Metabolism of Brain and Liver Sulfatides*

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The concentrations of lipid-solvent soluble sulfur in rat brain and liver have been reported by Gaitonde and Richter (2). The extraction procedure used by these authors resulted in lipid extracts containing nonlipid contaminants and proteolipid proteins. Folch et al. (3, 4) have described methods for removal of these materials from lipid extracts. In the experiments to be reported, these methods of lipid purification were used. The sulfatide-sulfur was determined, after oxidation of the lipids, by the reduction method reported by Archer (5), a method which circumvents the removal of phosphate necessary in the barium sulfate and benzidine sulfate methods (6, 7). These experiments were set up to ascertain the role of age, species differences, dietary protein intake, and liver cell degeneration on the concentration and metabolism of brain and liver sulfatides.

The present communication reports the detailed experiments which show incorporation in vivo of L-methionine-S\(^{35}\) and sulfate-S\(^{35}\) into rat brain and liver sulfatides. The concentration of sulfatide-sulfur in the brain is a function of age; however, the specific activities decreased with age. The brain and liver sulfatide-sulfur concentration is dependent upon dietary protein intake. On the other hand, the liver sulfatide-sulfur specific activity reached a plateau and the liver sulfatide-sulfur specific activity began decreasing. Since it was desirable to measure the S\(^{35}\)-incorporation of both the brain and liver sulfatides in the same animal, the 1-hour time interval between injection and decapitation was selected.

When specific activities were determined, all animals received 45 \(\mu\)c of isotope (L-methionine-S\(^{35}\) or sulfate-S\(^{35}\)). The radioactive measurements were made with an ultratrin, gas flow Geiger tube (Tracerlab TGC-14) and the specific activity of sulfatide-sulfur was calculated. The specific activity is defined as counts per minute per aliquot \(\times\) body weight per microgram of sulfatide sulfur per aliquot \(\times\) dose in counts per minute.

**Extraction and Purification of Lipids**—The methods of Folch et al. (3) were used for the extraction and purification of the lipids and for the removal of the proteolipid-proteins (4). The animals were killed by decapitation; the brains and livers were removed in toto and homogenized in absolute methanol at ice-bath temperature with the use of a teflon pestle. Sufficient chloroform and methanol were added to the homogenates to yield a 2:1 (volume for volume) mixture of chloroform and methanol with a final volume 20 times the volume of the fresh tissue (3). The tissues were homogenized within 5 minutes after decapitation.

The lipid extracts were washed once by the addition of 0.04% CaCl\(_2\) and once with the “pure solvent upper phase” containing a mixture of chloroform, methanol, and 0.04% of CaCl\(_2\) (3). The pure solvent phases were prepared in a separatory funnel by mixing chloroform, methanol, and 0.04% CaCl\(_2\) in the proportions 8:4:3 by volume. After the separation of the phases, the upper constituted the “pure solvent upper phase” and the lower represented the “pure solvent lower phase.” The washed lower phase lipid extract containing essentially all the tissue lipids other than strandin was taken to dryness, the residue taken up in “pure solvent lower phase,” and again taken to dryness. This cleaved the proteolipids and rendered the protein insoluble sulfur was investigated. The compositions of the diets have been reported previously (8, 9).

1. The time course of the incorporation of S\(^{35}\) from L-methionine-S\(^{35}\) and sulfate-S\(^{35}\) into rat brain and liver sulfatides was investigated.

2. An investigation was made of the variation of the concentrations and specific activities of rat brain and liver sulfatide-sulfur with age. Animals ranging in age from 22 days to 6 months were used. The sulfatide sulfur concentrations were also determined on one group of 6-month-old female rats.

3. The effect of dietary protein concentration on the concentrations and specific activities of rat brain and liver sulfatide-sulfur was investigated. The compositions of the diets have been reported previously (8, 9).

4. A comparison was made of the elution patterns of S\(^{35}\)-labeled, acetone insoluble liver and brain lipids from silicic acid columns with the use of the methods of Hanshan et al. (10). All rats were male albino animals of the Sprague-Dawley strain maintained on Purina Laboratory chow pellets.

The intraperitoneal route was chosen for the administration of the isotope. Preliminary studies had shown that 1 hour after a single injection of L-methionine-S\(^{35}\) into the rat the specific activities of brain and liver sulfatide-sulfur were 90% and 70%, respectively, of those at 3 hours, at which time the brain sulfatide-sulfur specific activity reached a plateau and the liver sulfatide-sulfur specific activity began decreasing. Since it was desirable to measure the S\(^{35}\)-incorporation of both the brain and liver sulfatides in the same animal, the 1-hour time interval between injection and decapitation was selected.

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in hot 2:1 (volume for volume) chloroform-methanol (4). The material soluble in the hot chloroform-methanol was made up to a measured volume and the sulfatide-sulfur specific activity determined with the use of an aliquot of this solution of purified lipid, free of proteolipid-protein.

