Golgi Apparatus of Rat Kidney

PREPARATION AND ROLE IN SULFATIDE FORMATION*

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SUMMARY

A procedure has been developed for the isolation of morphologically recognizable, largely intact, Golgi apparatus from rat kidneys. The fraction contains both uridine diphosphogalactose: N-acetylgalactosamine galactosyltransferase and 3'-phosphoadenosine 5'-phosphosulfate: cerebroside sulfotransferase activities enriched 30- to 80-fold over the original homogenate. Comparison of the Golgi-rich fraction with plasma membranes, endoplasmic reticulum, mitochondria, and nuclei isolated from rat kidney indicates that the Golgi apparatus is the main locus of both of these enzymes in kidney cells. Golgi apparatus from rat liver, although rich in galactosyltransferase, appears to be devoid of the sulfotransferase.

The cerebroside sulfotransferase of kidney Golgi forms sulfatide from added galactocerebrosides. Both hydroxyfatty acid and normal fatty acid-containing cerebrosides are sulfated. The enzyme is activated almost maximally by the addition of as little as 0.1% Triton X-100. The presence of 60 mM Mn2+ stimulates the activity about 3-fold. The apparent Km for cerebrosides was 1.3 x 10^-4 M, while that for 3'-phosphoadenosine 5'-phosphosulfate was 2.1 x 10^-3 M.

These results show that the Golgi apparatus in kidney cells can function to modify glycolipids as well as glycoproteins.

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One of the major advances in cell fractionation in recent years has been the development of isolation procedures for the Golgi apparatus of the mammalian hepatocyte (1-5). This has been followed by extensive enzymatic and chemical characterization (1, 3, 6, 7). The Golgi apparatus has been shown to be biochemically unique as compared to the other membranes of the liver cell in that it is the sole locus of UDP-galactose: N-acetylgalactosamine galactosyltransferase activity (1, 3, 7, 8) and has a unique protein profile on polyacrylamide gel electrophoresis as compared to other cell organelles of liver (3, 9). It is involved in the secretion of serum albumin (10, 11) and of serum very low density lipoproteins (12-14). It is presumed to modify glycoproteins that it secretes by catalyzing the addition of some of the terminal sugars of the carbohydrate chains (15).

The present study was undertaken with a number of objectives in mind. First, we wanted to see if methods for the isolation of the Golgi apparatus from liver which we had devised (1, 3, 16) could be applied to other tissues such as kidney where little or nothing is known about the role of the Golgi apparatus. Second, it is important to know how comparable the properties of Golgi apparatus are in various tissues of the same species. Third, we wished to investigate the possible role of the Golgi apparatus in glycosphingolipid formation, and kidney was particularly suited for such a study.

Glycosphingolipids are minor components of the lipids of most mammalian tissues other than brain. They are thought to be constituents primarily of plasma membranes and to be involved in the immunological specificity exhibited by various tissues (for a review of the biological significance of glycosphingolipids see Ref. 17). Most studies on the biosynthesis of sphingolipids have been carried out in brain, where the appearance of cerebrosides and sulfatides closely parallels the process of myelination. Studies on the biosynthesis of galactosyl and glucosyl ceramides generally have used brain microsomes as a source of enzyme (for review see Ref. 18). Sulfatide can be formed in vivo by the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate to galactosyl ceramide. The enzyme involved is PAPS:cerebroside sulfotransferase and has been described in rat brain (19) and rat kidney (20) microsomes. Sulfatides are not present to a significant extent in liver (21). In kidney, sulfatides have been shown to be enriched in the outer medulla where sodium transport is also enriched (22).

Little is known of the exact subcellular localization of any of the enzymes involved in cerebroside and sulfatide formation. Microsomes are a heterogeneous collection of vesicles which consist of various proportions of endoplasmic reticulum, Golgi apparatus, and plasma membrane depending on the tissue under study. Cerebroside sulfotransferase is highest in a light, smooth microsome fraction from brain and is not present to a significant extent in myelin (19). These results led us to investigate the localization of this enzyme in the Golgi apparatus. A preliminary report of this work has been published (23).

