THE FINE STRUCTURE OF THE CEREBROSIDE OCCURRING IN GAUCHER'S DISEASE*

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Gaucher's disease is a metabolic disorder characterized by the accumulation of excessive quantities of glucocerebrosidase in the reticuloendothelial system. Brady et al. have demonstrated that individuals with this disease have deficient amounts of glucocerebrosidase, one of the enzymes required for complete degradation of the lipid of the red cell membrane. The characteristic cell of Gaucher's disease has numerous membrane-enclosed sacs containing long slender tubules (Fig. 1). Cerebroside extracted with lipid solvents from the tissues of patients with this disease has a similar tubular form. Coiling of these tubules is not apparent in sectioned material but can be demonstrated in negatively stained preparations.2 3

It is the purpose of this report to present the unique morphology, following physical methods of separation, of the tubules of Gaucher's disease cerebroside.
**Materials and Methods.**—Spleenic and/or liver tissues were obtained from six patients with Gaucher's disease, either at the time of splenectomy or at autopsy. These tissues were processed for light and electron microscopy, and were stored frozen. The thawed tissues could easily be prepared for negative staining by mincing small fragments in a solution of 1% ammonium acetate. Small drops of this solution were added to drops of 1% phosphotungstic acid, and 300-mesh coated and carbonized grids were used to support the stained material. This process was equally satisfactory when applied to form-aldehyde-fixed tissues. Shadowing techniques were employed in order to determine the direction of the helix noted in negatively stained preparations. Shadowing was carried out in a vacuum evaporator, using carbon platinum shadowed at an angle of approximately 10°. A Phillips 200 electron microscope was used throughout this study. Care was given during the printing phase of the shadowed forms in order to preserve the correct screw sense of the helix.4

Density gradient centrifugation was carried out in order to separate the tubules from other cellular membranes. For this procedure, tissue was ground with 2.5 times the volume of Tris buffer (at pH 7.6) containing 0.35 M sucrose, 0.025 M KCl, and 0.01 M MgCl₂. After initial centrifugation for 10 min at 27,000 × g in a Sorvall centrifuge, 10 ml of the supernatant was carefully applied to a 30-ml centrifuge tube containing 10 ml of 1.5 M sucrose layered on 10 ml of 2.0 M sucrose. After centrifugation for 17 hr at 75,000 × g, the white layer that had collected between the 0.3 and 1.5 M sucrose was separately collected, pelleted, and resuspended in Tris buffer without added sucrose, and 2 ml was applied to a 15-ml continuous gradient solution ranging from 0.3 to 1.5 M sucrose. The material was then centrifuged again for 17 hr at 78,000 × g. Thin-layer chromatography with a polar lipid solvent system (composition: 100 ml chloroform, 40 ml methanol, 6 ml distilled water) was carried out on the continuous gradient density fraction which contained predominantly tubular forms as determined by electron microscopy. Protein and phosphorus determinations were also performed on this fraction according to the methods of Lowry et al.5 and Bartlett and Shin.6,7 respectively. In an attempt to produce changes in the morphology of the cerebroside tubule, this fraction was also exposed overnight to 2.0 M urea, as well as to 1% triton, 0.1 M NaOH, and 0.1 M HCl.

**Results.**—(1) **Morphology:** Tubular forms are easily identified as long forms in the negatively stained preparations of the minced tissues or in the gradient density fractions. Frequently, several tubules are twisted together. The individual tubules have a width varying from 250 to 500 Å, and some attain a length of 5 μ. Each tubule appears to be made up of 10–12 fibrils gently twisted about the long axis of the tubule. Shadowing techniques reveal a helix with a right-handed screw sense (Figs. 2 and 3). Adjacent tubules are often noted to be twisted about each other (Fig. 4).

The tubular nature of the structure was best judged from Gaucher's disease tissues that had been embedded and sectioned, with attention directed to transverse sections of the tubules. Such views reveal a round to oval structure consisting of 10–12 units distributed around the periphery of a tubule with a diameter of about 350 Å (Fig. 5).

The distance between the fibrils noted in negatively stained preparations is approximately 80 Å, or about twice the length of a cerebroside molecule.

(2) **Purification:** The results of the discontinuous and the continuous sucrose gradient separations are shown in the accompanying diagram (Fig. 6). The material collected from the upper layer of the continuous gradient in the range of 0.70–0.85 M sucrose was considered to be mostly free of the other cellular membranes that were present in the lower or 1.0-M sucrose region.

