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THE SYNTHESIS OF LIPIDS FROM [1-^{14}C]ACETATE BY ISOLATED RAT BRAIN CAPILLARIES

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Lipid biosynthesis was investigated in isolated cerebral microvessels obtained from adult Sprague–Dawley rats using [1-^{14}C]acetate as precursor. All lipid classes were labelled by [1-^{14}C]acetate. Neutral lipids incorporated about 50% of radiolabelled acetate, among which free fatty acids and triglycerids showed the highest level of incorporation. Moreover, about 4% of radioactivity was found in cholesterol fraction. In phospholipid fraction, phosphatidylcholine and phosphatidylethanolamine were the main radiolabelled phospholipids. [1-^{14}C]acetate was also incorporated into sulphatides and cerebrosides. The presence of bovine serum albumin in incubation medium modified the percentage of incorporation in different lipid fractions.

Endothelial cells of cerebral capillaries are known to play an important role in the transport of circulating substances from blood to brain and release of substances from brain to blood [2, 10]. Tight junctions between the plasma membrane of adjacent cells result in the formation of a continuous barrier between blood and brain. The flux of fluids and solutes takes place by a membrane-associated transport system or through the lipid matrix of plasma membrane [10]. Maintenance of plasma membrane lipid composition is necessary to the cell membrane permeability and cellular active transport systems. Only limited data have been reported on the contribution of endothelial cells from non-nervous tissues to the synthesis of their membrane lipids [14], and there is no available data concerning cerebral capillary endothelial cells. This study was undertaken to investigate the biosynthesis of lipids by rat brain capillaries using [1-^{14}C]acetate as precursor.

Several methods are available for the isolation of brain capillaries [4, 7, 13]. However, the application of these procedures for routine use is limited by a requirement for large amounts of starting tissues, by a considerable length of time in their work-up and by contamination from red blood cells or other material. In this work we have chosen two methods different in their work-up time to compare the possible differences in lipid biosynthesis.

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Brain capillaries were prepared from cerebral cortex of adult Sprague–Dawley rats from both sexes according to methods described by Goldstein et al. [4] or Hjelle et al. [7]. According to the method of Goldstein et al., the capillaries were isolated by mild homogenization in a glass homogenizer with a Teflon pestel (0.25 mm clearance, A. Thomas Co., Philadelphia, PA) and purified by Dextran flotation and glass beads filtration. In the method described by Hjelle et al. the cerebral hemispheres were cleaned and homogenized by hand with 5 up and down strokes in a smooth glass tube equipped with a tapered, serrated Teflon plunger. The homogenate was then poured onto the 86 μm pore size, nylon sieve (Ets A. Desjobert, Paris, France). This step was done twice.

Isolated capillaries were then washed with a modified Kreb's glucose buffer containing (in mM): 1.3 $MgCl_2$, 4.0 $NaHCO_3$, 5.4 KCl, 13 glucose, 15.7 $NaH_2PO_4 \cdot H_2O$ and 111.2 NaCl [11], which was adjusted to pH 7.4. The buffer contained 1% bovine serum albumin (BSA) when the incubation was performed in the presence of BSA. The brain capillaries were pre-incubated at 37°C for 30 min after bubbling with oxygen. After pre-incubation, [1-^{14}C]acetic acid sodium-salt (20 μCi/ml) from CEA (France) was added to the medium. The incubation was continued for another 2 h under the same conditions. At the end of this period the incubation was stopped by adding the ice-cold buffer. The capillaries were washed twice with Kreb's buffer to ensure the removal of free [1-^{14}C]acetate. Lipids were extracted with chloroform–methanol (2:1) [3] and sonication [12]. An aliquot of the lipid extract was counted to obtain the total incorporation of acetate into capillary lipids. Another aliquot was separated into neutral lipids by thin-layer chromatography (TLC) (TLC SilicaGel Plates 60 F_{254}, Merck) using hexane–ether–acetic acid (75:23:2) as developing solvent. Separation of phospholipids into subclasses was achieved using solvent system described by Vitiello and Zanetta [15]. Lipids were visualized by iodine vapor. Distribution of radioactivity among lipid subclasses was determined by scraping lipid zones from chromatographic plates into counting vials to which was added 10 ml of Beckman scintillation solution. TLC of lipid brain microvessels showed the spots corresponding to sphingolipids and glyoclipids. To ensure the presence of these lipid fractions in brain capillary endothelial cells, alkaline hydrolysis was performed [6]. An aliquot of lipid extract was incubated in the presence of methanolic alkaline for 30 min at 37°C to hydrolyse the phospholipids. Glycolipids were then separated by washing the incubation medium with Folch solution, and their presence was verified by TLC. Protein content of the capillaries was determined according to Lowry et al. [8].