The abbreviations used are: PAPS, 3'-phosphoadenosine 5'-phosphosulfate; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; VLDL, serum very low density lipoproteins.
EXPERIMENTAL PROCEDURES

Materials—Male Holtzman rats, 200 to 250 g, fed ad libitum were used. They were killed by decapitation, exsanguinated, and the kidneys quickly removed, decapsulated, and collected in ice-cold 0.25 M sucrose (9). Carrier PAPS (supplied by New England Nuclear Corp., Boston, Mass.) was purchased from New England Nuclear Corp. and diluted with carrier to a specific activity of approximately 1 mCi per mmole. Carrier PAPS was prepared from ATP and Na₂SO₄ by the method of Hodson and Schiff (24) using extracts of Chlorella pyrenoidosa as a source of sulfate-activating enzyme. UDP Gal was obtained from Calbiochem, Los Angeles, Calif. UDP-Gal uniformly labeled with MC in vivo was purchased from New England Nuclear Corp., Boston, Mass. It was diluted with carrier to a specific activity of about 1 mCi per mmole.

For column chromatography of lipid extracts, the silicic acid used was Unisil, 100 to 200 mesh, from Clarkson Chemical Co., Inc., Williamport, Pa. For thin layer chromatography of lipid components, plain silica gel (Research Specialties Co., Richmond, Calif.) containing 10% magnesium silicate was used (25). "Sulfospray" was obtained from Supelco, Inc., Bellefonte, Pa.

Preparation of Cell Fractions—All sucrose solutions were prepared using "Ultra-Pure" grade (Mann Research Laboratories). Sucrose gradients were adjusted to the desired concentration of sucrose (per cent sucrose = w/w solution) using a Bausch and Lomb 21865 refractometer (including the acceleration time). The rotor is then decelerated to 4,000 rpm, the contents displaced by pumping 55% sucrose into the periphery, and 20-ml fractions collected from the center.

Fractions are combined as described in individual experiments and diluted with 1/3 volume cold distilled water. The upper fractions are centrifuged at 30,000 rpm for 1 hour to recover the Golgi. Plasma membrane- and mitochondria-rich fractions are prepared from the zonal gradient at the same time as Golgi-rich fractions (see Fig. 1). Fractions rich in plasma membranes are diluted with an equal volume of cold distilled water and sedimented at 10,000 rpm for 10 min in the JA-20 rotor of the Beckman J-21 centrifuge. The upper white portion of the pellet is resuspended in 0.25 M sucrose containing 0.01 M Hepes, pH 7.4, and 0.001 M EDTA using a Dounce homogenizer with an A pestle. The membranes are re-centrifuged as before and the entire washing process is repeated four times. Mitochondria-rich fractions from the zonal gradient are combined and the mitochondria are recovered by centrifuging in the JA-20 rotor at 16,000 rpm for 10 min. The upper light portion of the pellet is discarded and the lower brown portion is resuspended in sucrose-Hepes-EDTA and recentrifuged as before. The washing procedure is repeated twice more. All pellets were suspended finally in 0.25 M sucrose. For purposes of comparison, nuclei, plasma membranes, mitochondria, and microsomes were prepared from rat kidneys by the method of Stein et al. (26) as modified by Fleischer and Kervina (27). The microsomes were further fractionated into a smooth and rough fraction by a modification of the method of Dallner (28).

Measurement of Enzyme Activities—PAPS:cerebroside sulfotransferase was measured by our modification of the assay conditions used by Farrell and McKhann (19). The assay mixture (0.1 ml) contained the following (in micromoles) in the order in which they are added: imidazole chloride, pH 7.0, 12; mercaptoethanol, 6; manganese chloride, 6; Triton X-100, 0.5 mg; protein, 50 to 200 μg; ATP, 3; mixed bovine cerebrosides, 20 μg; and [35S]-PAPS, 0.27. Mixed bovine cerebrosides (Supelco Inc., Bellefonte, Pa.) were first suspended at 10 mg per ml in water containing 1% (w/v) Triton X-100 using a small Potter-Elvehjem homogenizer. Incubations were carried out at 37° for 1 hour. The reaction was stopped by the addition of 0.03 ml of chloroform-methanol 9:1 (v/v). The contents were mixed by vortexing and the tubes were allowed to stand 15 min at room temperature. Non-lipid radioactivity was removed by washing once with 0.6 ml of 0.034% MgCl₂ followed by three washes consisting of 1 ml each of "upper phase" (29). The final lower phase was removed, taking care to leave behind no material at the interface, and dried 10 min under nitrogen. Non-lipid radioactivity was measured by washing once with 0.6 ml of 0.034% MgCl₂ followed by three washes consisting of 1 ml each of "upper phase" (29). The final lower phase was removed, taking care to leave behind no material at the interface, and dried 10 min under nitrogen. The lipid products were then extracted with 3.76 ml of 2.5-diphenyloxazole (PPO), 0.376 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP), and 3.3 ml of Triton X-100, was added to each vial. The samples were counted finally in a Packard TriCarb liquid scintillation spectrometer.