Radioactivity Measurements—Proteolipid-protein-free lipids (10 mg) was placed in a steel planchet and the radioactivity determined with an ultrathin window, gas flow Geiger tube (Tracerlab TGC-14). Measured aliquots of the injected dose were counted with 10 mg of nonradioactive lipids.

Oxidation of Lipids—The lipids were oxidized and the oxidation products evaporated to dryness by the methods described by Lees and Folch (11). The size of the combustion tube and the amount of oxidant (3:1 aqua regia) were increased to accommodate 50 to 60 mg of purified, proteolipid-protein-free lipids.

Sulfur Determination—The reduction method reported by Archer (5) was used for the determination of sulfur. One modification was necessary; 57% hydriodic acid was used in the preparation of the reduction mixture in place of a 2:1 mixture of 57% and 70% hydriodic acid. This concentration of hydriodic acid has been used successfully by Luke (12) for the reduction of oxidized forms of sulfur to sulfide.

Nitrogen Determination—Total tissue nitrogen was determined on an aliquot of the methanolic homogenate by a semimicro-Kjeldahl method.

Chromatography—Acetone-insoluble, proteolipid-protein-free lipids were prepared and chromatographed on silicic acid-Hyflo Super-Cel (Fisher Scientific) columns by the methods of Hanahan et al. (10). Preliminary experiments had shown that the acetone-insoluble lipids contained approximately 90% of the radioactivity found in the original purified, proteolipid-protein-free lipids.

RESULTS AND DISCUSSION

Incorporation of L-Methionine-S35 and Sulfate-S35—The time course of the incorporation of radioactivity from L-methionine-S35 and sulfate-S35 into rat brain and liver sulfatides are shown in Figs. 1, 2, and 3. When injected intraperitoneally, L-methionine-S35 was the better source of sulfatide label in both the brain and liver (Figs. 1 and 2). However, if these isotopic compounds were injected intracranially, the sulfatide was the better label source for brain sulfatides (Fig. 3). Even though the intracranially injected sulfate-S35 gave greater incorporation of label, this route of administration and, consequently, this isotopic compound was not used for the determination of brain sulfatide-sulfur specific activity because of the difficulty encountered in penetration of the skulls of older animals.

The slow incorporation of intraperitoneally injected sulfate-S35 into brain sulfatides was probably due to either a rapid excretion of the isotope by the kidney or the inability of any appreciable amount of sulfate to be transferred across the blood-brain barrier. The blood-brain barrier would have little effect upon the availability of L-methionine-S35 to the brain, for Clouet and Richter (13) and Kamin and Handler (14) have observed that the blood-brain barrier offered very little hindrance to the passage of methionine either into or out from the brain.

Radin et al. (15) have demonstrated the cerebroside sulfatide ester labeled with either galactose-C14 or sulfate-S35 exhibited very little if any decrease in radioactivity with time after a single injection of isotope. Data in Fig. 3 show that the specific activity of brain sulfatide-sulfur did not decrease during the 10-day period after a single injection of sulfate-S35. This evidence indicates that brain sulfatides have a very slow turnover.

The slow turnover of brain lipid constituents is not unique to sulfatides. Nicholas and Thomas (16) have shown that cholesterol retained appreciable quantities of radioactivity in both brain and spinal cord 1 year after the administration of acetate-C14. Davidson and Dobbie (17) found that phospholipids of whole rat brain exhibited very slow turnover during a 70-day period after the injection of a single dose of phosphate-P32.

Effect of Age on Concentration and Specific Activity—Table I gives the concentrations and specific activities of rat brain and liver sulfatide-sulfur as a function of age. Brain sulfatide-sulfur concentration increased with age, and old animals (180 days
old) were still able to incorporate label into the sulfatides, but at a diminished rate. The total incorporation of S\(^{35}\) per unit of brain tissue decreases with age. The liver sulfatide-sulfur concentrations of the 22-day-old rats were significantly higher (p values < 0.02) than those for the older-aged animals which were not different from one another. The specific activities of the liver sulfatide-sulfur showed no measurable change with age.

Effect of Dietary Casein—The brain and liver sulfatide-sulfur concentrations paralleled the dietary casein intake (Table II). Since proteins are the major source of dietary sulfur, the observed results are probably due to a quantitative lack of sulfur available for sulfatide synthesis. The low concentrations observed with the 5% casein diet supports the hypothesis that protein metabolism takes precedence over other metabolic functions of methionine when animals are subjected to conditions leading to fatty livers (18). The low specific activities observed with the 25% casein diet were probably due to dilution of the L-methionine-S\(^{35}\) by an increased amino-acid pool size. The total incorporation of S\(^{35}\) by intraperitoneal injection.

The animals were killed 1 hour after the intraperitoneal injection of 45 \(\mu\)g of L-methionine-S\(^{35}\). Figures in parentheses indicate the number of animals.