Isolated brain capillaries were examined by phase-contrast microscopy (Fig. 1) and found to be free from contamination by other brain structures. The average yield of protein from capillaries prepared by method of Hjelle et al. was significantly lower than that of preparation done according to Goldstein et al. [4] (0.858 ± 0.1 mg, 3.4 ± 0.18 mg, respectively, based on 13.12 ± 0.16 g of starting material).

This difference was due to a great loss during filtration. The incorporation was expressed in cpm/mg protein throughout the study. Incorporation of [1-^{14}C]acetate in isolated cerebral capillaries was influenced by the duration of preparation and

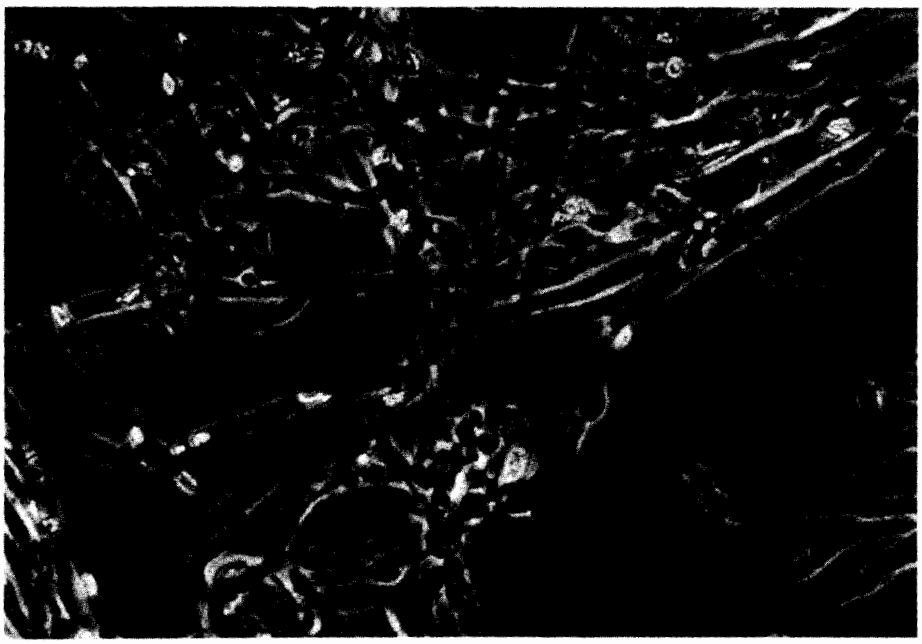

Fig. 1. Photomicrograph of isolated rat brain capillaries. × 360.

method of isolation, i.e. there was more incorporation per milligram of protein in the capillaries isolated by the method of Hjelle et al. [7] (8516 ± 619.45; 5857.2 ± 1106.4).

Incorporation of [1-^{14}C]acetate in different lipid fractions was not significantly different in two preparations.

The non-polar lipids (> 50%) and phospholipids (> 27%) were the major lipids

TABLE I

THE INCORPORATION OF [1-^{14}C]ACETATE INTO NEUTRAL LIPIDS OF ISOLATED RAT BRAIN CAPILLARIES

Results are the mean of 5 experiments and expressed as percentage of incorporation ± S.E.M. Comparison were made using Student's *t*-test. No significant difference observed between values obtained by each method in the absence of BSA in incubation medium. Significative differences (*$P < 0.05$, **$P < 0.001$) were observed when incubation was done in the presence of BSA. Results are percentage of radioactivity in identified spots (approximately 80% of total radioactivity).