Galactosyltransferase (16) and sucrase-xylosephosphorylase (20) were determined as described previously. Glucose 6-phosphate was determined according to the method of Swanson (31) except that incubations were made for 8 and 10 min and the phosphate released measured by the method of Chen et al. (32). Total ATPase was measured as described previously (30) except that the assay mixture also contained 100 μmoles of NaCl and 10 μmoles of KCl. Subsequent losses of ATPase were taken as the difference between total ATPase and the same activity measured in the presence of 1 mm ouabain and 2 mm KCl.

Other Analyses—Protein was estimated by the Lowry procedure (33) using crystalline bovine serum albumin as a standard. Phosphorus was determined by the method of Chen et al. (32).

The [35S]labeled sulfolipid formed as a product of the sulfotransferase was characterized using column and thin layer silicic acid and chromatography. The lower phase containing the lipid products was evaporated to dryness, taken up in 0.3 ml of chloroform, and fractionated using a silicic acid column (1.2 x 3 cm). The column was eluted with 30 ml of chloroform (neutral lipid fraction), followed by 80 ml of acetone (glycolipid fraction), and finally with 30 ml of methanol (phospholipid fraction). Aliquots of each fraction were evaporated to dryness and counted as described for the sulfotransferase assay. The glycolipid fraction was further fractionated by two-dimensional thin layer chromatography according to the method of Rouser et al. (25). Before chromatography, 200 μg of a cerebroside plus sulfatide fraction from bovine brain (a gift of Dr. George Rouser) was added as carrier. The plates were run in the first direction using 1.0% chloroform, 2.5% ethanol, 6; manganese chloride, 6; Triton X-100, 0.5 mg; protein, 0.05 ml of 1.4-bis[2-(5-phenyloxazolyl)]benzene (POPOP), and 3.3 ml of Triton X-100, was added to each vial. The samples were counted finally in a Packard TriCarb liquid scintillation spectrometer.

Electron Microscopy—For best morphological preservation, aliquots of the fractions containing Golgi apparatus were fixed immediately after isolation from the zonal gradient, before dilution and centrifugation to concentrate the Golgi. One half milliliter of 25% glutaraldehyde made up in 0.25 M sodium cacodylate, pH 7.4, was added to 5 ml of the combined fractions (about 0.5 to 1.0 mg of protein). The other cell fractions were fixed by treating an aliquot (containing about 0.5 mg of protein) with 4% glutaraldehyde in 0.25 M sucrose and 0.2 M sodium cacodylate, pH 7.4. After standing overnight in the refrigerator, the samples were centrifuged at 10,000 rpm and the supernatant discarded. The pellets were washed by suspension in 1 ml of 0.25 M sucrose and recentrifugation. The washing procedure was repeated twice. The final pellets were then fixed with 1% osmium tetroxide, dehydrated, embedded, and sectioned as described previously (1).
RESULTS

Fractionation of rat kidney homogenates by procedures similar to those which have proved effective in isolating liver Golgi apparatus resulted in the distribution of protein and enzymic activities shown in Fig. 1 and Table I. The upper portion of the gradient is the Golgi-rich fraction. It is enriched in both cerebroside sulfotransferase and galactosyltransferase and poor in glucose 6-phosphatase, ouabain-sensitive ATPase, and succinate-cytochrome c reductase. Farther down the gradient, this profile of enzymic activities is reversed. The ratio of galactosyltransferase to the sulfotransferase in the upper parts of the gradient appears to be fairly constant, giving further support to the conclusion that both activities are present in the same subcellular organelle.