**TABLE I**

Concentration and specific activity of rat brain and liver sulfatide-sulfur as function of age

<table>
<thead>
<tr>
<th>Age</th>
<th>Sulfatide-sulfur concentration</th>
<th>Specific activity</th>
<th>Sulfatide-sulfur concentration</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td>(\mu)g S/g wet tissue</td>
<td>(\mu)g S/mg N</td>
<td>(\times 10^4)</td>
<td>(\mu)g S/g wet tissue</td>
</tr>
<tr>
<td>22 (6)</td>
<td>62.4 ± 3.7</td>
<td>3.82 ± 0.34</td>
<td>0.329 ± 0.088</td>
<td>15.3 ± 4.2</td>
</tr>
<tr>
<td>45 (5)</td>
<td>75.3 ± 7.3</td>
<td>4.13 ± 0.44</td>
<td>0.292 ± 0.024</td>
<td>10.0 ± 1.5</td>
</tr>
<tr>
<td>50 (5)</td>
<td>88.3 ± 8.0</td>
<td>4.65 ± 0.55</td>
<td>0.252 ± 0.024</td>
<td>9.7 ± 2.0</td>
</tr>
<tr>
<td>65 (5)</td>
<td>96.9 ± 5.5</td>
<td>5.94 ± 0.52</td>
<td>0.252 ± 0.024</td>
<td>9.7 ± 1.8</td>
</tr>
<tr>
<td>117 (4)</td>
<td>101.8 ± 12.2</td>
<td>5.41 ± 0.61</td>
<td>0.001 ± 0.007</td>
<td>9.2 ± 0.9</td>
</tr>
<tr>
<td>180 (4)</td>
<td>153.1 ± 19.5</td>
<td>8.59 ± 0.98</td>
<td>0.346 ± 0.029</td>
<td>117 (4)</td>
</tr>
</tbody>
</table>

* The mean ± the standard deviation is given.
† Female rats.

**TABLE II**

Concentration and specific activity of rat brain and liver sulfatide-sulfur as function of dietary protein concentration

<table>
<thead>
<tr>
<th>Diet (% casein)</th>
<th>Brain</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu)g S/g wet tissue</td>
<td>Specific activity</td>
<td>(\times 10^4)</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>5%</td>
<td>98.6 ± 14.4</td>
<td>127.0 ± 9.3</td>
</tr>
<tr>
<td>5%</td>
<td>5.19 ± 0.59</td>
<td>7.08 ± 0.77</td>
</tr>
<tr>
<td>25% casein</td>
<td>0.256 ± 0.024</td>
<td>0.149 ± 0.044</td>
</tr>
</tbody>
</table>

* The mean ± the standard deviation is given.
† The probability derived from the test of significance applied in respect to the variable measured.

Because of the limited data, the linearity of this increase is proposed with reservation since only one tissue for each age was analyzed.

**Liver Cell Degeneration—**Liver cell degeneration by bromobenzene administration had no effect on the sulfatide-sulfur concentration of rat liver (Table V). This probably indicates that the sulfatides are associated with the more stable structural components of the cell.

**Sulfic Acid Chromatography—**The acetone-insoluble lipid fractions prepared from rat brain and liver tissues after the injection of either sulfate-S\(^{35}\) or L-methionine-S\(^{35}\) were chromatographed on silicic acid with the use of the methods of Hanahan et al. (10). The radioactivity elution patterns shown in Fig. 4-A and B demonstrate the presence of at least two labeled components.
**TABLE IV**

**Human brain and liver sulfatide-sulfur concentrations**

Tissues exhibiting no pathology were stored in the cold and extracted 8 to 15 hours post-mortem. The brain tissue samples extracted consisted of half white and half grey matter taken from the cortex and the subcortical white matter.

<table>
<thead>
<tr>
<th>Age</th>
<th>Brain</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>years</td>
<td>µg S/g wet tissue</td>
<td>µg S/g wet tissue</td>
</tr>
<tr>
<td>3 days</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>28 years*</td>
<td>124</td>
<td>10.2</td>
</tr>
<tr>
<td>39 years</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>60 years</td>
<td>241</td>
<td>9.5</td>
</tr>
</tbody>
</table>

* Female.

**TABLE V**

**Rat liver sulfatide-sulfur concentration in chemically-induced liver cell degeneration**

Liver cell degeneration was induced in 117-day-old male rats by the intraperitoneal injection of bromobenzene (1 X 10^-3 moles per 100 g of body weight in corn oil) after being deprived of food for 24 hours (19). After injection, food and water were available at all times. The animals were killed by decapitation 38 hours after injection. Controls were treated identically without the bromobenzene. Figures in parentheses indicate the number of animals.

<table>
<thead>
<tr>
<th>Liver sulfatide-sulfur µg S/g wet tissue µg S/mg N</th>
<th>Control (4)</th>
<th>Experimental (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg S/g wet tissue</td>
<td>8.8 ± 1.9</td>
<td>9.5 ± 0.7</td>
</tr>
<tr>
<td>µg S/mg N</td>
<td>0.412 ± 0.074</td>
<td>0.332 ± 0.151</td>
</tr>
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* The mean ± the standard deviation is given.
† The probability derived from the test of significance applied in respect to the parameter measured.

**REFERENCES**

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