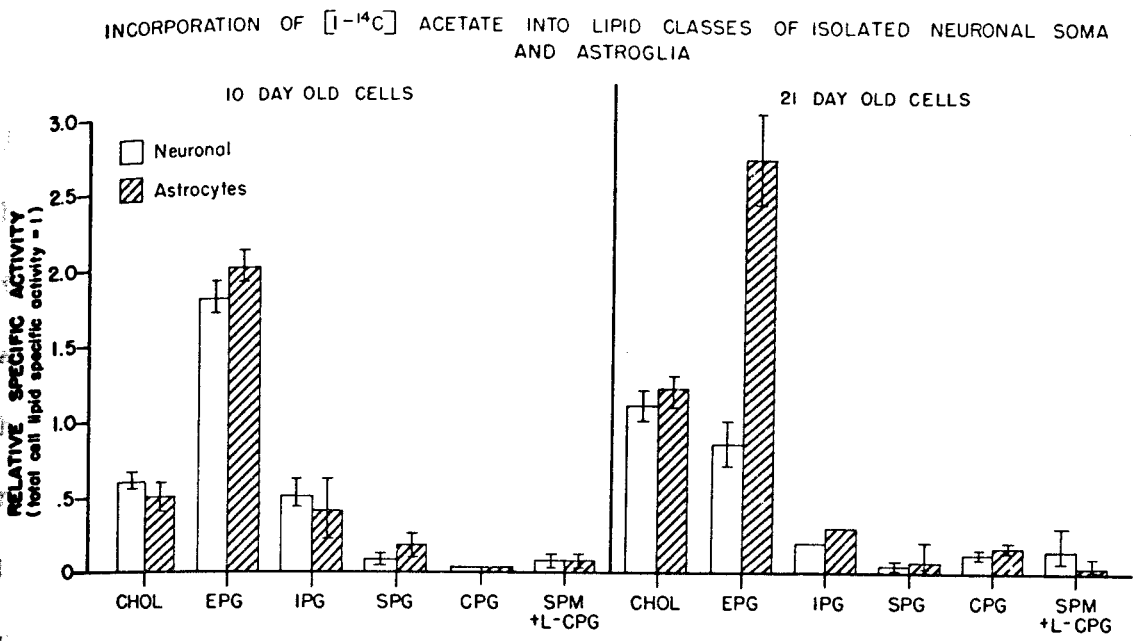
INCORPORATION OF (1-$^{14}$C) ACETATE INTO NEURONS AND ASTROCYTES OF DEVELOPING RAT BRAIN[1]

| | 10 days | 21 days |
|---|---|---|
| **SLICE INCUBATION** | | |
| NEURONS | 5.2±1.2 (3)[2] | 1.5±0.25 (5) |
| ASTROCYTES | 7.9±1.0 (3) | 0.6±0.05 (5) |
| **CELL INCUBATION** | | |
| NEURONS | 9.0±3.0 (2) | 37.0±8.0 (7) |
| ASTROCYTES | 18.0±6.0 (2) | 5.5±0.8 (7) |

1. C.P.M/nM lipid phosphorus
2. Number of experiments

Cells were incubated as described in Figure 9. Slices were incubated in 10 ml of the same medium with 10 µCi of (1-$^{14}$C) acetate, the cells were subsequently isolated and the total lipid specific activities determined. The incubations were for one hour.

Figure 10




Isolated cells were incubated as is described in Figure 9. The lipids were isolated, lipid classes separated by thin layer chromatography, and the specific activity of each fraction determined as described in the text. The results are expressed as relative specific activity (total cell lipid specific activity = 1).
(L-CPG = lysocholine phosphoglyceride).

tent. The incorporation of the (1-$^{14}$C) acetate into ethanolamine phosphoglycerides (EPG), compared to the other lipid fractions, decreased in neurons while in the astrocyte fraction it increased.

A significant finding was the relative lack of incorporation of the (1-$^{14}$C) acetate into the CPG fraction compared to the EPG fraction. Usually, the CPG fraction is the more active of the two (Dhopeshwarkar et al, 1971a; Cuzner et al, 1966; Kanazawa et al, 1972 and Claton and Rowe, 1966). In fact, when the phosphoglycerides of the slice incubated cells were fractionated (Table 2), the CPG fraction was more active than the EPG fraction, a result to be expected on the basis of work from other laboratories. Since the isolated cells had a metabolic pattern different from that of intact tissue, it was necessary to investigate if this was due to the isolation procedure before further experiments were performed with the isolated cells. The possibility that a contaminant with high specific activity was migrating with the EPG fraction in thin layer chromatography was ruled out by pooling the EPG fraction eluted from the thin layer plates of several experiments and running this EPG fraction on two dimensional thin layer (Figure 11). Nine spots were obtained, four of which were ninhydrin positive. The majority of the radioactivity was in the actual EPG spot as determined by chromatography of an au-

Table 2

COMPARISON OF SLICE AND CELL INCUBATED INCORPORATION OF (1-$^{14}$C) ACETATE INTO ETHANOLAMINE AND CHOLINE PHOSPHOGLYCERIDES OF ISOLATED NEURONS AND ASTROCYTES

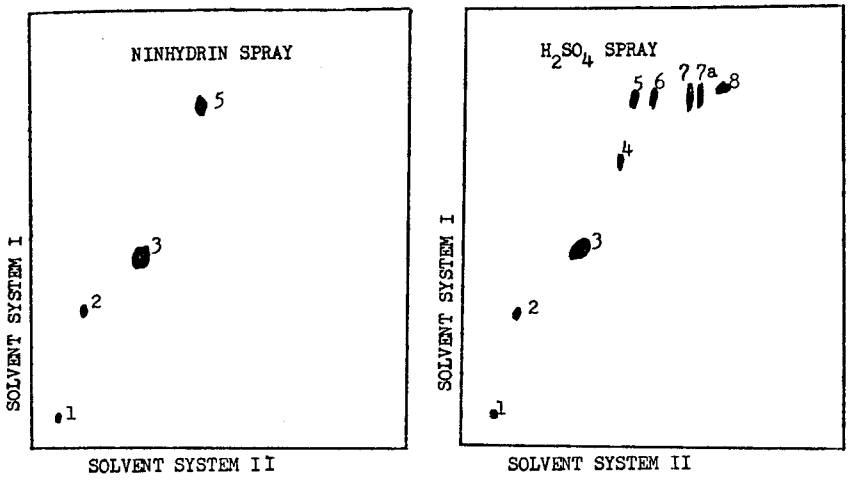
| | Slice Incubation | | Cell Incubation | |
|---|---|---|---|---|
| | Neurons | Astrocytes | Neurons | Astrocytes |
| EPG | 0.39* | 0.87 | 3.05 | 3.16 |
| CPG | 1.06 | 1.50 | 0.05 | 0.09 |

*Relative specific activity (total cell phospholipid specific activity = 1).

Ten day old tissue was incubated as described in Table 1. Average of 3 experiments.

# Figure 11

TWO DIMENSIONAL THIN LAYER CHROMATOGRAPHY OF ETHANOLAMINE PHOSPHOGLYCERIDE FROM RAT BRAIN CELLS INCUBATED WITH [1-$^{14}$C] ACETATE




SOLVENT SYSTEM I: $CHCl_3$:$CH_3OH$:28% $NH_4OH$::65:35:5

SOLVENT SYSTEM II: $CHCl_3$:$CH_3COCH_3$:$CH_3OH$:$CH_3COOH$:$H_2O$::10:4:2:2:1

| SPOT NUMBER | %RADIOACTIVITY |
|---|---|
| 1 | 0.0 |
| 2 | 1.9 |
| 3 | 71.2 |
| 4 | 6.1 |
| 5 | 10.5 |
| 6 | 6.8 |
| 7 | 0.9 |
| 7a | 0.8 |
| 8 | 1.8 |

thenic standard on a duplicate plate. Thus contamination of the radioactivity of the EPG fraction was ruled out.

In order to determine which step, if any, in the cell isolation procedure was causing the high EPG, low CPG specific activities, six different incubations were performed on brain and liver (Table 3). They were (A) control, incubation for 1 hr. in Krebs-Ringer medium with 10 uCi (1-$^{14}$C) acetate, (B) control with added cofactors, (C) pre-incubation in HAP - the media of the cell isolation procedure followed by incubation as in (A), (D) pre-incubation in HAP followed by incubation as in (B), (E) pre-incubation in HAP with 1% trypsin then incubated as in (A), and (F) pre-incubation in HAP with 1% trypsin, then incubated as in (B). Pre-incubation in HAP alone resulted in a greater incorporation of (1-$^{14}$C) acetate into the EPG fraction than in the CPG fraction (columns C, D, E, Table 3). Addition of cofactors (see Table 3) to the incubation media greatly stimulated the incorporation of (1-$^{14}$C) acetate into the EPG fraction of both brain and liver trypsin slices (Column F, Table 3), a situation resembling that of the isolated cells. Thus, the unusual pattern of incorporation found in the isolated cells is most likely due to the trypsinization used in the isolation procedure, and while the results found for incorporation into total lipids of isolated cells is similar to the slice incubated material, conclusions must be

TABLE 3

INCORPORATION OF (1-$^{14}$C) ACETATE INTO LIPID FRACTIONS OF RAT BRAIN UNDER VARIOUS INCUBATION CONDITIONS

| | (A) Control | (B) Control+ Cofactors | (C) HAP | (D) HAP Cofactors | (E) HAP+Tr | (F) HAP+Tr-- Cofactors |
|---|---|---|---|---|---|---|
| BRAIN | | | | | | |
| Total Lipids | 78.4 | 40.3 | 9.1 | 5.4 | 7.8 | 3.7 |
| Ethanolamine Phosphatides | 5.2 | 1.5 | 1.7 | 1.1 | 1.4 | 6.5 |
| Choline Phosphatides | 13.0 | 2.0 | 1.0 | 0.7 | 0.7 | 0.7 |
| Neutral Lipids | 210.0 | 125.0 | 36.0 | 15.0 | 30.9 | 6.0 |
| LIVER | | | | | | |
| Total Lipids | 27.6 | 9.8 | 3.2 | 1.5 | 2.0 | 3.0 |
| Ethanolamine Phosphatides | 4.9 | 1.0 | 1.7 | 1.4 | 4.6 | 8.8 |
| Choline Phosphatides | 4.3 | 1.5 | 1.4 | 0.4 | 0.2 | 0.4 |
| Neutral Lipids | 81.0 | 22.6 | 15.3 | 3.5 | 4.5 | 4.2 |

*cpm/mg. lipid (x-$10^{-2}$) 10 day old tissue

Incubation Conditions:

(A) 123mM NaCl; 6mM KCl; 1.5mM $MgSO_4$; 12mM Glucose; 20mM phosphate buffer, pH 7.4 and 10 uCi (1-$^{14}$C) Sodium Acetate. Total volume = 5 ml

(B) Same as (A) plus cofactors--8mg. ATP; 2mg. NADH; 1mg. NADPH and 1 mg CoASH.

(C) Pre-incubated in 5% glucose; 5% fructose; 1% Albumin; 10mM phosphate buffer pH 6.0 and incubated in medium A.

(D) Same as (C), except incubated in medium B.

(E) Pre-incubated in medium C with 1% trypsin added to the pre-incubation media, and incubated in medium A.

(F) Same as (E), incubated in medium B.

drawn with caution since the trypsin pre-incubation does affect metabolic activity. Control experiments such as this one are necessary before any work is done with isolated cells.

Similar experiments were performed with (1-$^{14}$C) linolenate as the precursor (Table 4) and it can be seen that the trypsin pre-incubation does not have such a marked effect upon the incorporation of this tracer into phospholipids as it does with (1-$^{14}$C) acetate. These phenomena may be due to specific inactivation of one of the enzymes involved in the choline phosphoglyceride synthetic pathway and/or a decrease in the pool size of saturated diglycerides necessary for CPG synthesis by inhibition of de Novo fatty acid synthesis. These possibilities have been discussed in more detail elsewhere (Cohen and Bernsohn, 1974).

C. In Vitro Incorporation of (1-$^{14}$C) Linoleic and Linolenic Acid into Neuronal Soma and Astroglia of Rat Brain and Fatty Acid Composition.

1. Phospholipids

When rat brain slices were incubated with (1-$^{14}$C) linolenic acid and the cells subsequently isolated (Table 5), it was found that the phospholipids from 21 day old neurons were more radioactive than those from the astroglial fraction of the same age. With ten day old slices, the situation was reversed, the glial phospholipids had a specific activity greater than

TABLE 4

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOLIPID FRACTIONS OF RAT BRAIN UNDER VARIOUS INCUBATION CONDITIONS

| | (A) Control | (B) Control+ Cofactors | (C) HAP | (D) HAP-- Cofactors | (E) HAP+Tr | (F) HAP+Tr-- Cofactors |
|---|---|---|---|---|---|---|
| BRAIN | | | | | | |
| Total Phospholipids | 7.0* | 6.6 | 6.9 | 6.3 | 3.7 | 3.1 |
| Ethanolamine Phosphatides | 4.8 | 4.3 | 5.1 | 5.8 | 3.3 | 1.9 |
| Choline Phosphatides | 7.1 | 8.5 | 7.6 | 7.1 | 4.7 | 4.0 |
| LIVER | | | | | | |
| Total Phospholipids | 4.0 | | 3.2 | 4.8 | 3.2 | 3.3 |
| Ethanolamine Phosphatides | 1.9 | | 1.3 | 3.5 | 1.7 | 1.2 |
| Choline Phosphatides | 2.3 | | 2.2 | 3.8 | 3.9 | 3.9 |

*CPM/mg. Phospholipid ($\times 10^{-3}$)

Legend same as for Table 3 but the labeled precursor was (1-$^{14}$C) linolenate.