The presence of cerebroside sulfotransferase is not linked to the presence of galactosyltransferase in all tissues. In brain homogenate, a high sulfotransferase is present together with a low level of galactosyltransferase, and in liver a high galactosyltransferase activity is present whereas no sulfotransferase is detectable (Table I). The Golgi apparatus isolated from rat liver also does not exhibit sulfotransferase activity (23).

Since the amount of protein in Fraction 1 was rather low, Fractions 1 and 2 were combined with some sacrifice of purity. The combined fractions, generally tubes 2 to 10 from the gradient, as well as the plasma membrane-rich and mitochondrial-rich fractions, were studied by electron microscopy. A typical field of the combined Fractions 1 + 2 which are rich in both galactosyltransferase and in sulfotransferase is shown in Fig. 2A. The morphology of this fraction clearly identifies it as being derived predominantly from the Golgi apparatus. The plasma membrane-rich fraction consists of large smooth vesicles (Fig. 2C) and the mitochondrial fraction is morphologically identifiable although many of the mitochondria are swollen and fragmented (Fig. 2D).

The Golgi apparatus fraction illustrated in Fig. 2A appears to be about 70% pure as judged morphologically. From the glucose 6-phosphatase activity (Table II) it can be estimated to contain about 25% endoplasmic reticulum. From the ouabain-sensitive ATPase activity (Table I), it appears to be about 5% contaminated with plasma membranes. It also contains about 7% mitochondrial contamination as measured by succinate-cytochrome c reductase activity (Table II).

About 15% of the total sulfotransferase and about 10% of the galactosyltransferase of the homogenate is found in the combined Fractions 1 + 2. If these enzymes are only present in the Golgi apparatus it would mean the yield of Golgi by this procedure is 10 to 20% and that Golgi constitutes about 1% of the protein of the kidney homogenate.

### Table I

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Total Protein (mg)</th>
<th>Total Phosphorus (µg/mg protein)</th>
<th>Cerebroside Sulfotransferase*</th>
<th>Galactosyl Transferase*</th>
<th>Glu-6 P'ase</th>
<th>Succ-Cyt. c Reductase</th>
<th>Total ATP'ase</th>
<th>Quabain-Sensitive ATP'ase</th>
</tr>
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<tr>
<td>1</td>
<td>6.0</td>
<td>29.4</td>
<td>28.0</td>
<td>579</td>
<td>.055</td>
<td>.039</td>
<td>.035</td>
<td>.000</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>28.7</td>
<td>22.0</td>
<td>467</td>
<td>.077</td>
<td>.109</td>
<td>.061</td>
<td>.065</td>
</tr>
<tr>
<td>3</td>
<td>11.1</td>
<td>27.9</td>
<td>12.1</td>
<td>347</td>
<td>.102</td>
<td>.126</td>
<td>.082</td>
<td>.13</td>
</tr>
<tr>
<td>4</td>
<td>12.3</td>
<td>25.9</td>
<td>7.9</td>
<td>158</td>
<td>.151</td>
<td>.209</td>
<td>.116</td>
<td>.26</td>
</tr>
<tr>
<td>PM-rich</td>
<td>20.4</td>
<td>20.7</td>
<td>0.5</td>
<td>14</td>
<td>.079</td>
<td>.311</td>
<td>.142</td>
<td>.52</td>
</tr>
<tr>
<td>Mito-rich</td>
<td>73.0</td>
<td>11.7</td>
<td>0.0</td>
<td>0.5</td>
<td>.008</td>
<td>.816</td>
<td>.025</td>
<td>.02</td>
</tr>
<tr>
<td>Kidney Homog.</td>
<td>6,150</td>
<td>-</td>
<td>0.26</td>
<td>9.7</td>
<td>.065</td>
<td>.256</td>
<td>.20</td>
<td>-</td>
</tr>
<tr>
<td>Brain Homog.</td>
<td>-</td>
<td>-</td>
<td>0.41</td>
<td>1.9</td>
<td>.012</td>
<td>.241</td>
<td>.19</td>
<td>-</td>
</tr>
<tr>
<td>Liver Homog.</td>
<td>-</td>
<td>-</td>
<td>0.00</td>
<td>8.4</td>
<td>.085</td>
<td>.161</td>
<td>.19</td>
<td>-</td>
</tr>
</tbody>
</table>

* Nanomoles per hour per mg of protein at 37°; all other activities expressed as micromoles per min per mg of protein at 32° except glucose 6-phosphatase which was carried out at 37°. Fractions obtained as described under "Experimental Procedures" and Fig. 1.

