Cerebroside Sulfate (Sulfatide A) in Some Organs of the Rat and in a Mast Cell Tumor*

JACK PETER GREEN† and J. D. ROBINSON, Jr.

From the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut

(Received for publication, November 25, 1959)

A noteworthy characteristic of cerebroside sulfate (2) (sulfatide A (3)) is its continual accumulation in the brain of rats (4), human beings (5), and mice (6). Recent experiments (7) with radioactive galactose and sulfate showed that cerebroside sulfate in rat brain, unlike other galactolipids, turns over slowly, if at all.

Sulfolipids have been described in organs other than brain, including skeletal muscle (8), submaxillary gland (8), testis (8), liver (8, 9), lung (10), kidney (11), and, inferentially (12), in spleen, blood, and adrenal gland. The identity of the sulfolipids extracted from these organs as cerebroside sulfate has not been established. In this paper evidence is presented that cerebroside sulfate is present in kidney, liver, spleen, and possibly, heart of the rat, and also is present in neoplastic murine mast cells.

The turnover rate of cerebroside sulfate in these organs of the rat and in the mast cells was compared with that in the brain. The intracellular distribution of this compound in brain, kidney, and liver was also determined.

EXPERIMENTAL

EXTRACTION AND FRACTIONATION PROCEDURES—Rats weighing 150 g were injected intraperitoneally with 1 mc of S35-sulfate, obtained from the Oak Ridge National Laboratories. At stated time intervals, the animals were decapitated, the blood was collected from the Oak Ridge National Laboratories. At stated time intervals, the animals were decapitated, the blood was collected and, after elution with methanol-petroleum ether (1:1), in a liquid scintillation counter. Galactose was measured by an anthrone method (17). To detect galactose chromatographically, extracts were first hydrolyzed in 6 N HCl in a sealed tube for 1 hour in a boiling water bath.

To measure sulfate, the sample of cerebroside sulfate was placed in a sealed tube containing 1 ml of glacial acetic acid and 1 ml of 6 N HCl and kept in a boiling water bath for 48 hours. Acid was carefully removed from the hydrolysate by repeated heating; the neutral hydrolysate was taken to dryness and the residue taken up in 0.5 ml of H2O. To the aqueous extract was added 0.5 ml of 0.1 M acetate buffer (pH 4.0), 1 ml of a mixture of chloroform-methanol (2:1), 10 mg of barium chloranilate (18) and 1 ml of ethanol. The mixture was shaken for 15 minutes, centrifuged and the supernatant solution read in a Beckman DU spectrophotometer at a wave length of 530 μm. This method reliably detects 40 μg of inorganic sulfate. Optical density at 530 μm is linearly related to the concentration of inorganic sulfate up to at least 200 μg.

Reversed phase paper chromatography was carried out in diisobutyl ketone-acetic acid-water (40:25:5), a solvent system useful in the resolution of phosphatides (19); the Whatman No. 3 paper used was impregnated with either silicic acid (19) or silicic acid (20). Rf values were the same in papers coated with either substance. Heparin was chromatographed in isopropyl alcohol-0.04 M ammonium formate (14). Other solvent systems for paper chromatography were n-propyl alcohol-ethyl acetate-water (70:10:20) (21), butanol-ethyl acetate-water (2:1:1), and aqueous 2,4-lutidine (22); the latter was prepared by diluting 67 ml of 2,4-lutidine to 100 ml with water and allowing the mixture to stand for several minutes until a single phase formed.1 Radioactivity on the paper chromatograms was measured either directly with the aid of an end window Geiger-Müller counter or, after elution with methanol-petroleum ether (1:1), in a liquid scintillation apparatus. Some papers were stained with Azure A (14), ninhydrin (21), or alkaline silver nitrate (21).

RESULTS

PRESENCE OF CEREBROSIDE SULFATE IN TISSUES—Kidney, liver, and spleen of the rat and the mastocytoma from the mouse were extracted for cerebroside sulfate. The final extract was precipitated three times (3). Hydrolysates of these extracts were chromatographed on n-propyl alcohol-ethyl acetate-water (70:10:20) along with authentic glucose and galactose. Papers were stained with alkaline silver nitrate. Only galactose was detected.