**TABLE 5**

INCORPORATION OF VARIOUS PRECURSORS INTO LIPID FRACTIONS OF NEURONAL SOMA AND ASTROCYTES FROM DEVELOPING RAT BRAIN

| PRECURSOR | NEURONAL | ASTROGLIAL | NEURONAL | ASTROGLIAL |
|---|---|---|---|---|
| (1-$^{14}$C) ACETATE$^{+}$ | 5.2 (3)$^{*}$<br>(4.3-6.8)$^{**}$ | 7.9 (3)<br>(5.5-10.3) | 1.5 (4)<br>(1.3-1.7) | 0.6 (4)<br>(0.5-0.7) |
| (1-$^{14}$C) LINOLEATE$^{++}$ | 28.3 (2)<br>(22.0-34.6) | 17.2 (2)<br>(13.0-21.5) | 19.8 (2)<br>(17.0-22.6) | 12.1 (2)<br>(10.5-13.6) |
| (1-$^{14}$C) LINOLENATE$^{++}$ | 14.6 (3)<br>(11.8-20.2) | 25.3 (3)<br>(23.5-27.2) | 11.5 (2)<br>(10.8-12.3) | 7.4 (2)<br>(6.3-8.5) |

$^{*}$number of experiments
$^{**}$range of values
$^{+}$total lipid cmp/nM phospholipid phosphorus
$^{++}$phospholipid cpm/nM phospholipid phosphorus

Slices from six (21 day old rats) or 10 (10 day old rats) were incubated in 10 ml of the Krebs-Ringer medium with 7.5-10.0 uCi of the appropriate tracer, the cells isolated and the lipid or phospholipid specific activities determined.

TABLE 6

RATIOS OF NEURONAL TO ASTROGLIAL LIPID SPECIFIC ACTIVITIES AFTER INCUBATION OF DEVELOPING RAT BRAIN SLICES WITH LABELLED PRECURSORS

| Precursor | Ratio of Neuronal/Astroglial Lipid Specific Activities | |
|---|---|---|
| | 10 day | 21 day |
| (1-$^{14}$C) Acetate | 0.66 | 2.50 |
| (1-$^{14}$C) Linoleate | 1.64 | 1.63 |
| (1-$^{14}$C) Linolenate | 0.58 | 1.55 |

that of the neuronal phospholipids. This is a pattern similar to that found with (1-$^{14}$C) acetate, although from three to eleven times more linolenate was incorporated, depending on the cell type and the age. When the precursor was (1-$^{14}$C) linoleate, the neuronal phospholipids were more radioactive than the glial phospholipids from both 10 and 21 day old slices.

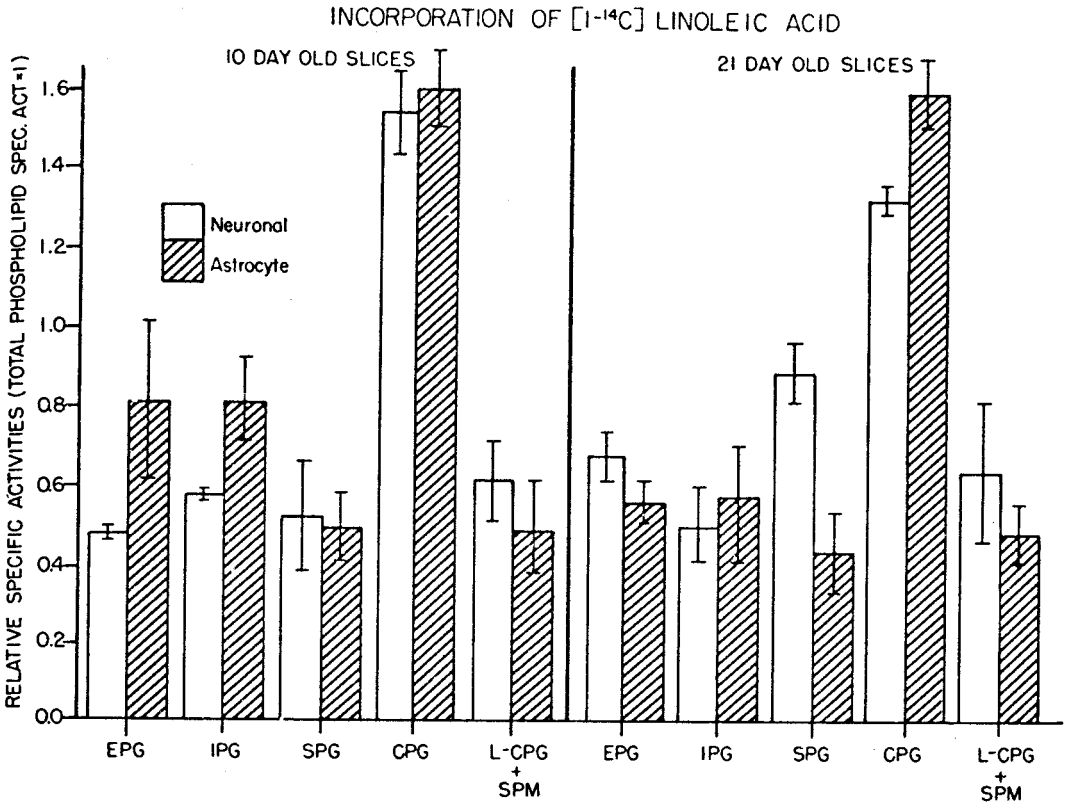
There was a general decrease in incorporation of all three precursors between the ten and twenty-one day old cells. Neuronal lipid specific activity dropped by 61% and the glial lipids by 91% with (1-$^{14}$C) acetate as the precursor. The corresponding decreases for phospholipid specific activity with (1-$^{14}$C) linolenate were 21% (neuronal) and 71% (glial), and with (1-$^{14}$C) linoleate the decreases in phospholipid specific activity were 30% for both cell types.

The neuronal to astroglial ratios of lipid specific activity (Table 6) demonstrate that at 21 days of age there was a similar ratio for the incorporation of the two fatty acids and for all three precursors the neuronal phospholipids had a greater specific activity than those of the glia. When ten day old cells, both acetate and linolenate,were incorporated more into the glial phospholipids than the neuronal. (1-$^{14}$C) linoleate had a similar ratio at both ages.

Inspection of the individual phospholipid classes after incorporation of (1-$^{14}$C) linoleate (Figure 12) revealed the highest relative specific activity in the CPG fraction, with the other phospholipids very close to each other in relative specific activity. Neuronal-glial differences were found in relative specific activity in the CPG fraction, with the other phospholipids very close to each other in relative specific activity. Neuronal-glial differences were found in EPG and IPG at 10 days of age and SPG and CPG at 21 days of age. The only significant changes between the two ages was an increase in neuronal SPG relative specific activity from 10 to 21 days of age.

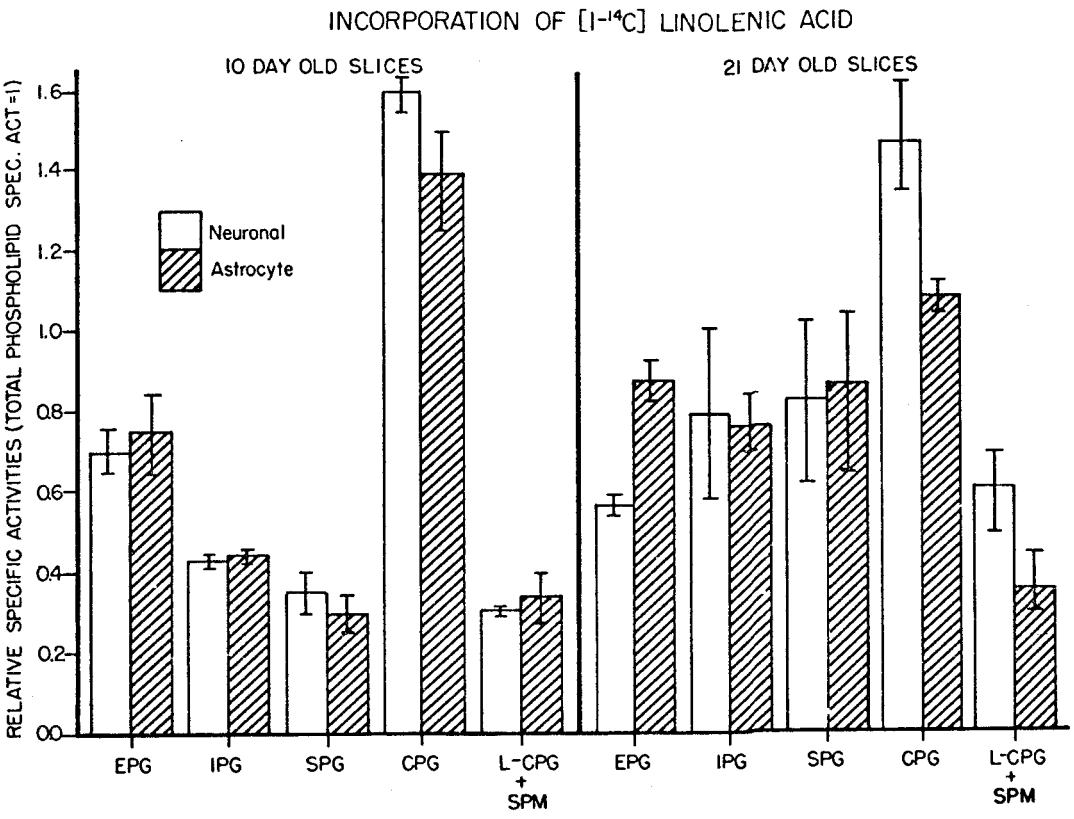
When the precursor was (1-$^{14}$C) linolenate (Figure 13) the highest relative specific activity in both cell fractions at both ages was also in the CPG fraction and the only significant neuronal-glial differences were in the CPG and EPG fraction at 21 days of age. At the older age, the differences between the relative specific activities of the various phospholipids was much less than in the 10 day old cells. The relative specific activities increased in the IPG and SPG fractions of both cells from ten to 21 days of age.

Figure 12




Rat brain slices were incubated as previously described, the cells isolated, the phospholipids were separated by TLC, and the specific activity of each phospholipid class determined. The results are expressed as relative specific activities (total cell phospholipid specific activity = 1). (L-CPG = lysocholine phosphoglyceride, SPM = sphingomyelin).

Figure 13




Legend same as for Figure 11

2. Fatty acids - incorporation

Fractionation by GLC of the phospholipid fatty acids of neuronal soma and astroglia after incorporation of (1-$^{14}$C) acetate revealed most of the radioactivity to be in palmitic, palmitoleic and stearic acids, and of the polyunsaturated fatty acids (PUFA), 22:4 had the highest percentage of radioactivity (Table 7). In ten day brain, both cell fractions had more radioactivity in the palmitic-palmitoleic fractions (49.5% for neurons and 32.0% for glia) than the other fatty acids followed by 22:4 (19.0% for neurons and 24% for glia). At 21 days of age, in the neuronal fraction, most of the radioactivity was in the stearic acid fraction (30.7%) followed by 22:4 (23%) and palmitic-palmitoleic acids (15.6). The glial fatty acid with the most radioactivity at this age was the stearic acid fraction (30.3%) followed by 16:0 +16:1 (23.3%) and 22:4 (20.0%). In both cell fractions from ten to twenty-one days of age, the percentage of radioactivity in the stearic acid fraction increases while decreasing in the palmitic-palmitoleic acid fraction and remaining about the same in 22:4.

Fractionation of the phospholipid fatty acids of the two cell types following incubation with (1-$^{14}$C) linolenate (Table 8) revealed over 85% of the radioactivity remained in the precursor fatty acid, 18:3. Similar results were found when

TABLE 7

INCORPORATION OF (1-$^{14}$C) ACETATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF NEURONAL AND GLIAL PREPARATIONS OF DEVELOPING RAT BRAIN

| FATTY ACID | 10-DAY BRAIN | | 21-DAY BRAIN | |
|---|---|---|---|---|
| | NEURONAL | GLIAL | NEURONAL | GLIAL |
| 14:0 | 6.0* | 13.3* | 4.7* | 1.5* |
| 16:0+16:1 | 49.5 | 32.0 | 15.6 | 23.3 |
| 18:0 | 11.3 | 6.9 | 30.7 | 30.3 |
| 18:1 | 3.5 | 3.2 | 4.7 | 8.9 |
| 18:2 | 1.9 | 1.3 | 3.6 | 1.5 |
| 18:3+2-:1 | 2.6 | 1.6 | 4.9 | - |
| 20:3 (n-9)+(n-6) | 1.8 | 0.6 | 2.2 | - |
| 20:4 | 2.1 | 3.1 | 5.7 | 3.8 |
| 20:4 (n-3) | - | 2.1 | 1.2 | 4.5 |
| 22:4 | 19.0 | 24.0 | 23.4 | 20.0 |
| 22:5 (n-6) | 0.8 | - | 4.0 | 3.9 |
| 22:5 (n-3) | 1.1 | 3.6 | 2.2 | 1.0 |
| 22:6 | 1.2 | 1.5 | 3.1 | 2.1 |

*Percentage of total fatty acid radioactivity.
Total cpm recovered was between 400 and 1000.