Rat brain and liver homogenates, prepared in the same manner as the kidney homogenate, are included for purposes of comparison.
Fig. 2. Electron micrographs of purified fractions obtained from the fractionation of rat kidney homogenate by zonal ultracentrifugation. A, Golgi-rich fraction (equivalent to Fraction 1 + 2 in Fig. 1 and Table I), × 35,000; B, higher magnification of the isolated Golgi apparatus, × 100,000; C, plasma membrane-rich fraction, × 20,000; D, mitochondrial fraction, × 10,000.
Table II

Distribution of "marker enzymes" in purified subcellular fractions of rat kidney

Average of four preparations from pooled rat kidneys ± standard deviations of the mean. Supernatant and nuclei are the mean of two experiments. Enzyme activities expressed as in Table I. Supernatant is the fraction of the homogenate which is not sedimentable at 104,000 × g for 1 hour. The Golgi apparatus fractions used were equivalent to Fractions 1 + 2 shown in Fig. 1 and Table I.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Amount phosphorus per mg protein</th>
<th>Cerebroside sulfotransferase</th>
<th>Galactosyl transferase</th>
<th>Glucose 6-phosphatase</th>
<th>Total ATPase</th>
<th>Succinate-cytochrome c reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>31.0 ± 1.5</td>
<td>0.31 ± 0.16</td>
<td>10.9 ± 5.1</td>
<td>0.055 ± 0.017</td>
<td>0.172 ± 0.054</td>
<td>0.272 ± 0.079</td>
</tr>
<tr>
<td>Nuclei</td>
<td>41.2 ± 4.0</td>
<td>0.19 ± 0.19</td>
<td>3.4 ± 0.6</td>
<td>0.103 ± 0.023</td>
<td>0.062 ± 0.025</td>
<td>0.043 ± 0.018</td>
</tr>
<tr>
<td>Rough microsomes</td>
<td>36.2 ± 0.9</td>
<td>0.39 ± 0.16</td>
<td>6.2 ± 4.1</td>
<td>0.191 ± 0.018</td>
<td>0.255 ± 0.035</td>
<td>0.013 ± 0.006</td>
</tr>
<tr>
<td>Smooth microsomes</td>
<td>28.4 ± 0.4</td>
<td>2.6 ± 0.6</td>
<td>54.5 ± 5.0</td>
<td>0.390 ± 0.064</td>
<td>0.968 ± 0.035</td>
<td>0.008 ± 0.004</td>
</tr>
<tr>
<td>Golgi apparatus</td>
<td>32.5 ± 3.1</td>
<td>25.6 ± 3.4</td>
<td>55.3 ± 17</td>
<td>0.077 ± 0.017</td>
<td>0.391 ± 0.139</td>
<td>0.052 ± 0.017</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>13.8 ± 0.3</td>
<td>0.16 ± 0.24</td>
<td>1.7 ± 0.9</td>
<td>0.013 ± 0.009</td>
<td>0.393 ± 0.045</td>
<td>0.746 ± 0.142</td>
</tr>
<tr>
<td>Plasma membranes</td>
<td>19.1 ± 2.9</td>
<td>0.32 ± 0.12</td>
<td>6.1 ± 5.4</td>
<td>0.031 ± 0.010</td>
<td>1.084 ± 0.250</td>
<td>0.129 ± 0.075</td>
</tr>
<tr>
<td>Supernatant</td>
<td>15.8 ± 0.5</td>
<td>0.00</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table III

Identification of products formed by cerebroside-sulfotransferase of rat kidney Golgi

A Golgi-rich fraction (200 µg of protein), equivalent to those described in Table II, was incubated as described under "Experimental Procedures" for the measurement of PAPS:cerebroside sulfotransferase activity. After washing with "upper phase" (29) to remove water-soluble radioactive substrates and products, the lower phase was first chromatographed on silicic acid columns. The "glycolipid" fraction was further fractionated on silicic acid thin layer chromatography (TLC) as described under "Experimental Procedures." Before thin layer chromatography, 200 µg of a cerebroside plus sulfatide fraction from bovine brain was added to the "glycolipid" fraction to enable the visualization of the cerebroside and sulfatide areas of the chromatogram.