In order to confirm the presence of cerebroside in these tubules, thin-layer
Fig. 2.—Phosphotungstic acid-stained tubule obtained from a Gaucher spleen showing the fibrils arranged in a gentle spiral along the length of the tubule. Flattening of the tubule probably enables fibrils on both sides of the tubule to be in focus. Magnification ×28,000.

Fig. 3.—A carbon platinum-shadowed tubule printed from a positive made from the original negative. The helix has a right-handed screw sense. The partially flattened appearance is most likely associated with the drying that precedes the shadowing. Magnification ×135,000.

chromatography was performed on the material from the upper zone of the continuous gradient. Glucose cerebroside accounted for more than 90 per cent of the dry weight of the material in this fraction when compared with known standards. The remainder produced a single spot, which migrated with a lecithin standard. Occasionally there was a second spot, which migrated with a phosphatidylethanolamine standard. A protein determination on one sample showed a concentration of 3.0 μg per milligram of dried material (0.3%). The phosphorus content was found to be less than 2.0 μg per 10.0 mg of dry material (0.02%). No morphologic changes in the tubules could be seen by treatment with 1 per cent tritonor 2.0 M urea or by exposure to extremes of pH from pH 1 to 14.

Discussion.—Numerous previous studies have described the electron-microscopic appearance of the tubules of Gaucher cells as they appeared in embedded
and sectioned tissues. These techniques fail to reveal the complex nature of the tubules observed in negatively stained preparations. These are not known to exist in other lipid storage diseases or in normal tissues. I have examined tissues from two cases of Neimann-Pick disease and one case of Fabry's disease, as well as control tissues not involved with a lipid storage state, and have failed to find tubular forms in any of the preparations. This lack of tubular structures in other lipid storage states or in normal tissues suggests that the negatively stained tubules can serve as a diagnostic feature of Gaucher's disease.

The purification procedures permitted a verification of the nature of the material by chromatography methods. The amounts of protein and phosphorus are in keeping with a high degree of lipid purity. The lack of alteration of morphology after exposures to a detergent or urea or extremes of hydrogen ion concentration suggests that a firm binding exists between the aggregated molecules.

The exact orientation of the cerebroside molecules which have aggregated to form the tubules awaits more precise investigation, such as X-ray diffraction studies. One possible model is depicted in Figure 7. The long chains of the fatty acid and the sphingosine comprise the hydrophobic end of the molecule, and glucose is attached to the hydrophilic end. If the hydrophobic ends of
two adjacent molecules were in contact and the hydrophilic ends were also in close contact, a periodicity of 80 Å might be expected, for each molecule of cerebrosides has a length of 40 Å. Ten or twelve such pairs of molecules would form the periphery of the cerebrosides tubule, and these molecules would develop their linear arrangement by attaching themselves to molecules arranged similarly in a lateral fashion, with a 20-30° tilt from the long axis. This would result in a gently spiraled tubular form.

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**Fig. 6.**—When sucrose gradient centrifugation is carried out with the supernatant solution of ground Gaucher's disease tissues, one or two bands appear at the interface of the 0.3 and 1.5 M sucrose solutions in the discontinuous gradient and in the 0.70-0.85 M and 1.0 M zones of the continuous gradient. The upper band of the continuous gradient contains fewer additional membranes than the lower band.

**Fig. 7.**—A model depicting the arrangement of the aggregated molecules of cerebrosides in the form of a tubule. Both lateral and transverse views are shown.
Although the amount of protein was small (0.3%) in the upper zone of the continuous gradient, where the tubules appear most purified, it is possible this amount plays some role in the structure of the tubule. Chemically purified cerebroside also has a tubular appearance, but the fine fibrils are lacking in negatively stained preparations. It is conceivable that protein may play a role in this delicate arrangement of the tubule, possibly by being associated with the hydrophilic zone.

Conclusion.—The tubules of the Gaucher cell have been studied by negative staining, and a unique substructure has been observed. They are composed of 10 or 12 fibrils which spiral in a right-handed screw sense along the length of the tubule. These fibrils are about 80 Å wide and possibly result from the alternating hydrophobic and hydrophilic portions of aggregated cerebroside molecules. Purification can be achieved by sucrose gradient centrifugation of ground tissue containing Gaucher cells. The lack of similar tubules in other lipid storage diseases or in normal tissues, coupled with the ease of preparing negatively stained tissue, suggests the possible role of this procedure in the diagnosis of Gaucher's disease.

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