Fatty acid radioactivity from slice incubated cells were analyzed as described in the Materials and Methods section.

TABLE 8

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF NEURONAL AND ASTROGLIAL PREPARATIONS FROM TEN DAY OLD RAT BRAIN

| FATTY ACID | NEURONAL SOMA | ASTROGLIA |
|---|---|---|
| 14:0 | 0.2* | 0.1* |
| 16:0 | 0.7 | 0.3 |
| 18:0 | 0.3 | 0.2 |
| 18:1 | 0.4 | 0.3 |
| 18:3+20:0 | 86.0 | 89.0 |
| 20:3 (n-6) | 3.8 | 3.0 |
| 20:4 (n-6) | 3.8 | 3.4 |
| 20:4 (n-3) | 1.4 | 1.1 |
| 20:5 (n-3)+22:3 (n-9) | 1.3 | 0.8 |
| 22:4 (n-6) | 0.8 | 0.6 |
| 22:5 (n-6) | 0.4 | 0.3 |
| 22:5 (n-3) | 0.5 | 0.2 |
| 22:6 (n-3) | 0.8 | 0.6 |

*percentage of total fatty acid radioactivity

Fatty acid radioactivity from slice incubated cells were analyzed as described in the Materials and Methods section. In all linolenate incorporation experiments, a minimum of 2000 cpm was recovered from the GLC.

linoleic acid was the precursor. Therefore, little or none of the linolenic acid was elongated and the small amount of radioactivity found in 20:3 and 20:4 was probably due to "tailing" since injection of a standard methylated linolenic acid gave a similar pattern, i.e. 90% of the radioactivity was collected in the 18:3 peak and 5-7% in the two following peaks.

3. Fatty acids - composition

The distribution of phosphoglyceride fatty acids was very similar in ten day old neuronal soma and astroglia from rat brain (Table 9). The major fatty acids are palmitic, stearic, oleic, arachidonic and docosahexenoic. There was slightly more palmitic acid in the astroglial phosphoglycerides, while the neuronal soma phosphoglycerides contained more arachidonic acid.

Similar results were found for calf brain neuronal soma and astroglial phosphoglyceride fatty acids (Table 10), namely more palmitic acid in the astrocytes with the neurons having slightly more arachidonic acid than the astroglia. Hamberger and Svennerholm (1971) found a very similar fatty acid pattern for neurons and glia isolated from adult rabbit brain.

The phosphoglyceride fatty acid pattern of the oligodendroglial fraction (Table 10) resembles myelin more than that of the other two cell types. The major fatty acids of phosphoglyceride fatty acids are palmitic, stearic and oleic acids

TABLE 9

DISTRIBUTION OF PHOSPHOGLYCERIDE FATTY ACIDS IN NEURONAL SOMA AND ASTROGLIA OF TEN DAY OLD RAT BRAIN

| FATTY ACID | NEURONAL SOMA | ASTROGLIA |
|---|---|---|
| 12:0 | 1.9* | 1.1 |
| 14:0 | 2.4 | 1.8 |
| 16 ald | 2.7 | 2.2 |
| 16:0 | 20.8 | 27.6 |
| 18 ald | 1.8 | 2.0 |
| 18:0 | 16.9 | 14.6 |
| 18:1 | 14.4 | 13.9 |
| 18:2 | 4.3 | 2.8 |
| 18:3+20:0 | 1.8 | 1.7 |
| 20:1+20:2 | 3.0 | 4.0 |
| 20:3 (n-6) | 2.2 | 2.1 |
| 20:4 (n-6) | 14.8 | 10.6 |
| 20:4 (n-3) | 1.6 | 0.5 |
| 20:5 (n-3) | 0.5 | 0.3 |
| 22:4 (n-6) | 1.8 | 3.1 |
| 22:5 (n-6) | 0.3 | 0.8 |
| 22:5 (n-3) | 0.2 | 0.5 |
| 22:6 (n-3) | 8.4 | 10.3 |
| Saturated and Monounsaturated | 60.9 | 63.2 |
| Polyunsaturated | 38.9 | 36.7 |

+ Number of Experiments

*Percentage of total fatty acids

Fatty acids were analyzed as described in the Materials and Methods section.

TABLE 10

DISTRIBUTION OF PHOSPHOGLYCERIDE FATTY ACIDS IN CELLS FROM DEVELOPING CALF BRAIN

| FATTY ACID | NEURONAL SOMA[3]* | ASTROGLIA[4] | OLIGODEN-DROGLIA[3]* | MYELIN** |
|---|---|---|---|---|
| 14:0 | 2.2+ | 2.1+ | 3.2+ | |
| 16:0 | 20.6 | 24.9 | 26.6 | 17.5+ |
| 18:0 | 14.5 | 17.8 | 18.9 | 20.8 |
| 18:1 | 16.7 | 19.4 | 27.6 | 30.5 |
| 18:2 | 4.8 | 3.1 | 5.0 | 2.3 |
| 18:3+20:1 | 2.9 | 1.3 | 2.1 | 3.3 |
| 20:2 | 1.4 | 0.9 | - | - |
| 20:3 (n-6) | 3.6 | 1.4 | 0.8 | 2.3 |
| 20:4 (n-6) | 13.6 | 10.6 | 8.5 | 9.8 |
| 20:5 (n-3) +22:3 (n-9) | 1.9 | 1.2 | 1.0 | - |
| 22:4 (n-6) | 4.9 | 3.2 | 1.5 | 5.0 |
| 22:5 (n-6) | 1.7 | 3.1 | 1.8 | 4.0 |
| 22:5 (n-3) | 0.4 | 1.3 | - | 0.4 |
| 22:6 (n-3) | 9.9 | 9.7 | 3.1 | 3.8 |
| Saturated and Monounsaturated | 54.0 | 64.2 | 76.3 | 68.8 |
| Polyunsaturated | 45.1 | 35.8 | 23.8 | 25.3 |
| Ratio $\frac{(n-6)}{(n-3)}$ fatty acids | 2.3 | 1.7 | 4.1 | 5.0 |

*Number of experiments.

+Percentage of total.

**Data from Hamberger and Svennerholm, 1971 (Rabbit Myelin). Fatty acids were analyzed as described in the Materials and Methods Section.

with less arachidonic and docosahexanoic acid in the oligodendroglia than the neurons and astrocytes. There was relatively little polyunsaturated fatty acids in the myelin and oligodendroglia. The highest proportions of n-6 fatty acids was found in the oligodendroglial fraction followed by myelin, neurons and astroglia.

D. In Vitro Incorporation of Fatty Acids into Neuronal Soma, Astroglia and Oligodendroglia of Calf Brain.

Since it requires 50-60 grams of white matter to obtain sufficient oligodendrocytes for a lipid analysis, it is not practical to incubate slices of white matter for incorporation into oligodendrocytes. Several hundred milliliters of incubation media would be required as would an expensive amount of radioactive tracer in order to have a sufficiently high concentration and specific activity. The best system would be the incubation of the isolated cells in order to allow a small incubation volume. Because the trypsin pre-incubation did not have the same radical effect on fatty acid incorporation as it did on acetate incorporation (Tables 3 and 4), it was decided to incubate the isolated cells with (1-$^{14}$C) stearic, linoleic and linolenic acid (Table 11) in order to compare the incorporation of the three fatty acids into the phospholipids of the three cell types.

TABLE 11

INCORPORATION OF (1-$^{14}$C) FATTY ACIDS INTO ISOLATED

NEURONAL SOMA, ASTROGLIA AND OLIGODENDROGLIA FROM CALF BRAIN

| Fatty Acid | (1-$^{14}$C)Stearic | | | (1-$^{14}$C) Linoleic | | | (1-$^{14}$C) Linolenic | | |
|---|---|---|---|---|---|---|---|---|---|
| | Neurons | Astro-glia | Oligoden-droglia | Neurons | Astro-glia | Oligoden-droglia | Neurons | Astro-glia | Oligoden-droglia |
| Exp. 1 | 8.0* | 10.0 | 76.8 | 11.9 | 29.7 | 124.3 | 6.4 | 14.2 | 140.9 |
| Exp. 2 | 5.6 | 12.0 | 69.0 | 20.0 | 37.7 | 384.4 | 20.5 | 37.8 | 319.4 |
| Exp. 3 | 5.5 | 10.4 | 130.0 | 3.9 | 19.6 | 649.6 | 4.4 | 14.9 | 300.3 |
| Exp. 4 | | | | 3.0 | 3.5 | 213.0 | | | |
| Exp. 5 | | | | | | | 12.2 | 9.9 | 254.3 |

| | (1-$^{14}$C) Acetate | | |
|---|---|---|---|
| | Neurons | Astro-glia | Oligoden-droglia |
| Exp. 6 | 1.0 | 1.9 | 31.0 |
| Exp. 7 | 0.6 | 0.6 | 5.5 |

*CPM/nM phospholipid phosphorus
Isolated cells were incubated in 0.5 ml of the Krebs-Ringer medium with 1% albumin and 2.5 uCi of the fatty acid or 10 uCi of (1-$^{14}$C) acetate. After one hour, incubation, the lipids were extracted and analyzed as previously described.

Experiments 1, 2 and 3 represent 3 separate cell isolations, each of which was divided into 3 aliquots for incubation with (1-$^{14}$C) stearic acid, (1-$^{14}$C) linoleic acid or (1-$^{14}$C) linolenic acid. The results were rather startling; with all three fatty acids, the oligodendroglial phospholipids had a specific activity from four to 166 times greater than that of the phospholipids of the neurons and astrocytes. Within the one hour incubation period, both of the essential fatty acids, linoleic and linolenic, were more rapidly incorporated into the oligodendroglial phospholipids than was stearic acid. The oligodendroglia phospholipids were approximately 10-24 times more active than the neuronal and 6-12 times more active than the astroglial phospholipids in the incorporation of (1-$^{14}$C) stearic acid. With linoleic acid the values varied over a much wider range, the oligodendroglia phospholipids were 10-166 and 4-61 times more radioactive than the neuronal and astroglial phospholipids respectively. Where the precursor was (1-$^{14}$C) linolenic acid, the oligodendroglia phospholipids incorporated from 20 to 68 times more than the neuronal and 9 to 25 times more than the astroglial phospholipids. The astroglia appeared to incorporate more of the three precursors than the neuronal phospholipids, but the differences were not as great as with the oligodendrocytes; the astroglia were only at most four times more active than the neurons, and in some experiments

the values were nearly equal. (1-$^{14}$C) acetate was also incorporated into the three cell types (Experiments 6 and 7) for the basis of comparison, and with this precursor, the oligodendroglial phospholipids were from 9 to 31 times more active than those of the neurons and astrocytes. These results resemble those found for ten day old rats where the astrocytes incorporated more (1-$^{14}$C) linolenic acid and (1-$^{14}$C) acetate into phospholipid than did the neuronal soma, but contrasts the data for (1-$^{14}$C) linoleate because with that precursor, the neuronal phospholipids were more active than the astroglial at both ages. The variations between experiments (as much as 11 fold difference with (1-$^{14}$C) linoleic acid incorporation into the astroglia ) are probably due in part to differences in the age of the animals, since changes between ages as great as 11 fold were found for (1-$^{14}$C) acetate and 3 fold for fatty acid incorporation into rat brain cells of different ages (Table 5). Unfortunately, it was not possible to obtain the age of the animal at the time of death, and the manner of dissection from the skull precluded obtaining brain weights.

When the phosphoglyceride fatty acids were fractionated by GLC and the peaks counted for radioactivity, the results were rather disappointing. Although the (1-$^{14}$C) linolenate was readily incorporated into the phosphoglycerides of all three cell types, little or none of it was elongated (Table 12). One

TABLE 12

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF ISOLATED CELLS FROM DEVELOPING CALF BRAIN

| FATTY ACID | NEURONAL SOMA | ASTROCYTES | OLIGODENDROCYTES |
|---|---|---|---|
| 14:0 | 0.3* | 0.2* | - |
| 16:0 | 1.6 | 0.7 | 0.2 |
| 18:0 | 0.6 | 0.5 | 0.2 |
| 18:1 | 0.5 | 0.6 | 0.5 |
| 18:3+20:0 | 85.1 | 87.2 | 83.9 |
| 20:3 (n-6) | 3.5 | 3.4 | 2.9 |
| 20:4 (n-6) | 3.6 | 3.3 | 8.3 |
| 20:4 (n-3) | 1.0 | 1.0 | 0.9 |
| 20:5 (n-3) +22:3 (n-9) | 1.1 | 1.1 | 0.9 |
| 22:4 (n-6) | 1.3 | 0.7 | 1.2 |
| 22:5 (n-6) | 0.2 | 0.3 | 0.1 |
| 22:5 (n-3) | 0.5 | 0.3 | 0.2 |
| 22:6 (n-3) | 0.7 | 0.6 | 0.6 |

*Percent of total fatty acid radioactivity.