A. Silicic acid column chromatography

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Counts per min</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform (NL)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acetone (GL)</td>
<td>8910</td>
<td>98</td>
</tr>
<tr>
<td>Methanol (PL)</td>
<td>210</td>
<td>2</td>
</tr>
<tr>
<td>Recovery</td>
<td>97%</td>
<td></td>
</tr>
</tbody>
</table>

B. Two-dimensional TLC of glycolipid fraction

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Counts per min</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Hydroxy FA* cerebrosides</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Normal FA cerebrosides</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hydroxy FA sulfatides</td>
<td>1058</td>
<td>41</td>
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<tr>
<td>Normal FA sulfatides</td>
<td>1443</td>
<td>56</td>
</tr>
<tr>
<td>Recovery</td>
<td>97%</td>
<td></td>
</tr>
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</table>

* FA, fatty acid.

In order to verify that cerebroside sulfotransferase activity was localized predominantly in the Golgi apparatus from rat kidney, we compared the activities of purified subcellular organelles prepared by more conventional means with our Golgi apparatus-rich fraction. The results are summarized in Table II. In all the fractions studied, only smooth microsomes had significant levels of both sulfotransferase and galactosyltransferase activities. Both activities appeared to be about 10% of the level found in the Golgi apparatus fraction. This is most likely due to contamination of this fraction with fragments of the Golgi apparatus. Rough microsomes, which are more clearly representative of endoplasmic reticulum, contain only about 1% of the level of activity found in the Golgi apparatus. The plasma membrane fraction prepared by the more conventional methods (20, 27), like the plasma membrane-rich fraction prepared from our zonal gradients, is poor in both sulfotransferase and galactosyltransferase activities. The only major cell fraction not studied with regard to the localization of galactosyltransferase and sulfotransferase was the lysosomal fraction.

Table III summarizes the results obtained when the product of the sulfotransferase reaction was chromatographed on silicic acid columns and thin layer plates. Chromatography of the lipid extract on silicic acid columns yielded 98% of the initial counts in the glycolipid fraction. Two-dimensional thin layer chromatography of the glycolipid fraction to which carrier brain cerebrosides and sulfatides were added revealed that 97% of the counts chromatographed with the added sulfatides, and that both normal fatty acid sulfatides and hydroxy fatty acid sulfatides are formed in about equal amounts.

The effect of pH and the type of buffer used on the activity of the sulfotransferase in the purified Golgi fraction is shown in Fig. 3. Maximum activity is observed at pH 7 to 7.25 using imidazole. There are sharp falloffs in activity above and below this pH range. The use of Hepes instead of imidazole results in a typical saturation curve. A Lineweaver-Burk plot of the data yielded an apparent Eₐ of 1.3 × 10⁻⁴ M for cerebroside, assuming a molecular weight of 800 for mixed brain cerebro-
Fig. 3 (left). Effect of varying pH of the buffer used on the PAPS:cerebroside sulfotransferase activity of isolated kidney Golgi apparatus. •••••, imidazole-HCl; Δ—Δ, K-Hepes; ○—○, Tris-HCl. The assay conditions are described under "Experimental Procedures." In all cases, the final buffer concentrations were 0.12 M.

Fig. 4 (center). Effect of varying concentrations of divalent cations on PAPS:cerebroside sulfotransferase activity of isolated kidney Golgi apparatus. The cations were added in the form of their chloride salts: Δ—Δ, Mn^2+; ○—○, Mg^2+; □—□, Ca^2+. Other constituents added as described under "Experimental Procedures." The amount of protein used was 61 μg.

Fig. 5 (right). Effect of varying Triton X-100 concentration on PAPS:cerebroside sulfotransferase activity of isolated kidney Golgi apparatus. Other constituents added as described under "Experimental Procedures." The amount of protein used was 60 μg.