Class of lipid	Method of Hjelle (− BSA)	Method of Goldstein	
		− BSA	+ BSA
Diglycerides	3.8 ± 0.2	4.9 ± 0.5	4.8 ± 0.4
Triglycerides	11.2 ± 0.9	12.6 ± 1.6	3.1 ± 0.2*
Free fatty acids	38.8 ± 1.5	31.6 ± 1.6	5.5 ± 0.2**
Cholesterol	4.3 ± 0.2	4.0 ± 0.3	5.9 ± 0.8
Cholesteryl esters	2.0 ± 0.2	2.1 ± 0.3	1.3 ± 0.1

TABLE II

THE INCORPORATION OF [1-^{14}C]ACETATE INTO PHOSPHOLIPIDS AND GLYCOLIPIDS OF ISOLATED RAT BRAIN CAPILLARIES

Results are the mean of 5 experiments and expressed as percentage of incorporation ± S.E.M. Comparison were made using Student's *t*-test. No significant difference observed between values obtained by each method in the absence of BSA in incubation medium. Significative differences. (*$P<0.001$, **$P<0.010$ were observed when incubation was done in the presence of BSA. Results are percentage of radioactivity in identified spots (approximately 80% of total radioactivity).

Class of lipid	Method of Hjelle (−BSA)	Method of Goldstein	
		−BSA	+BSA
Sphingomyeline	2.2±0.4	4.3±0.2	7.2±1.1
Phosphatidylcholine	15.3±0.8	16.0±0.4	34.7±1.0*
Phosphatidylserine	3.2±0.3	2.7±0.2	10.3±0.9**
Phosphatidylinositol	3.2±0.2	5.2±0.8	6.6±0.4
Phosphatidylethanolamine	7.6±0.4	6.4±0.4	11.0±0.4**
Phosphatidic acid	2.2±0.5	2.3±0.4	3.7±0.6
Cerebrosides	3.6±0.2	4.4±0.5	2.9±0.3
Sulphatides	2.5±0.3	3.6±0.4	2.3±0.3

labelled from [1-^{14}C]acetate. Table I shows the distribution of relative radioactivity in non-polar lipid fraction. The highest level of incorporation was seen in free fatty acids and triglycerids. Moreover, incorporation in the cholesterol fraction represented about 4% of the total incorporation.

Table II shows the incorporation of [1-^{14}C]acetate in phospholipids and glycolipid fractions. [1-^{14}C]acetate was incorporated in all phospholipids, but the highest level of incorporation was seen in phosphatidylcholine and phosphatidylethanolamine. Alkaline hydrolysis confirmed the incorporation of [1-^{14}C]acetate in the glycolipid fraction. Radiolabelled glycolipids represented more than 5% of the total incorporation.

When incubation was performed in the presence of BSA, the percentage of incorporation in different lipid fractions was modified. The incorporation of [1-^{14}C]acetate in total neutral lipids was significantly reduced. This reduction was seen in free fatty acids and triglyceride fractions. Consequently, the relative incorporation in phospholipid fraction was significantly increased. The increment in phospholipid fraction was observed in phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine.

The available data on lipid metabolism in isolated cerebral capillaries concern fatty acid oxidation [1, 5]. Therefore, it seemed important to study their lipid biosynthesis. This report demonstrates the lipid biosynthesis in brain capillaries from [1-^{14}C]acetate. As in endothelial cells of umbilical vein in culture [14], radiolabelled acetate was incorporated into all lipid fractions, but compared with these cells there was more incorporation into phosphatidylcholine and phosphatidylethanoline and less in other phospholipid fractions. Also different from endothelial cells of umbilical vein,

there was more incorporation into free fatty acids and monoglyceride but less into diglyceride and triglyceride. These data show that there are some differences in lipid metabolism of endothelial cells from different organs. The percentage of incorporation of radiolabelled acetate into phospholipids of isolated brain microvessels compared well with their cellular levels reported by Selivonchick and Roots [13] and Matheson et al. [9]. Isolated cerebral capillaries were incubated in the absence of serum albumin since brain extracellular space contains minute quantity of serum albumin. However, brain capillaries in vivo are in contact with blood from the luminal plasma membrane of endothelial cells. It was presumed that due to high affinity of blood serum albumin to fatty acids there may be some release of newly synthesized fatty acids from the cells to the extracellular medium. Therefore, incubation was also performed in the presence of serum albumin. Concerning the results of the incorporation of radiolabelled acetate in the presence of BSA, experiments are in progress to determine the role of serum albumin in changing the percentage of incorporation in different lipid fractions.

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