Fatty acid radioactivity was analyzed as described previously following incubation of isolated cells as in Table 11.

would expect elongation to occur since little 18:3 is found in the cell phosphoglycerides (Table 10). Eighty-four to eighty-seven percent of the radioactivity remained in the linolenate peak. The oligodendrocyte data demonstrated a very slight amount of elongation in that the 20:4 (n-6) peak, which probably also contains 20:4 (n-3) a product in the elongation of linolenate, contained 8.3% of the radioactivity. When rat (Table 13) and calf brain cells (Table 14) were incubated for 20 hours in synthetic tissue culture media, similar results were obtained in that over 80% of the fatty acid radioactivity was still in linolenate, except for the oligodendrocytes which again contained a large amount of radioactivity in the 20:4 (n-6) peak (Table 14). Even slices (from brain and Liver), homogenates, trypsinized slices and the starting cell suspension did not elongate the linolenate to any significant extent (Tables 14, 15, 16), so the lack of elongation was not due to the trypsinization step involved in cell isolation. Two basic explanations are possible, either the elongation of linolenic acid is a slow process and the cells do not remain viable long enough or something is inhibiting the elongation system in vitro. A possibility for the latter is that free fatty acids are know to inhibit elongation (Mohrhauer et al, 1967).Excision of the tissue coupled with slicing may release phospholipases which would the increase concentration of free fatty acids within the cells,thus inhibiting elongation.

TABLE 13

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS INCUBATED IN SYNTHETIC CULTURE MEDIUM(NCTC 135)

| FATTY ACID | NEURONAL SOMA | ASTROGLIA |
|---|---|---|
| 14:0 | - | - |
| 16:0 | 0.6* | 0.4* |
| 18:0 + 18:1 | 1.8 | 0.6 |
| 18:2 | 0.8 | 0.5 |
| 18:3 + 20:1 | 89.3 | 88.3 |
| 20:2 + 20:3 | 1.0 | 7.7 |
| 20:4 (n-6) | 3.5 | 0.7 |
| 20:5 (n-3) +22:3 (n-9) | 0.6 | 0.7 |
| 22:4 (n-6) | 0.8 | 0.2 |
| 22:5 (n-6) | 0.2 | 0.1 |
| 22:5 (n-3) | 0.3 | 0.2 |
| 22:6 (n-3) | 0.7 | 0.3 |

*percentage of total fatty acid radioactivity

Isolated brain cells were incubated for 24 hours in synthetic tissue culture medium(NCTC 135 Grand Island Biological Company, Grand Island, N.Y.) with 1 uCi of (1-$^{14}$C) linolenate. Following incubation phospholipid fatty acid radioactivity was analyzed in the usual way.

TABLE 14

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF CALF BRAIN CELLS INCUBATED IN CULTURE MEDIUM

| FATTY ACID | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA |
|---|---|---|---|
| 14:0 | 1.0* | 0.4* | - |
| 16:0 | 1.6 | 3.3 | 0.5* |
| 18:0 | 1.0 | 1.8 | 0.8 |
| 18:1 | 1.1 | 1.8 | 0.3 |
| 18:2 | 0.5 | 0.6 | 0.4 |
| 18:3 + 20:1 | 79.1 | 81.9 | 70.8 |
| 20:2 | 3.5 | 1.7 | 1.0 |
| 20:3 | 2.3 | 0.5 | 1.3 |
| 20:4 (n-6) | 2.3 | 4.1 | 19.1 |
| 20:5 (n-6) + 22:3 (n-9) | 3.0 | 1.4 | 1.5 |
| 22:4 (n-6) | 1.5 | 0.8 | 2.3 |
| 22:5 (n-6) | 1.5 | 0.5 | 0.9 |
| 22:5 (n-3) | 0.6 | 0.6 | 0.1 |
| 22:6 (n-3) | 1.3 | 0.4 | 0.9 |

*percentage of total fatty acid radioactivity

Isolated brain cells were incubated for 24 hours in synthetic tissue culture medium (NCTC 135 Grand Island Biological Company, Grand Island, N.Y.) with 1 uCi of (1-$^{14}$C) linolenate. Following incubation phospholipid fatty acid radioactivity was analyzed in the usual way.

TABLE 15

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO SLICES AND HOMOGENATES OF RAT BRAIN AND LIVER

| FATTY ACID | SLICES | | | HOMOGENATE | |
|---|---|---|---|---|---|
| | 10 day brain | 10 day liver | 21 day brain | 10 day brain | 21 day brain |
| 14:0 | 0.1 | 0.1 | 0.0 | 0.1 | 0.0 |
| 16:0 | 0.5 | 0.1 | 0.0 | 0.0 | 0.1 |
| 18:0 | 0.5 | 0.9 | 0.8 | 0.1 | 0.4 |
| 18:1 | 0.1 | 4.2 | 0.1 | 0.1 | 0.1 |
| 18:2 | - | - | 0.4 | 0.3 | 0.5 |
| 18:3 +20:1 | 79.4 | 85.4 | 81.4 | 79.4 | 84.3 |
| 20:2 +20:3 (n-6) | 6.8 | 3.4 | 8.8 | 8.8 | 4.1 |
| 20:4 (n-6) | 6.1 | 2.1 | 4.5 | 3.9 | 3.5 |
| 20:5 (n-3) +22:3 (n-9) | 2.0 | 1.3 | 1.4 | 3.4 | 2.2 |
| 22:4 (n-6) | 0.9 | 0.6 | 0.7 | 0.9 | 0.6 |
| 22:5 (n-6) | 0.4 | 0.5 | 0.2 | 0.5 | 0.1 |
| 22:5 (n-3) | 0.8 | 0.7 | 0.6 | 1.0 | 0.4 |
| 22:6 (n-3) | 0.8 | 0.8 | 0.7 | 0.7 | 0.6 |

250-350 mg slices from rat brain or liver were incubated either intact or as a homogenate in 5 ml of the Krebs-Ringer medium 8 mg ATP, 2 mg NADH, 1 mg NADPH, 1 mg CoAsh with 1 uCi (1-$^{14}$C) linolenate. The lipids were extracted and the phosphoglyceride fatty acids analyzed as described previously.

TABLE 16

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN TISSUE INCUBATED IN CULTURE MEDIUM

| FATTY ACID | SLICES | TRYPSINIZED SLICES | CELL SUSPENSION |
|---|---|---|---|
| 14:0 | - | 0.1* | 0.2* |
| 16:0 | 0.7* | 0.4 | 3.1 |
| 18:0 | 0.7 | 0.7 | 2.3 |
| 18:1 | - | 0.6 | 0.6 |
| 18:2 | 0.7 | 0.2 | 0.8 |
| 18:3 + 20:1 | 85.8 | 87.2 | 68.9 |
| 20:2 | 2.6 | 2.0 | 2.1 |
| 20:3 | 0.7 | 0.8 | 0.8 |
| 20:4 (n-6) | 3.7 | 2.8 | 6.1 |
| 20:5 (n-3) + 22:3 (n-9) | 0.7 | 2.2 | 3.8 |
| 22:4 (n-6) | 1.9 | 0.7 | 1.7 |
| 22:5 (n-6) | 0.7 | 0.4 | 1.1 |
| 22:5 (n-3) | 0.7 | 0.6 | 4.4 |
| 22:6 (n-3) | 0.7 | 0.8 | 4.4 |

*percentage of total fatty acid radioactivity

One ten day old rat brain was sliced, and incubated as such or first trypsinized in HAP as described or trypsinized and a cell suspension prepared by sieving. The latter was centrifuged at 600xg to prepare the cell suspension. The incubations were in 5 ml of culture medium(NCTC 135) with 1 uCi of (1-$^{14}$C) linolenate. The fatty acids were analyzed as described before.

E. *In vivo* Incorporation of (1-$^{14}$C) linolenate into Neuronal Soma, Astroglia and Oligodendroglia of Rat Brain.

Due to the results regarding elongation *in vitro*, in order to investigate the elongation of linolenic acid it was necessary to find a system where elongation would take place. This was done *in vivo* by intracerebral injections of the linolenate. Since large amounts of white matter are necessary in order to obtain an oligodendroglial fraction, there were two alternatives. The first was injection of a large animal, such as a calf with the isotope and sacrifice at the proper time, or secondly, injection of a large number of small animals, such as rats, then sacrifice and dissect the white matter from the brains. The first was not possible due to cost and handling of the animal and the second was not practical due to the time involved for injection and dissection of the hundreds of rat brains necessary for each experiment. Consequently a third method was devised to measure oligodendrocyte incorporation and elongation of (1-$^{14}$C) linolenate indirectly.

The use of a carrier substance is not new in biochemistry. For example, in gas-liquid or thin-layer chromatography in order to visualize peaks or spots with samples that contain little material but high radioactivity, cold carrier is added, the peak or spot collected and the radioactivity determined.

The carrier is used simply to visualize the material. The disadvantage is that unless the proportion of sample to carrier is known the specific activity of the sample cannot be determined.

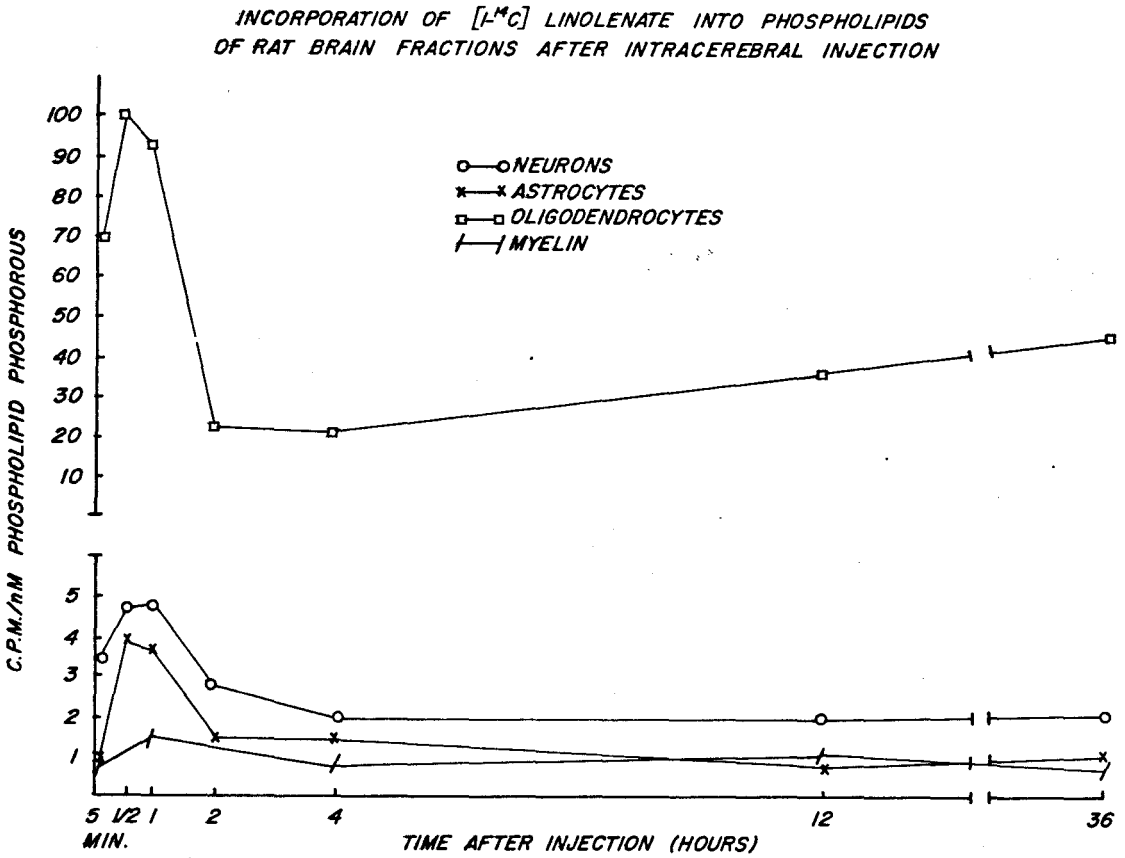
In order to be able to collect rat oligodendrocytes off a gradient, calf brain white matter was used as a carrier. The rat brain and calf white matter were minced together in a proportion of about 1 to 5-7 (see Materials and Methods section) and the material treated as calf white matter for the preparation of oligodendrocytes. Thus when rats were injected intravertebrally with (1-$^{14}$C) linolenate and the oligodendrocytes isolated in this manner, the radioactivity in the oligodendrocyte fraction was due to the rat brain cells. The lipids were extracted from this fraction, the fatty acids analyzed for radioactivity by GLC and the amount of elongation at the various times after injections was determined. While the specific activity of the lipids was diluted by the cold calf brain, the percent distribution of radioactivity among the fatty acids was unchanged. In this way the amount of elongation in the rat oligodendrocytes could be compared to the neuronal and astroglial fractions when the latter two cell types were prepared in the usual way.