Table IV
Analysis of reaction products formed after incubation of rat kidney fractions with UDP-[14C]galactose

In the Dowex 2 method, galactose released is obtained by measuring the amount of radioactivity which passes through Dowex 2 when the sample is incubated with UDP-[14C]Gal in the absence of added N-acetylglucosamine. Galactose transferred is taken to be the increase in the radioactivity passing through Dowex 2 when N-acetylglucosamine (3 nmoles/75 μl of assay mixture) is present in the assay mixture (1). It is assumed that galactose released is the same acceptor. In the chromatography method, the products of the reaction are separated by chromatography on Whatman DE-81 paper (see "Experimental Procedures" section for details). In this assay radioactivity migrating as a monosaccharide is considered "released" while radioactivity migrating as a disaccharide is "transferred."

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Assay method</th>
<th>N-Acetylglucosamine</th>
<th>Released</th>
<th>Transferred</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>nmol galactose/μg protein</td>
<td></td>
</tr>
<tr>
<td>Golgi</td>
<td>Dowex 2</td>
<td>—</td>
<td>192</td>
<td>533</td>
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<tr>
<td></td>
<td>Chromatography</td>
<td>+</td>
<td>138</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>166</td>
<td>65</td>
</tr>
<tr>
<td>Plasma membrane-rich</td>
<td>Dowex 2</td>
<td>—</td>
<td>40</td>
<td>53</td>
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<td>Chromatography</td>
<td>+</td>
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<td>106</td>
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The products of the galactosyltransferase reaction were investigated further by chromatography. After incubation of the kidney fractions with UDP-[14C]Gal in the presence and absence of added N-acetylglucosamine as acceptor for the transferase enzyme, the reaction mixture was chromatographed to separate monosaccharide and disaccharide reaction products. The results are summarized in Table IV (see also Fig. 6). In the absence of added acceptor, some hydrolysis of UDP-Gal occurs in the Golgi fraction but a much higher level of hydrolysis occurs in the endoplasmic reticulum fraction, as well as a second peak migrating in the disaccharide region. The level of this second peak formed by the endoplasmic reticulum fraction is not stimulated by the addition of N-acetylglucosamine as it is in the Golgi fraction. Good agreement is obtained between the chromatographic estimation of the amount of disaccharide formed by the Golgi or by the plasma membrane fraction and the values estimated by difference using Dowex 2 columns. In the case of endoplasmic reticulum, however, the high rate of hydrolysis makes it difficult to measure small rates of transfer by difference, as is required in the Dowex 2 method. The presence of a second peak migrating in the disaccharide region both in the absence or presence of acceptor also makes the estimation of transferase activities difficult using the chromatographic method. It was recently reported by Bergeron et al. (7) that homogenates and endoplasmic reticulum fractions of liver, when assayed for galactosyltransferase activity using chromatography on DEAE-paper, exhibited an unknown peak intermediate in mobility between free galactose and N-acetyllactosamine. This peak disappeared when the incubated reaction mixture was extracted with 2 volumes of chloroform-methanol, 2:1 (v/v), before chromatography of the aqueous phase. We investigated the effect...
FIG. 6. Chromatography of the reaction mixture on DEAE-paper after incubation of: A, kidney Golgi fraction, or B, kidney rough endoplasmic reticulum with UDP-[^14]C]Gal in the galactosyltransferase assay. []—[] without added N-acetylglucosamine; O—O, with added N-acetylglucosamine; △—△, with added N-acetylglucosamine but extracted with 2 volumes of a mixture of chloroform and methanol, 2:1 (v/v), to remove lipid-soluble material. Galactose (1.5 moles) and N-acetyllactosamine (3 moles) were chromatographed simultaneously as standards. After chromatography they were located by spraying with aniline phthalate (see “Experimental Procedures”).

mixture of removal of such organic solvent-soluble components on the chromatography of the reaction products of the galactosyltransferase assay on both the Golgi apparatus fraction and rough endoplasmic reticulum. The results are illustrated in Fig. 6. In the presence of N-acetylglucosamine as acceptor, the main reaction product of the galactosyltransferase reaction in the Golgi apparatus is a peak which migrates with the same relative mobility as N-acetyllactosamine. Extraction of the reaction mixture with 2 volumes of chloroform-methanol, 2:1, does not affect the level of this reaction product. Rough endoplasmic reticulum, on the other hand, when incubated with UDP-[^14]C]Gal, forms a reaction product which migrates in the disaccharide region. The amount of this product is not enhanced by the presence of N-acetylglucosamine in the reaction mixture. Furthermore, extraction of the mixture with chloroform-methanol, 2:1, eliminates this product from the aqueous phase. A small amount of radioactive material remains in the N-acetyllactosamine region of the chromatogram. It amounts to only 0.6% of the activity per mg of protein found in the purified Golgi apparatus fraction, and is probably due to contamination of the fraction with Golgi.