1 J. Awapara, personal communication.

† Senior Research Fellow, United States Public Health Service.
Table I

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Galactose:Sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.04</td>
</tr>
<tr>
<td>Liver</td>
<td>0.90</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.1</td>
</tr>
<tr>
<td>Mastocytoma</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Brain</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debris and nuclei</td>
<td>33</td>
<td>38</td>
<td>20</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>50</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Microsomes</td>
<td>10</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td>Supernatant fraction</td>
<td>7</td>
<td>11</td>
<td>39</td>
</tr>
</tbody>
</table>

Chloroform-methanol extract—Experiments were carried out to determine at which step in the extraction procedure the extract was free of S35-labeled compounds other than cerebroside sulfate. The chloroform-methanol extracts of brain, kidney, liver, spleen, and heart of the rat and of the mastocytoma were washed with water (Step 1 of the procedure for extracting cerebroside sulfate (3)). With all tissues, reversed phase paper chromatography of the organic phase showed a single radioactive spot at Rf 0.50, corresponding to known cerebroside sulfate. Furthermore, in neither the butanol nor the lutidine system was a radioactive spot other than cerebroside sulfate revealed.

The aqueous phase was also radioactive and this activity was chromatographed in butanol-acetic acid-water, a solvent system in which cerebroside sulfate travels with the solvent front. A radioactive, ninhydrin-positive spot was detected at Rf 0.18 and 0.32, corresponding to cysteic acid and taurine. In the aqueous lutidine system, both radioactivity and ninhydrin reactivity were found at Rf 0.39 and 0.44, corresponding to the known values for taurine and cysteic acid, respectively. Chromatography of the aqueous phase in admixture with taurine and cysteic acid indicated the identity of the S35-labeled compounds in the aqueous phase as taurine and cysteic acid. No choline sulfate (23) or ethanolamine sulfate could be detected.

Turnover of Cerebroside Sulfate—Since cerebroside sulfate was the only S35-labeled compound present in the washed chloroform-methanol extracts of tissues after the administration of S35-sulfate, an aliquot of this extract was used to measure cerebroside sulfate turnover. The turnover of cerebroside sulfate in brain, kidney, liver, spleen, and heart of the rat and in the mastocytoma of the mouse is shown in Figs. 1 and 2. In brain, the maximal incorporation of the isotope occurred 2 days after injection of S35-sulfate, after which time the activity remained constant through the 16th day, but slowly fell thereafter; thus, on the 32nd day, the level was still 1/2 of that found on the 2nd day.

In comparison, in liver, spleen, and heart the concentration of labeled cerebroside sulfate was highest at 12 hours, with only a small fraction present at 48 hours. After 4 days, the radioactivity had virtually disappeared. In kidney, however, incorporation of S35-sulfate was highest 24 hours after injection of S35-sulfate and measurable radioactivity remained on the 32nd day. The mast cells showed maximal incorporation 12 hours after administration of S35-sulfate and the activity fell rapidly.
Distribution of Cerebroside Sulfate—Table II shows that cerebroside sulfate was distributed differently in the three organs studied. In brain the mitochondrial fraction was richest, whereas in the liver it was the supernatant, particle-free fraction; in the kidney the microsomal fraction had the same amount as the nuclei and debris, whereas the content of the mitochondria and microsomes was quite low. To test the possibility that the distribution of cerebroside sulfate within cells of different organs is a function of time, cerebroside sulfate was extracted from all three organs at intervals of 12, 24, and 48 hours, respectively, after the injection of S35-sulfate. The distribution was the same at all three periods. Calculations of the intracellular distribution of cerebroside sulfate, based on nitrogen content or mass of the various fractions (24), did not significantly alter the relationship.