However, the specific activity of the oligodendrocyte phospholipids could not be determined due to the presence of the calf brain carrier, a clear disadvantage since it was

desirable to compare the specific activities of the phospholipids of the three cell types, both *in vivo* and *in vitro*. In order to arrive at the phospholipid specific activity of the rat oligodendrocyte fraction, a calculation based on the dilution of the rat myelin by the calf brain myelin was used. This dilution factor, when applied to the phospholipid specific activity of the mixed rat and calf oligodendrocytes yielded the specific activity of the rat brain oligodendrocyte phospholipids. As mentioned in the Material and Methods section, the use of the dilution factor presupposes some assumptions which are not provable at this time. However, while the dilution of myelin may not equal the dilution of oligodendrocytes, it will be within at least ± 100% and thus the number arrived at for the phospholipid specific activity of the oligodendrocytes will give an indication of how active the oligodendrocytes are.

Figure 14 presents the specific activities of the phospholipids from the neuronal soma, astroglial, myelin and oligodendroglia (after multiplication by the dilution factor) fractions of 21 day old rat brain, 5 min., 0.5, 1, 2, 4, 12 and 36 hrs after intracerebral injection of (1-$^{14}$C) linolenate. At all time periods, the highest phospholipid specific activity was in the oligodendrocytes followed by the neurons, the astrocytes and the myelin fractions (except at 12 hrs where the myelin fraction had a slightly higher specific activity than

FIGURE 14




Twenty-one day old rats were injected intracranially with 0.75 uCi of (1-$^{14}$C) linolenate as the albumin complex. At the appropriate time after injection, the animal was sacrificed and the cells prepared as described. The oligodendrocytes were prepared as by mixing rat brains with calf brain white matter. Phospholipid specific activities were determined as described. The oligodendrocyte phospholipid specific activity was calculated by using the dilution factor as specified in the text.

the astrocytes). One hour after injection the neuronal and astroglial phospholipids had specific activities of 4.7 and 3.6 cpm/nM phospholipid phosphorus respectively, and the neuronal to glial ratio was 1.3. The ratio is in excellent agreement for that found in the in vitro incubation (1.5), and the actual specific activities agree well with the values for the cells in vitro (Table 5), especially considering the different variables in the two studies in vitro and in vivo, the concentration of tracer, the condition of the cells and the diffusion of the substrate into the tissue. At all other time periods the neuronal phospholipids were from 1.2 (.5 hr) to 3.7 (5 min) times more active than those of the astrocytes. The least active fraction, myelin, had about 0.20 to 0.25 the activity of the neuronal phospholipids. At one hour after injection, the oligodendrocyte phospholipids had a specific activity of 93 cpm/nM phospholipid phosphorus. This value is relatively close to the in vitro incubation of calf oligodendrocytes (Table 11) considering the different conditions in vivo and in vitro (species and age differences plus those mentioned previously). The agreement of the in vitro and in vivo results is evidence for the validity of the use of the dilution factor. The phospholipid specific activities of the oligodendrocyte and astrocyte fractions peaked at 0.5 hr while those of the neuronal and myelin fraction peaked at 1 hr after

injection. From one to two hours after injection, there was a sharp decrease in the specific activities of all of the fractions and then from two hours until 36 hours, a very slight decrease in the neurons, astrocyte and myelin fraction and an increase in the oligodendrocyte phospholipid specific activity.

Despite the fact that the oligodendrocyte is the cell presumed responsible for the formation and maintenance of the myelin sheath (Bunge 1968, 1970), no product-precursor relationship was obtained between these two fractions. However, given the large mass of myelin (50-70) mMoles phospholipid phosphorus recovered from the rat in these studies) and the small mass of oligodendrocytes (1-2 mMoles phospholipid phosphorus recovered of both rat and calf oligodendrocytes), any lipid transferred from the oligodendrocytes into the myelin would be greatly diluted by pre-existing myelin lipids and hence the specific activity of the myelin fraction could never reach that of the oligodendrocytes.

Ten day old neurons and astrocytes (not shown on figure) had phospholipid specific activities of 2.4 and 5.0 and 3.9 and 5.0 cpm/nM phospholipid phosphorus at 0.5 and 2 hours after injection respectively, agreeing with the <u>in vitro</u> data (Table 5)where astrocytes incorporated approximately twice the linolenate into phospholipids than did the neurons.

The percent distribution of radioactivity between the

neutral, glyco- and phospholipids is given in Tables 17-22. Even at 5 minutes after injection, there was only 30% of the radioactivity in the neutral lipid fraction, which contains the free fatty acids, indicating that the linolenate is rapidly incorporated into phospholipid as soon as it comes in contact with the tissue. This is similar to results described by Sun (1973), namely a very short half life for free fatty acids in brain. The radioactivity in the neutral lipid fraction dropped to about half of the 5 min value within 0.5 hr after injection and from then on there was a steady decrease. The radioactivity in the glycolipid fraction remained at about a steady 2% except in myelin where it was higher (6-7%) at the longer time periods. As the radioactivity in the neutral lipid fraction decreased, there was a proportional increase in the radioactivity of the phospholipids. The myelin and oligodendrocyte fractions were not found to be any more similar in radioactivity distribution than the other cell fractions. The pattern of distribution of radioactivity between the neutral glyco- and phospholipids in the ten day old neurons and astrocytes was very similar to that of 21 day old rats (Table 23).

The distribution of the radioactivity among the ethanolamine and choline phosphoglycerides is shown in Figure 15 and for these and the rest of the phospholipids in Tables 24 to 31. The choline phosphoglyceride function was initially more active

Table 17

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO LIPIDS OF RAT BRAIN AFTER INTRACEREBRAL INJECTION

Twenty-one Day Old Rats

5 Minutes After Injection

| Fraction | Neuronal Soma | Astroglia | Oligodendroglia | Myelin |
|---|---|---|---|---|
| Neutral Lipids | 30.8* | 32.8* | 27.5* | 28.0* |
| Glycolipids | 2.1 | 1.7 | 0.3 | 2.2 |
| Phospholipids | 67.0 | 65.3 | 72.1 | 69.7 |
| Total Lipid Radioactivity | 39.9** | 52.2** | 6.9** | 46.1** |

*Percent of total lipid radioactivity.
**Total lipid CPM x $10^{-3}$.

Legend for Tables 17-23

The lipids from the rat brain cells following intracerebral injection were fractionated by column chromatography as described in the Material and Methods section. The results are expressed as percent of the total lipid radioactivity for each cell type and time point, and the total radioactivity in CPM x $10^{-3}$ is given. The radioactivity in the oligodendrocyte is that of the mixed oligodenrocyte preparation of the dilution factor.

Table 18

INCORPORATION OF (1- C) LINOLENATE INTO LIPIDS OF RAT BRAIN AFTER INTRACEREBRAL INJECTION

Twenty-one Day Old Rats

30 Minutes After Injection

| Fraction | Neuronal Soma | Astroglia | Oligodendroglia | Myelin |
|---|---|---|---|---|
| Neutral Lipids | 16.7* | 20.6* | 19.3* | 16.5* |
| Glycolipids | 2.7 | 1.9 | 2.7 | 3.2 |
| Phospholipids | 82.4 | 77.4 | 78.0 | 80.3 |
| Total Lipid Radioactivity | 56.1 | 43.1 | 11.8 | 93.1 |

*Percent of total lipid radioactivity.
**Total lipid CPM x $10^{-3}$.

Table 19

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO LIPIDS OF RAT BRAIN AFTER INTRACEREBRAL INJECTION

Twenty-one Day Old Rats

1 Hour After Injection

| Fraction | Neuronal Soma | Astroglia | Oligodendroglia | Myelin |
|---|---|---|---|---|
| Neutral Lipids | 16.0* | 15.0* | 19.6* | 15.0* |
| Glycolipids | 2.4 | 2.5 | 3.1 | 4.5 |
| Phospholipids | 82.5 | 82.5 | 77.3 | 80.3 |
| Total Lipid Radioactivity | 38.8** | 39.8** | 6.5** | 95.4** |

*Percent of total lipid radioactivity.
**Total lipid CPM x $10^{-3}$.

Table 20

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO LIPIDS OF RAT BRAIN AFTER INTRACEREBRAL INJECTION

Twenty-one Day Old Rats

Two Hours After Injection

| Fraction | Neuronal Soma | Astroglia | Oligodendroglia | Myelin |
|---|---|---|---|---|
| Neutral Lipids | 11.4* | 15.1* | 13.6* | 14.5* |
| Glycolipids | 0.7 | 0.7 | 1.1 | 5.2 |
| Phospholipids | 87.8 | 84.1 | 85.2 | 80.2 |
| Total Lipid Radioactivity | 17.1** | 18.3** | 1.3** | 19.2** |

*Percent of total lipid radioactivity.
**Total lipid CPM x $10^{-3}$.

TABLE 21

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO LIPIDS OF RAT BRAIN AFTER INTRACEREBRAL INJECTION

Twenty-one Day Old Rats

Four Hours After Injection

| Fraction | Neuronal Soma | Astroglia | Oligodendroglia | Myelin |
|---|---|---|---|---|
| Neutral Lipids | 8.6* | 9.6* | 10.2* | 12.7* |
| Glycolipids | 2.8 | 2.6 | 1.0 | 4.9 |
| Phospholipids | 88.7 | 87.9 | 88.8 | 80.0 |
| Total Lipid Radioactivity | 20.6** | 16.3** | 3.2** | 42.2** |

*Percent of total lipid radioactivity.
**Total lipid CPM x $10^{-3}$.

TABLE 22

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO LIPIDS OF RAT BRAIN AFTER INTRACEREBRAL INJECTION

Twenty-one Day Old Rats

Thirty-six Hours After Injection

| Fraction | Neuronal Soma | Astroglia | Oligodendroglia | Myelin |
|---|---|---|---|---|
| Neutral Lipids | 6.0* | 10.4* | 6.8* | 10.7* |
| Glycolipids | 0.8 | 1.8 | 0.6 | 6.6 |
| Phospholipids | 93.1 | 87.6 | 92.2 | 82.4 |
| Total Lipid Radioactivity | 11.0** | 17.0** | 2.5** | 49.1** |

*Percent of total lipid radioactivity.
**Total lipid CPM x $10^{-3}$.

TABLE 23

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO LIPIDS OF RAT BRAIN AFTER INTRACEREBRAL INJECTION

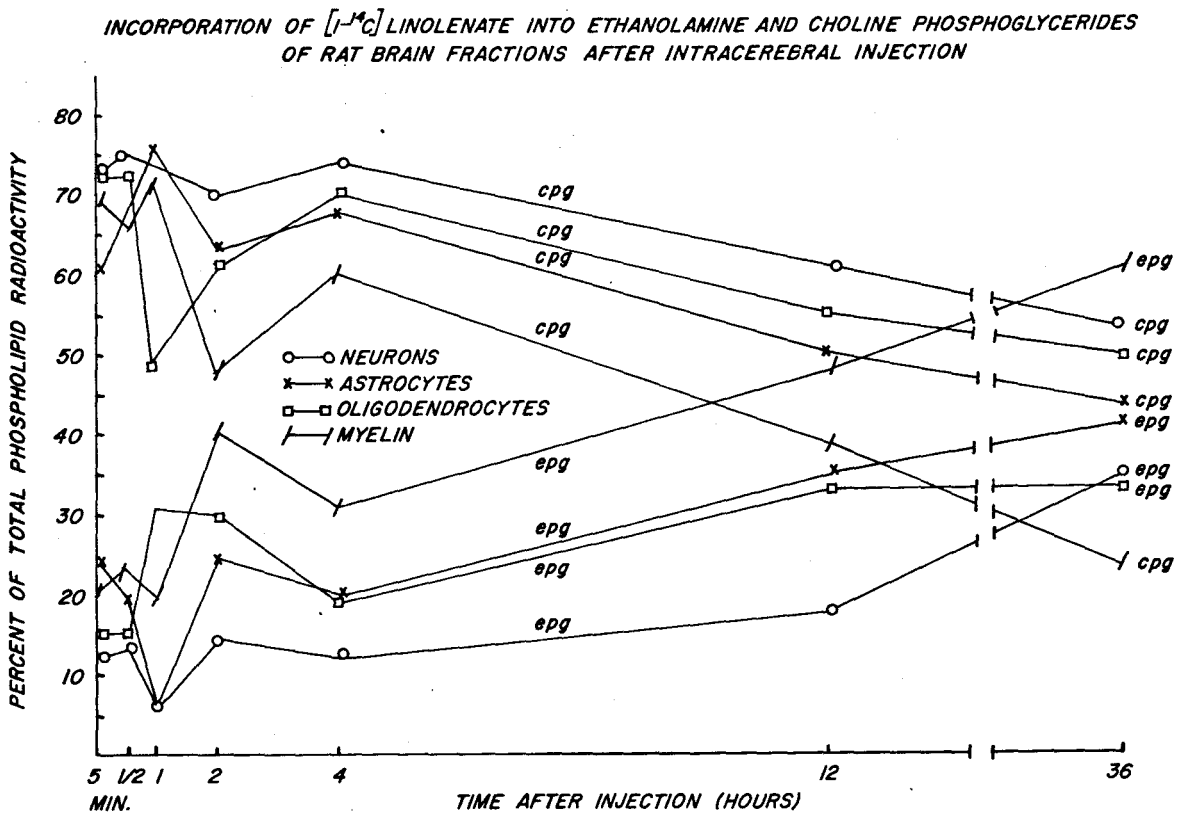
Ten Day Old Rats

| FRACTION | Time After Injection 1/2 Hour | 1 Hour | 2 Hours |
|---|---|---|---|
| Neuronal | | | |
| Neutral Lipids | 19.9* | 14.4* | 12.7* |
| Glycolipids | 1.2 | 1.3 | 1.3 |
| Phospholipids | 78.9 | 84.1 | 85.6 |
| Total Lipid Radioactivity | 35.6** | 62.4** | 58.6** |
| Astroglial | | | |
| Neutral Lipids | 26.7* | 19.1* | 17.8* |
| Glycolipids | 0.6 | 1.7 | 1.6 |
| Phospholipids | 72.3 | 79.0 | 80.5 |
| Total Lipid Radioactivity | 61.9** | 42.1** | 64.9** |