DISCUSSION

The procedures we have developed for the isolation of purified Golgi apparatus from rat liver (3, 16) are applicable, with minor modifications, to the isolation of Golgi apparatus from rat kidney. Our method has the following useful features: (a) it can be used on animals in a normal metabolic state; (b) it yields practically intact Golgi apparatus rather than fragments from different parts of the apparatus; and (c) it can be applied to other tissues with minor modifications and thus allows the comparison of Golgi apparatus from different cell types within the same organism.

The isolated Golgi apparatus of kidney cells, like that of liver, consists of a number of flattened cisternae with attached anasto-
ences are apparent in the two tissues, possibly in the control of the sulfotransferase in rat is different in the brain and the kidney (20). The enzyme in brain rises sharply and remains at a fairly constant level in the adult. A second sulfotransferase is lower than normal in the brains of mouse. 

The finding that PAPS-cerebroside sulfotransferase is localized mainly in the Golgi apparatus of rat kidney, brings to light a new functional aspect of the Golgi apparatus in mammalian cells. It is the first demonstration that Golgi can modify not only mucopolysaccharides and glycoproteins, but glycolipids as well. It is also interesting to note that the sulfatides formed there are probably destined not for secretion, but to be used as constituents of the cell membranes, themselves.

Sulfatides occur in all the cell fractions of kidney but are most abundant in Golgi apparatus and plasma membranes. Little is known about how the sulfatides, which are not freely soluble in the cell cytoplasm, are transported from the Golgi apparatus to the plasma membrane of the cell. Some evidence of a mechanism involving a carrier protein in the cytoplasm which picks up newly formed sulfatide from the Golgi and transfers it to the plasma membrane has been found in brain. In these experiments, the transfer in vitro of newly formed sulfatide from microsomes to myelin prepared from rat brain was investigated. It was shown that a lipoprotein fraction isolated from the soluble fraction of brain accepts [35S]sulfatide from microsomes (41). Similar results have been reported for chick embryo sciatic nerves in tissue culture (42). These results, however, do not rule out the possibility that the lipoprotein in the soluble fraction was originally present in the cisternae of the Golgi apparatus, and appeared in the cytoplasmic fraction due to disruption of the Golgi during the homogenization procedures.

The PAPS-cerebroside sulfotransferase of kidney Golgi apparatus appears to be similar to the enzyme described in some detail by Farrell and McKhann from brain microsomes (19). Both enzymes have a sharp pH optimum of 7.0 to 7.2 in imidazole buffer, and are stimulated by addition of ATP, Triton X-100, divalent cations, and exogenous cerebroside. Both enzymes will transfer sulfate to added psychosine but not to added glucocerebrosides (19, 23). The enzyme from kidney Golgi is stimulated by manganese > magnesium > calcium. The response of the brain enzyme to added manganese was not studied by Farrell and McKhann, but the response to magnesium was greater than the response to calcium for this enzyme. The concentration of divalent cations necessary for maximum activity of the two enzymes appear to be quite different, however. Maximum activity of the Golgi preparation occurred at 60 mM Mn++, whereas the brain preparation showed maximum activity at 20 mM Mg++.

Although the sulfotransferase in brain and kidney appears to have very similar properties in in vitro assays, important differences are apparent in the two tissues, possibly in the development of mechanism involved in its expression. For example, the development of the sulfotransferase in rat is different in the brain and the kidney (20). The enzyme in brain rises sharply to a peak of activity at about 20 days postpartum, which correlates well with the time of maximum myelination in rat brain. After this point, the activity drops almost as rapidly to about one-third the maximum value which persists in the adult. The kidney enzyme, however increases steadily after kidney weight and remains at a fairly constant level in the adult. A second indication of different control mechanisms is the report that the sulfotransferase is lower than normal in the brains of mouse neurological mutants "Jimpy" and "Quaking" whereas the activity in the kidney is unimpaired (43).

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