Failure to Show Degradation of Cerebroside Sulfate—An aqueous suspension of cerebroside sulfate-S35 was incubated for 3 hours with homogenates of either 5 g of the solid mastocytoma or 2 g of rat brain. In neither case was there any detectable degradation of cerebroside sulfate. The presence of cerebroside sulfate in mast cells, which are rich in heparin (14), suggested that these cells might have the capacity to transfer S35-sulfate from cerebroside sulfate to ψ-heparin, but incubation of homogenates of the mast cell tumor with labeled cerebroside sulfate and ψ-heparin resulted in no labeling of the heparin.

DISCUSSION

The preparations of cerebroside sulfate from the various organs, like brain cerebroside sulfate, yielded galactose on hydrolysis, had the same galactose-sulfate ratios as brain cerebroside sulfate, and had Rf values on paper chromatography identical to brain cerebroside sulfate. These observations strongly imply that the compound in the various organs is cerebroside sulfate. Turnover studies revealed several salient differences between brain and other organs. Brain required 48 hours for maximal deposition of S35-sulfate into cerebroside sulfate, whereas kidney required 24 hours, and the other organs and the mast cells only 12 hours. An analogous difference between brain and other organs in the incorporation of radioactive phosphate into phospholipids (25) is partly attributable to slow passage of the isotope into brain (26-28), but primarily due to a slow rate of incorporation of the isotope into the lipid (28). Similarly, the relatively slow incorporation of sulfate into the cerebroside by brain may be, in part, a reflection of the slow penetration of sulfate into brain (29).

A more remarkable difference between brain and the other organs was the exceedingly slow turnover rate found in brain. In this organ, as has been shown by others (7), cerebroside sulfate does not turn over for at least 16 days. However, at 24 days there was a measurable loss of radioactivity and this was more marked at 32 days. By contrast, cerebroside sulfate in other organs in and the mast cells had a half-life of 10 to 96 hours. The exceedingly slow turnover of cerebroside sulfate in brain is not a characteristic of other galactolipids (7). However, the brain phospholipid fraction resembles brain cerebroside sulfate in that it turns over very slowly in both adult (25, 28) and young brain (25, 28, 30), as contrasted with a relatively rapid turnover in other organs (25, 28, 31).

An interesting finding was the distribution of the major portion of cerebroside sulfate in different cellular fractions of the three organs examined: in brain, it sediments with mitochondria; in kidney, with microsomes; and in liver it remained in the supernatant fraction. These observations are consonant with the observation (9) that in brain it is the particulate fraction that incorporates S35-sulfate into a sulfolipid, whereas in liver it is the supernatant fraction.

The finding of S35-labeled taurine and cysteic acid after the administration of S35-sulfate warrants comment. The reaction has been noted before (32, 33) and attributed to the bacterial flora (32), but it appeared possible that in mammalian tissue a pathway may exist for forming S35-cysteic acid from S35-sulfate by sulfonylation and subsequent amimation of pyruvate. However, homogenates of either brain or mast cells in a fortified system were unable to convert either S35-sulfate or pyruvate-2-C14 to cysteic acid or taurine; moreover, taurine and cysteic acid extracted from mast cells grown in culture with S35-sulfate in the medium were not labeled.

SUMMARY

The turnover of cerebroside sulfate (sulfatide A) in brain, kidney, liver, spleen and heart of the rat and in a mast cell tumor of the mouse has been measured. The compound turned over rapidly in the mast cell and in all organs of the rat except the brain. Fractionation of brain, kidney, and liver demonstrated that the distribution of cerebroside sulfate differs in these organs: in brain, the substance sedimented with mitochondria; in kidney, with microsomes; whereas in liver it remained in the soluble fraction. No cerebroside sulfate could be detected in rat blood cells or plasma.

Acknowledgments—The authors are grateful to Dr. Margaret Day for providing the mast cell tumor and to Miss Bella Wu for helpful technical assistance.

REFERENCES

10. Sammartino, U., Biochim. Z., 124, 234 (1921).

2. J. P. Green, and J. D. Robinson, unpublished experiments.
Cerebroside Sulfate (Sulfatide A) in Some Organs of the Rat and in a Mast Cell Tumor
Jack Peter Green and J. D. Robinson, Jr.


Access the most updated version of this article at http://www.jbc.org/content/235/6/1621.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/235/6/1621.citation.full.html#ref-list-1