*Percent of total lipid radioactivity.
**Total lipid CPM x $10^{-3}$.

than ethanolamine phosphoglyceride (Figure 15) but the trend was toward a decrease in the radioactivity of the CPG fraction and an increase in that of the ethanolamine phosphoglyceride fraction. There were major changes in this direction between 0.5 and 1 hour in the oligodendrocyte fraction and between 1 and 2 hours in the neuron, astrocyte and myelin fractions. By twelve hours after injection, the radioactivity in the myelin ethanolamine phosphoglyceride fraction had surpassed that of the choline phosphoglyceride fraction, while for the three cell types it was probably after 36 hours that this occurred. It would appear that the (1-$^{14}$C) linolenate was first incorporated into the choline phosphoglycerides fraction and then transferred to the ethanolamine phosphoglycerides after elongation. Since the specific radioactivity for each of the four fractions is nearly constant from two to thirty-six hours after injection (Figure 14), the rise in the proportion of radioactivity in the ethanolamine phosphoglyceride fraction cannot be explained by preferential uptake of the (1-$^{14}$C) linolenate by the ethanolamine phosphoglyceride fraction. This is similar to the results reported by Yavin and Menkes (1974), for brain culture, who also found the choline phosphoglyceride fraction begin to decrease in radioactivity four hours after administration of (1-$^{14}$C) linolenate to the cultures with a concomitant rise in the ethanolamine phosphoglyceride radio-

FIGURE 15




Legend for Figure 16 and Tables 18-25:

Phospholipids were separated by thin layer chromatography as described. Following development of the TLC plate the individual phospholipid spots were scraped off into counting vials and the radioactivity determined by liquid scintillation counting. The results were expressed as percent of total phospholipid radioactivity.

TABLE 24

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Five Minutes-21 Day Old Rats

| FRACTION | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| EPG | 12.3* | 24.6* | 15.1* | 20.7* |
| IPG | 9.1 | 8.1 | 10.0 | 6.6 |
| SPG | 5.0 | 5.7 | 1.5 | 3.2 |
| CPG | 72.8 | 61.1 | 72.0 | 69.1 |
| SPM + L-CPG | 0.8 | 0.5 | 1.3 | 0.4 |

*Percent of Total Phospholipid Radioactivity.

TABLE 25

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

One Half Hour-21 Day Old Rats

| FRACTION | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| EPG | 12.7* | 19.5* | 15.0* | 23.0* |
| IPG | 8.3 | 6.4 | 2.3 | 2.9 |
| SPG | 1.6 | 5.1 | 8.1 | 6.0 |
| CPG | 75.1 | 66.8 | 72.6 | 66.7 |
| SPM + L-CPG | 2.2 | 2.1 | 2.0 | 1.4 |

*Percent of Total Phospholipid Radioactivity.

TABLE 26

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

One Hour-21 Day Old Rats

| FRACTION | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| EPG | 5.9* | 5.6* | 31.1* | 19.2* |
| IPG | 0.9 | 5.9 | 7.3 | 4.1 |
| SPG | 7.6 | 8.5 | 11.6 | 3.8 |
| CPG | 74.0 | 75.9 | 48.5 | 72.5 |
| SPM + L-CPG | 1.7 | 4.1 | 1.5 | 0.5 |

*Percent of Total Phospholipid Radioactivity.

TABLE 27

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Two Hours--21 Day Old Rats

| FRACTION | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| EPG | 14.1* | 24.6* | 30.3* | 39.9* |
| IPG | 5.1 | 7.9 | 4.6 | 7.4 |
| SPG | 6.3 | 3.2 | 4.6 | 3.8 |
| CPG | 70.5 | 62.9 | 60.5 | 48.6 |
| SPM + L-CPG | 4.0 | 1.2 | 0.2 | 0.3 |

*Percent of Total Phospholipid Radioactivity.

TABLE 28

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Four Hours-21 Day Old Rats

| FRACTION | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| EPG | 11.8* | 20.2* | 19.5* | 31.2* |
| IPG | 6.4 | 4.4 | 6.7 | 3.2 |
| SPG | 3.0 | 3.9 | 1.1 | 4.2 |
| CPG | 73.9 | 67.5 | 69.9 | 59.9 |
| SPM + L-CPG | 4.9 | 4.0 | 2.5 | 1.4 |

*Percent of Total Phospholipid Radioactivity.

TABLE 29

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Twelve Hours-21 Day Old Rats

| FRACTION | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| EPG | 18.1* | 34.7* | 32.5* | 47.8* |
| IPG | 7.1 | 9.3 | 7.1 | 4.9 |
| SPG | 8.4 | 4.1 | 3.9 | 8.0 |
| CPG | 60.9 | 50.6 | 55.1 | 38.9 |
| SPM + L-CPG | 5.4 | 1.2 | 1.4 | 0.4 |

*Percent of Total Phospholipid Radioactivity.

TABLE 30

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Thirty-Six Hours-21 Day Old Rats

| FRACTION | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| EPG | 34.6* | 41.6* | 33.7* | 61.3* |
| IPG | 4.6 | 5.9 | 11.0 | 6.6 |
| SPG | 4.6 | 8.2 | 4.1 | 7.4 |
| CPG | 54.3 | 43.7 | 50.0 | 24.2 |
| SPM + L-CPG | 2.0 | 0.6 | 1.1 | 0.4 |

*Percent of Total Phospholipid Radioactivity.

TABLE 31

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FRACTIONS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Ten Day Old Rats

| | One Half Hour | | One Hour | |
|---|---|---|---|---|
| FRACTION | NEURONAL SOMA | ASTROGLIA | NEURONAL SOMA | ASTROGLIA |
| EPG | 20.1* | 25.8* | 13.8* | 18.6* |
| IPG | 10.3 | 16.1 | 3.6 | 8.1 |
| SPG | 1.8 | 1.3 | 13.0 | 2.1 |
| CPG | 67.3 | 56.0 | 67.0 | 69.6 |
| SPM + L-CPG | 0.4 | 0.7 | 2.6 | 1.6 |

Two Hours

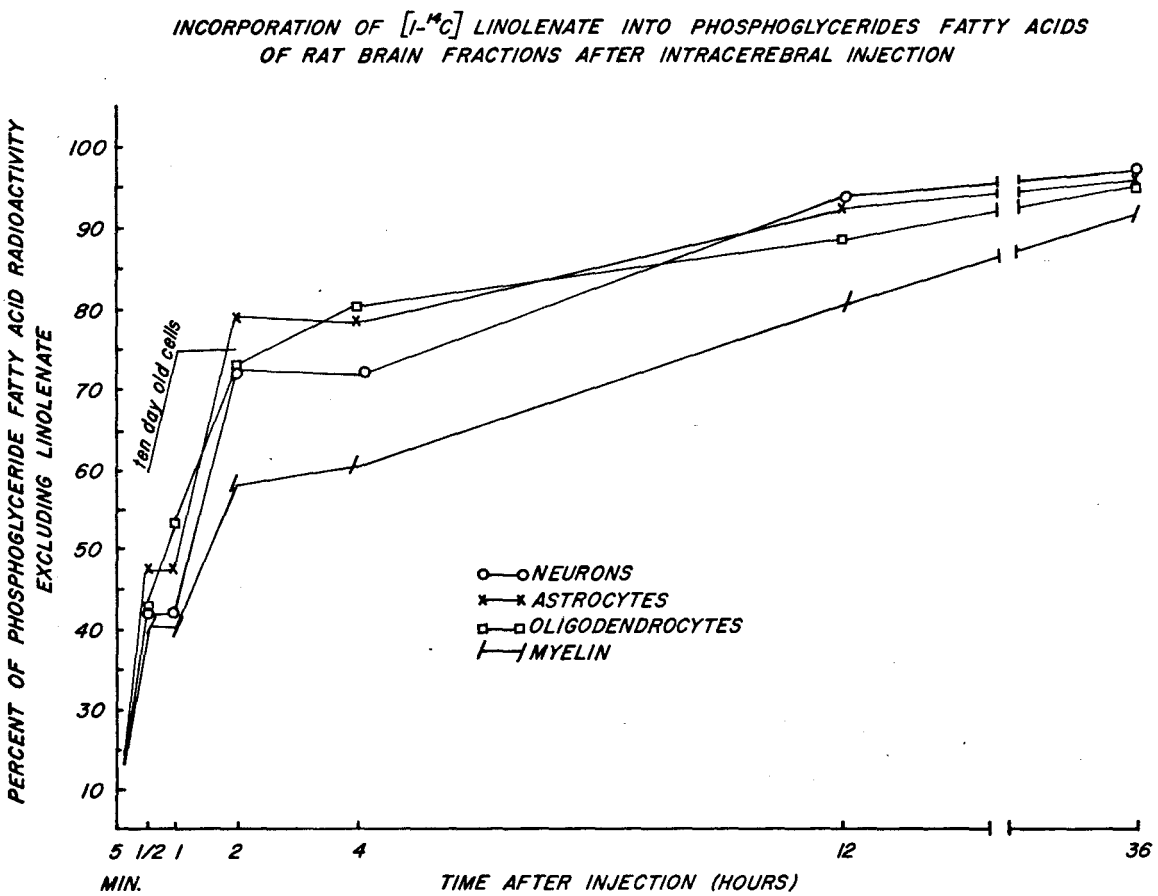
| | NEURONAL SOMA* | ASTROGLIA* |
|---|---|---|
| EPG | 19.3 | 27.6 |
| IPG | 9.2 | 11.6 |
| SPG | 2.2 | 2.0 |
| CPG | 67.7 | 58.1 |
| SPM + L-CPG | 1.5 | 0.6 |

*Percent of Total Phospholipid Radioactivity.

activity. By eight hours after (1-$^{14}$C) linolenate administration, the ethanolamine- and choline phosphoglyceride fractions had nearly equal radioactivity in their experiments. The remaining phosphoglyceride fractions (Table 24-31) contained little of the radioactivity compared to EPG and CPG and by 36 hours after injection IPG and SPG were nearly equal in radioactivity.

The rate of conversion of the linolenate to other fatty acids is shown in Figure 16 wherein the percentage of non-linolenate phosphoglyceride fatty acid radioactivity is plotted against time after injection. At five minutes after injection 75% of the fatty acid radioactivity was still linolenate; approximately 25% has been converted to other fatty acids. A small amount of degradation and synthesis utilizing the degradation products had occurred since approximately 2.5% of the fatty acid radioactivity was in palmitic acid (1.3% in the oligodendroglia fraction). At five minutes approximately 20% of the fatty acid radioactivity was in elongated products, with the 20:4 and 20:5 + 22:3 fractions having the most, 5-8% each (Table 32). Within thirty minutes after injection 40-45% of the linolenate had been elongated or degraded and neutralized (Figure 16 and Table 33). The astrocytes had converted the most (47%) and myelin the least (40%) of the linolenate to other fatty acids. The three cell fractions have approximately

FIGURE 16




Phosphoglyceride fatty acid radioactivity was analyzed as described in the Materials and Methods section. The percent of fatty acid radioactivity excluding the starting tracer, (1-$^{14}$C) linolenate, was calculated and plotted against time after injection.

TABLE 32

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Five Minutes-21 Day Old Rats

| FATTY ACID | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| 14:0 | 0.2* | 0.1* | 0.1* | - |
| 16:0 | 2.6 | 2.4 | 1.3 | 2.4* |
| 18:0 | 0.6 | 0.7 | 0.1 | 1.0 |
| 18:1 | 0.3 | - | 0.3 | - |
| 18:2 | - | 0.3 | 0.2 | - |
| 18:3 + 20:1 | 75.6 | 74.9 | 77.0 | 77.3 |
| 20:2 | 2.7 | 5.9 | 3.2 | 4.2 |
| 20:3 | 0.7 | 0.6 | 0.6 | 0.6 |
| 20:4 (n-6) | 8.5 | 5.8 | 7.6 | 6.5 |
| 20:5 (n-6) +22:3 (n-9) | 6.2 | 6.3 | 6.3 | 5.1 |
| 22:4 (n-6) | 0.8 | 0.6 | 1.2 | 1.0 |
| 22:5 (n-6) | 0.6 | 0.5 | 0.5 | 0.2 |
| 22:5 (n-3) | 0.8 | 0.9 | 1.2 | 0.9 |
| 22:6 (n-3) | 0.4 | 1.0 | 0.3 | 0.9 |
| Total Elongation | 20.7 | 21.6 | 20.9 | 19.4 |
| Total De Novo | 3.7 | 3.2 | 1.8 | 3.4 |

*Percent of Total Fatty Acid Radioactivity.

Legend for Tables 26-32

Phosphoglyceride fatty acid radioactivity was analyzed by GLC as described.

35-37% of the fatty acid radioactivity in the elongated products, while myelin contains only 23% in the long chain fatty acids. Among the polyunsaturated fatty acids, 20:4 and 20:5 + 22:3 had the most radioactivity. Six to nine percent of the radioactivity in the cells and 17% in myelin phosphoglycerides was in 16:0 - 18:1 fatty acids, mostly in palmitic acid. Between one-half and one hour, there was a lag in conversion of the linolenate in all fractions except the oligodendroglia (Figure 16). Also, the radioactivity in 22:5 (n-3) began to increase, most likely at the expense of 20:5 or 22:3 (Table 34). There was another rapid conversion of the linolenate between one and two hours after injection, 70-80% of the radioactivity was in other fatty acids. Of the saturates, palmitic acid still contained the most radioactivity (7.6-11%), but in the polyunsaturated fatty acids fractions, the radioactivity of 22:5 (n-3) had nearly equalled or surpassed that of both the 20:4 and 20:5 x 22:3 fractions (Table 35). The radioactivity in 22:6 (n-3) was also beginning to increase.

At four hours after injection, there was the widest spread in the percentage of radioactivity in the linolenate of the four fractions, 23% in the astrocyte and 40% in the myelin phosphoglycerides (Table 36). From 45% (myelin) to 56% (neuronal soma) of the fatty acid radioactivity was in the polyunsaturated fatty acid fraction. From four to thirty-six hours

TABLE 33

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Thirty Minutes-21 Day Old Rats

| FATTY ACID | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| 14:0 | 0.2* | 0.2* | 0.2* | 0.1* |
| 16:0 | 3.9 | 7.3 | 4.2 | 10.4 |
| 18:0 | 1.6 | 1.6 | 1.7 | 5.0 |
| 18:1 | 0.6 | 0.4 | 0.3 | 1.3 |
| 18:2 | 0.1 | - | 0.2 | 0.4 |
| 18:3 + 20:1 | 58.7 | 53.0 | 57.1 | 59.9 |
| 20:2 | 1.5 | 3.0 | 1.3 | 1.7 |
| 20:3 | 0.7 | 0.4 | 0.8 | 0.9 |
| 20:4 (n-6) | 14.7 | 11.0 | 13.8 | 9.7 |
| 20:5 (n-6) +22:3 (n-9) | 10.6 | 15.5 | 12.0 | 6.3 |
| 22:4 (n-6) | 1.4 | 1.7 | 1.5 | 1.6 |
| 22:5 (n-6) | 1.4 | 0.7 | 1.4 | 0.6 |
| 22:5 (n-3) | 3.1 | 2.2 | 3.6 | 1.0 |
| 22:6 (n-3) | 1.4 | 2.7 | 1.8 | 1.1 |
| Total Elongation | 34.8 | 37.2 | 36.2 | 22.9 |
| Total De Novo | 6.3 | 9.5 | 6.4 | 16.8 |

*Percent of Total Fatty Acid Radioactivity.

TABLE 34

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

One Hour-21 Day Old Rats

| FATTY ACID | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| 14:0 | 0.2* | 0.2* | 0.3* | 0.4* |
| 16:0 | 6.4 | 8.0 | 6.4 | 5.5 |
| 18:0 | 1.6 | 1.9 | 2.6 | 2.6 |
| 18:1 | 0.5 | 0.6 | 1.0 | 0.5 |
| 18:2 | 0.2 | 0.2 | - | 0.3 |
| 18:3 + 20:1 | 58.6 | 53.8 | 46.1 | 64.7 |
| 20:2 | 1.5 | 1.4 | 1.9 | 1.5 |
| 20:3 | 0.6 | 0.8 | 1.3 | 1.0 |
| 20:4 (n-6) | 15.1 | 23.7 | 14.4 | 11.3 |
| 20:5 (n-6) +22:3 (n-9) | 9.0 | 1.0 | 15.2 | 6.3 |
| 22:4 (n-6) | 1.0 | 0.9 | 1.7 | 1.4 |
| 22:5 (n-6) | 0.8 | - | 0.9 | 0.6 |
| 22:5 (n-3) | 3.1 | 5.0 | 6.3 | 2.5 |
| 22:6 (n-3) | 1.4 | 2.3 | 2.0 | 1.5 |
| Total Elongation | 32.5 | 35.1 | 43.7 | 26.1 |
| Total De Novo | 8.7 | 10.7 | 10.3 | 9.0 |

*Percent of Total Fatty Acid Radioactivity.

TABLE 35

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Two Hours-21 Day Old Rats

| FATTY ACID | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA |
|---|---|---|---|
| 14:0 | - | - | - |
| 16:0 | 7.6* | 11.2* | 10.9* |
| 18:0 | 2.6 | 2.5 | 3.4 |
| 18:1 | | 1.1 | |
| 18:2 | 0.1 | 0.5 | - |
| 18:3 + 20:1 | 27.5 | 20.8 | 26.1 |
| 20:2 | 1.0 | 1.4 | - |
| 20:3 | 0.6 | 1.2 | - |
| 20:4 (n-6) | 19.1 | 12.1 | 15.1 |
| 20:5 (n-6) +22:3 (n-9) | 15.9 | 17.2 | 19.3 |
| 22:4 (n-6) | 1.3 | 1.3 | 1.7 |
| 22:5 (n-6) | 3.1 | 1.7 | 2.5 |
| 22:5 (n-3) | 16.5 | 19.1 | 13.4 |
| 22:6 (n-3) | 4.8 | 9.8 | 7.6 |
| Total Elongation | 62.3 | 63.8 | 59.5 |
| Total De Novo | 10.2 | 14.9 | 14.3 |

*Percent of Total Fatty Acid Radioactivity.

TABLE 36

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Four Hours-21 Day Old Rats

| FATTY ACID | NEURONAL SOMA | ASTROGLIA | MYELIN |
|---|---|---|---|
| 14:0 | - | 0.2* | 0.3* |
| 16:0 | 11.2 | 17.9 | 9.1 |
| 18:0 | 2.3 | 4.8 | 5.8 |
| 18:1 | 1.6 | 1.2 | |
| 18:2 | 0.1 | 0.1 | - |
| 18:3 + 20:1 | 28.6 | 22.8 | 39.7 |
| 20:2 | 1.6 | 0.2 | 0.6 |
| 20:3 | 0.4 | 0.5 | 0.2 |
| 20:4 (n-6) | 23.4 | 11.4 | 16.3 |
| 20:5 (n-6) +22:3 (n-9) | 11.3 | 9.3 | 12.5 |
| 22:4 (n-6) | 1.7 | - | 2.4 |
| 22:5 (n-6) | 3.7 | 2.0 | 1.3 |
| 22:5 (n-3) | 8.9 | 11.7 | 7.7 |
| 22:6 (n-3) | 5.1 | 17.8 | 4.1 |
| Total Elongation | 56.1 | 52.0 | 45.1 |
| Total De Novo | 15.1 | 24.1 | 15.2 |

*Percent of Total Fatty Acid Radioactivity.

after injection there was a gradual rise in the non-linolenate fatty acid radioactivity. Only 6-11% of the radioactivity of the cell phosphoglyceride fatty acids was still in the linolenate fraction at 12 hours, 18.8% in the myelin fraction (Table 37). Radioactivity in the 22:6 (n-3) fractions was approaching that of the 22:5 (n-3) fraction. Over 70% of the radioactivity of the cell phosphoglyceride fatty acids was in the elongated products, 58.3% in myelin. At 36 hours after injection (Table 38) the four fractions have nearly identical amounts of radioactivity remaining in linolenate (3-8%). In the neuronal soma most of the radioactivity was in 22:5 (n-3) followed by 22:6, 20:3+20:4, 16:0 and 20:5+ 22:3 while in the oligodendroglia the pattern was 22:6> 22:5 (n-3)>18:0> 16:0> 20:4> 20:5 + 22.3 and in myelin phosphoglyceride fatty acids 22:6> 22:5 (n-3) > 20:3 + 20:4> 18:0 >20:5 + 22:3> 16:0, indicating differences in the elongation patterns of the various fractions. Yavin and Menkes (1974) also found 22:6, 21:5 and 20:5 to be the major products formed from 18:3 in their cell cultures and little radioactivity was in the short chain fatty acids. Ten day old neuronal soma and astroglia (Figure 16 and Tables 39 and 40)appeared to elongate more rapidly than 21 day old cells.

In the three cell fractions and myelin, between 5 minutes and 36 hours, the fatty acid radioactivity goes in a pattern

TABLE 37

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Twelve Hours-21 Day Old Rats

| FATTY ACID | NEURONAL SOMA | ASTROGLIA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|---|
| 14:0 | 0.2* | 0.4* | - | - |
| 16:0 | 15.3 | 12.2 | 6.3* | 11.5* |
| 18:0 | 3.9 | 4.2 | 4.3 | 9.0 |
| 18:1 | 2.9 | 3.5 | 2.9 | 2.3 |
| 18:2 | 0.3 | 0.4 | - | 0.3 |
| 18:3 + 20:1 | 5.9 | 7.6 | 10.9 | 18.8 |
| 20:2 | 0.3 | 1.0 | - | 0.6 |
| 20:3 | 0.3 | 0.4 | - | 0.6 |
| 20:4 (n-6) | 10.4 | 9.2 | 12.7 | 13.9 |
| 20:5 (n-6) +22:3 (n-9) | 15.6 | 10.9 | 19.3 | 13.5 |
| 22:4 (n-6) | 1.7 | 1.2 | 1.7 | 2.6 |
| 22:5 (n-6) | 2.7 | 1.2 | 3.5 | 2.0 |
| 22:5 (n-3) | 27.0 | 26.0 | 24.8 | 13.5 |
| 22:6 (n-3) | 13.4 | 21.7 | 13.5 | 11.6 |
| Total Elongation | 71.4 | 71.6 | 75.5 | 58.3 |
| Total De Novo | 22.3 | 20.3 | 13.5 | 22.8 |

*Percent of Total Fatty Acid Radioactivity.

TABLE 38

INCORPORATION OF (1-$^{14}$C)LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

Thirty-six Hours-21 Day Old Rats

| FATTY ACID | NEURONAL SOMA | OLIGODENDROGLIA | MYELIN |
|---|---|---|---|
| 14:0 | 0.1* | 0.9* | 0.4* |
| 16:0 | 11.0 | 9.5 | 7.3 |
| 18:0 | 4.3 | 11.0 | 13.3 |
| 18:1 | 4.7 | | |
| 18:2 | - | - | 0.6 |
| 18:3 + 20:1 | 2.9 | 4.8 | 8.2 |
| 20:2 | - | - | - |
| 20:3 | 11.6 | - | 13.5 |
| 20:4 (n-6) | | 8.5 | |
| 20:5 (n-9) +22:3 (n-9) | 7.7 | 7.6 | 9.8 |
| 22:4 (n-6) | 1.6 | 1.9 | 2.5 |
| 22:5 (n-6) | 3.0 | 3.8 | 5.3 |
| 22:5 (n-3) | 31.2 | 24.3 | 18.2 |
| 22:6 (n-3) | 22.3 | 27.6 | 20.9 |
| Total Elongation | 77.4 | 73.7 | 70.2 |
| Total De Novo | 20.1 | 21.4 | 21.0 |

*Percent of Total Fatty Acid Radioactivity.

TABLE 39

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

One Hour-10 Day Old Rats

| FATTY ACID | NEURONAL SOMA | ASTROGLIA |
|---|---|---|
| 14:0 | 0.3* | 0.6* |
| 16:0 | 18.0 | 21.2 |
| 18:0 | 3.3 | 4.0 |
| 18:1 | 1.5 | 2.1 |
| 18:2 | 0.2 | 0.1 |
| 18:3 + 20:1 | 25.5 | 25.0 |
| 20:2 | 1.7 | 1.7 |
| 20:3 | 0.6 | 0.8 |
| 20:4 (n-6) | 15.1 | 8.2 |
| 20:5 (n-6) +22:3 (n-9) | 16.1 | 19.8 |
| 22:4 (n-6) | 1.2 | 1.0 |
| 22:5 (n-6) | 2.5 | 1.0 |
| 22:5 (n-3) | 9.7 | 9.1 |
| 22:6 (n-3) | 4.0 | 5.3 |
| Total Elongation | 50.9 | 46.9 |
| Total De Novo | 23.1 | 27.9 |

* Percent of Total Fatty Acid Radioactivity.

TABLE 40

INCORPORATION OF (1-$^{14}$C) LINOLENATE INTO PHOSPHOGLYCERIDE FATTY ACIDS OF RAT BRAIN CELLS AFTER INTRACEREBRAL INJECTION

10 Day Old Rats

| FATTY ACID | One Half Hour NEURONAL SOMA | ASTROGLIA | Two Hours NEURONAL SOMA |
|---|---|---|---|
| 14:0 | 0.3* | 0.4* | 0.5* |
| 16:0 | 15.1 | 15.0 | 18.8 |
| 18:0 | 3.6 | 2.8 | 4.5 |
| 18:1 | 0.7 | 1.1 | 1.7 |
| 18:2 | 1.5 | 0.2 | 0.4 |
| 18:3 + 20:1 | 40.7 | 40.8 | 26.0 |
| 20:2 | 0.2 | 2.8 | 1.3 |
| 20:3 | 0.6 | 0.7 | 12.5 |
| 20:4 (n-6) | 15.5 | 8.6 | 11.4 |
| 20:5 (n-6) +22:3 (n-9) | 13.1 | 20.1 | 1.2 |
| 22:4 (n-6) | 1.1 | 1.0 | 1.4 |
| 22:5 (n-6) | 1.1 | 0.6 | 2.7 |
| 22:6 (n-3) | 3.5 | 3.5 | 7.6 |
| 22:6 (n-3) | 2.9 | 2.4 | 10.2 |
| Total Elongation | 38.0 | 39.7 | 48.3 |
| Total De Novo | 19.7 | 19.3 | 25.5 |

*Percent of Total Fatty Acid Radioactivity.

from 18:3→ 20:4→ 20:5→ 22:5→ 22:6, which is expected from the known elongation pattern of linolenic acid (Sprecher, 1972).

The patterns of fatty acid conversion (Figure 16) and EPG-CPG radioactivity (Figure 15) resemble each other in that there were rapid changes within the first two hours followed by a period from 2-36 hours of gradual changes. It would appear then, that as the linolenate is elongated, the elongated products are transferred to the ethanolamine phosphoglycerides. This is not surprising since ethanolamine phosphoglyceride fatty acids are more unsaturated than the fatty acids found in the choline phosphoglyceride fraction and polyunsaturated fatty acids are preferentially incorporated into the ethanolamine phosphoglycerides (McMurray and McGee, 1972).

The data in Tabe 41 are the proportion of radioactivity in the carboxyl carbon of the fatty acids 16:0, 18:3, 20:4, 20:5, 22:5 (n-3) and 22:6 collected from the GLC of various fractions and times. Because of the low carboxyl radioactivity in the 22:6 and 22:5 (n-3) fractions, it is obvious that the radioactivity in these fatty acids was in the remainder of the carbon chain and hence are derived from the elongation of the radioactive 18:3. The data from 20:4 and 20:5 demonstrate that most of the radioactivity of these fatty acids was not in the carboxyl carbon. However, it is impossible to conclude how these acids were synthesized, probably because of the heterogeneous nature

of these fatty acid peaks (20:4 contains both 20:4 (n-6) and 20:4 (n-3) while 20:5 contains 22:3 (n-9), 20:5 (n-6) and 20:5 (n-3). Low R.C.A. (theoretically zero) would indicate elongation of 18:3, a R.C.A. of about 10% indicates de Novo synthesis and equal distribution of the radioactivity between the carbon atoms. A high R.C.A. indicates elongation of an endogenous non-radioactive fatty acid such as the essential fatty acid linoleic. Dhopeshworkar, et al (1971b) obtained similar results following feeding of (1-$^{14}$C) linolenate to rats and extraction and analysis of the brain fatty acids, except that their 20:4 had a very high R.C.A. (81.7%) indicating different mechanisms of degradation and elongation between fed and intracerebrally injected fatty acids. This is certainly an important area for future research.

TABLE 41

PERCENTAGE OF RADIOACTIVITY IN THE CARBOXYL CARBON OF BRAIN FATTY ACIDS AFTER INJECTION OF (1-$^{14}$C) LINOLENATE

| FATTY ACID | SOURCE | TIME AFTER INJECTION | % R.C.A.* |
|---|---|---|---|
| 16:0 | myelin | 1/2 hour | 2.8 |
| 16:0 | myelin | 12 hours | 11.0 |
| 16:0 | neurons | 1/2 hour | 5.3 |
| 18:3 | neurons | 1 hour | 98.0 |
| 18:3 | astrocytes | 1 hour | 100 |
| 18:3 | myelin | 1/2 hour | 87.0 |
| 18:3 | myelin | 1 hour | 91.5 |
| 20:4 | neurons | 5 min. | 9.2 |
| 20:4 | neurons | 1 hour | 9.3 |
| 20:4 | astrocytes | 4 hours | 13.0 |
| 20:4 | myelin | 5 min. | 3.3 |
| 20:5 | astrocytes | 4 hours | 9.3 |
| 20:5 | myelin | 1/2 hour | 3.3 |
| 22:5 (n-3) | myelin | 36 hours | 0.11 |
| 22:6 | myelin | 12 hours | 0.54 |
| 22:6 | myelin | 36 hours | 0.56 |

*% R.C.A. = relative carboxyl activity (radioactivity in -COOH X 100/radioactivity in total fatty acid

The fatty acid peaks from phosphoglycerides of rat brain cells of intracerebrally injected rats were collected by GLC and decarboxylated as described.

# IV Conclusions

## A. The Method of Brain Cell Isolation

It is significant for the future of neurochemistry that this study was able to duplicate the cell isolation procedure of Norton and Poduslo (1970) and obtain results similar to theirs. The investigation of neuronal and glial metabolism is an important area of research and for the bulk cell isolation methods to become accepted (as they are, since an increasing number of papers and reports at the American Society for Neurochemistry meetings utilize such a method) they must be readily reproducible from laboratory to laboratory. While some conditions (such as amount and time of trypsinization) must be altered from laboratory to laboratory, it is the conclusion of this study that the Norton and Poduslo method offers a practical isolation technique for the preparation of neurons, astrocytes and oligodendrocytes.

However, one must be cautious since the isolation procedures may alter the metabolism of the cells as this study demonstrated with the incorporation of (1-$^{14}$C) acetate into the choline phosphoglycerides (Table 3). Careful controls are necessary to determine the extent of damage, if any, to the particular pathway or enzyme under investigation in the isolated cells.

In this study, a method is presented for the investigation

of the *in vivo* oligodendrocyte metabolism of small animals, by using white matter from calf brain as a carrier and a mathematical calculation of the dilution factor. The validity of the method was indicated by the fact that the results obtained agree with those found in the *in vitro* studies and the theoretical prediction for the dilution factor was close to the actual experimental values.

B. The Neuron and Astrocyte

Previous work with neuronal soma and glial preparations has revealed that in the adult rat or rabbit, *in vivo* and *in vitro*, protein synthesis is occurring at a more rapid rate in the neuronal fraction than in the glia (Blomstrand and Hamberger, 1969, 1970; Rose, 1968, Hamberger et al, 1971; Tiplady and Rose, 1971). Likewise, studies of RNA metabolism of the two cell fractions has demonstrated a more rapid incorporation of labelled precursors into neuronal as compared to glial RNA (Volpe and Giuditta, 1967; Flangas and Bowman, 1970; Jarlstedt and Hamberger, 1971). This present work and that of Freysz et al, (1969) arrive at a similar conclusion with regard to lipids; a more rapid metabolism in the adult neuron than in the adult glia with respect to the incorporation of the lipid precursors investigated in this study, (Figure 9 and Table 5). The finding in this study of a more active astrocyte lipid metabolism at 10 days of age probably reflects the fact that, at

that age in the rat, the glia are still dividing and growing at a rapid rate and hence are rapidly synthesizing membranes, while the neurons have reached their adult number (Brizzee et al, 1964). From these results, it can be concluded that generally the neuronal soma have a more active metabolism than the neuroglia.

Both cell types appear to have the full compliment of lipid synthesizing enzymes; some pathways such as ganglioside biosynthesis (Radin et al, 1972; Jones et al, 1972) and the cytidine-dependent pathways for choline and ethanolamine phosphoglyceride biosynthesis (Goracci et al, 1973) may be primarily neuronal. This study found that both cell types have the ability both to synthesize all the phospholipid classes from the radioactive precursors acetate, stearic, linoleic and linolenic acid and elongate linolenic acid. While there were quantitative differences in the amounts of lipid synthesized which depended upon the age of the cells (Figure 9 and Table 5) very few qualitative differences were found in the pattern of incorporation into phospholipids (Figure 12, 13 and 15 and Tables 24-31) and elongation into fatty acids (Tables 7 and 32-40). The possibility of a localization of lipid biosynthetic pathways to one cell type and transfer of lipid to other cells was neither proved nor disproved by this study.

There are three processes in which the glia have been

found to be more active than the neurons, 1) generation of amino acids from glucose (Rose, 1968), 2) uptake and concentration of potassium from the medium (the glial $Na^+K^+$-ATPase was also more sensitive to potassium ions; Haljamae and Hamberger, 1971; Henn, et al, 1972) and 3) uptake of $\alpha$ aminobutyrate from the medium (Henn and Hamberger, 1971). The function of these cells may be both as a source of some metabolites for the neurons, since the glia generate amino acids but the neurons utilize them more rapidly, and also as regulators of the extracellular milieu of the neurons (see Bunge, 1970 for a discussion).

With regard to brain lipid metabolism, this study describes results similar to those of Yavin and Menkes (1974), namely that the elongation of fatty acids occurs before they are incorporated into EPG and the elongation of 18:3 to 20:5, 22:5 (n-3) and 22:6 (n-3).

C. The Oligdendrocyte and Myelin

Histochemical studies of the oligodendrocyte have indicated high levels of enzymes of the hexose monophosphate shunt (Lowry, 1955; Meyer and Meyer, 1964). This has been interpreted by Adams (1965) as being indicative of the rapid lipid metabolism of the oligodendrocyte in relationship to its maintenance of the myelin sheath, since this pathway supplies the NADPH necessary for lipid biosynthesis (see Bunge, 1968 for a discussion of the oligodendrocyte-myelin relationship). This

study found no product-precursor relationship between the oligodendrocyte and myelin, but this may have been due to the type of experimental protocol used. The fatty acid composition of the phospholipids of the oligodendrocytes and myelin fractions was similar (Table 10).

Norton and Poduslo (1973) calculated that during development each oligodendrocyte must synthesize more than three times its own weight of myelin each day. It would be necessary for each of these cells to be highly specialized for the synthesis of myelin membranes. This would explain the histochemical studies and the findings of the work presented in this paper: that the oligodendrocyte is a cell uniquely geared to the synthesis of lipids for the production of myelin.

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## BIOGRAPHICAL SKETCH

Steven Roger Cohen was born in Chicago, Illinois on June 10, 1947. He is a graduate of the Armstrong Elementary School and Sullivan High School of Chicago. His undergraduate work was done at Illinois Institute of Technology where he was awarded an Illinois State Scholarship and was on the Dean's List. He received a B.S. in Biology in June, 1969.

In September, 1969, he entered the Department of Biochemistry and Biophysics of Loyola University School of Medicine. He was the recipient of a National Science Foundation Traineeship for his four years of study at Loyola and during the fourth year, he was also awarded a Veterans Administration Hospital Research Fellowship. From September, 1971 to June 1972, while still pursuing fulltime research and study at Loyola, he taught introductory biology at the National College of Education - Urban Campus in Chicago, Ill.

Under the advisorship of Dr. Joseph Bernsohn, Principal Scientist of the Neuropsychiatric Research Laboratory of the Hines V.A. Hospital, Mr. Cohen investigated lipid metabolism of isolated brain cells. He has presented papers on his work at the Third Annual American Society for Neurochemistry Meetings in Seattle, Washington, March 1972, The International Symposium on Dietary Lipids and Postnatal Development in Milan, Italy, October 1972, the Fourth Annual ASN Meeting in Columbus, Ohio,

March 1973 and the Fifth Annual ASN Meeting in New Orleans, March 1974.

His research interests are in neurochemistry with the long range goals of research, teaching and writing in that area. He is also interested in philosophy, both as it applies to his own life and to the neurosciences.

Presently, Mr. Cohen is a Postdoctoral Fellow in the Department of Neurology of The Johns Hopkins University.

He is co-author of the following:

ABSTRACTS

S.R. Cohen and J. Bernsohn "Incorporation of (1-$^{14}$C) Acetate into lipids of Neuronal and Glial-enriched cell fractions of developing rat brain."
Transactions of the American Society for Neurochemistry, Third Annual Meeting, Seattle, Washington, March 1972.

J. Bernsohn and S.R. Cohen "The uptake of (1-$^{14}$C) linoleic acid into phospholipids of neuronal and glial-enriched fractions of developing rat brain."
International Symposium on Dietary Lipids and Postnatal Development, Milan, Italy, October 1972.

S.R. Cohen and J. Bernsohn "Incorporation of essential fatty acids into phospholipids of neuronal and astroglial enriched fractions of developing rat brain." Transactions of the American Society for Neurochemistry, Fourth Annual Meeting, Columbus, Ohio, March 1973.

J. Bernsohn and S.R. Cohen "Fatty acid composition of phospholipids of neuronal soma, astrocytes and oligodendrocytes isolated from calf brain." International Society for Neurochemistry. Fourth Meeting, Tokyo, Japan, August 1973.

S.R. Cohen and J. Bernsohn " In vivo incorporation of (1-$^{14}$C) linolenic acid into lipids of neuronal soma, astroglia and oligodendroglia of rat brain." Transactions of the American Society for Neurochemistry. Fifth Annual Meeting, New Orleans, La., March 1974.

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S.R. Cohen and J. Bernsohn "In vivo incorporation of (1-$^{14}$C) linolenic acid into lipids of neuronal soma, astroglia and oligodendroglia isolated from developing rat brain." In preparation.

APPROVAL SHEET

The dissertation submitted by Steven Roger Cohen has been read and approved by five members of the faculty of Loyola University of Chicago and one from the faculty of the University of Chicago.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval with reference to content, form and mechanical accuracy.

This dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

5/17/74
Date

Joseph Bernardin
Signature of Advisor