THE ROLE OF THE GOLGI COMPLEX IN SULFATE METABOLISM

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ABSTRACT

This investigation was designed to determine if sulfate metabolism is the function of a particular cell organelle, or whether the site of sulfation varies, depending upon the type of cell and the class of sulfated compound. Rats and mice were injected intravenously with inorganic sulfate labeled with $^{35}$S ($\text{H}_2\text{SO}_4$), and were then killed by vascular perfusion of fixative 5–30 min later. Several tissues were prepared for electron microscope autoradiography. 14 different types of specialized cells which incorporated the labeled sulfate were analyzed. In every case, the sulfate was initially detected in the smooth membranes and vesicles of the Golgi complex. Available evidence indicates that these cells were engaged in the synthesis of several different sulfated compounds, including mucopolysaccharides, glycoproteins, lipids, and steroids. These results lead to the generalization that the enzymes required for the transfer of inorganic sulfate to a variety of acceptor molecules are located in the Golgi complex.

Many different sulfated compounds have a widespread distribution in the animal body. These include sulfated mucopolysaccharides (MPS), glycoproteins, steroids, phenols, thyroxine derivatives, amines, and lipids (21, 37, 51). During biosynthesis of these molecules, sulfate is first activated by ATP in a two-step sequence requiring two separate enzymes. ATP-sulfurylase catalyzes the reaction between ATP and $\text{SO}_4^-$ to give adenosine 5'-phosphosulfate (APS). Then, APS is phosphorylated by ATP to form 3'-phosphoadenosine 5'-phosphosulfate (PAPS), catalyzed by the enzyme, APS-phosphokinase. Next, the activated sulfate is transferred to the acceptor molecule by a class of enzymes called sulfotransferases. These appear to be specific for each acceptor (18, 21).

Is sulfate metabolism the function of a particular cell organelle, or does the site of sulfation vary, depending upon the type of cell and the class of sulfated compound? This question can be answered by using electron microscope autoradiography to localize radioactivity in cells exposed for very short periods to $^{35}$S-labeled inorganic sulfate ($\text{H}_2\text{SO}_4$). Reports on only three types of cells have appeared so far. In goblet cells of the rat colon, labeled sulfate is first detected in the stacked lamellae of the Golgi complex (35). In embryonic rat cartilage cells, sulfate is initially concentrated in vesicles in the Golgi complex (20). Similarly, in mouse pancreatic acinar cells, sulfate is incorporated into the small vesicles and agranular membranes of the Golgi complex (6).

In this type of experiment, the site at which the sulfate is concentrated within the cell is revealed, but the nature of the sulfated substance is not. In fact, the material sulfated by pancreatic acinar cells has not yet been identified. In embryonic cartilage, the sulfated products are the acid MPS, chondroitin sulfates A and C (38). Goblet cells also produce sulfated MPS, as well as sulfated glycoproteins (44).
In the work described below, 14 additional types of cells which utilize sulfate in a variety of biosynthetic reactions have been analyzed by electron microscope autoradiography at short intervals after exposure to $^{35}$S-labeled sulfate.

**MATERIALS AND METHODS**

The experimental animals consisted of C57 Bl/6J mice, 5-9 wk old, 15-17 g body weight, and Sherman rats, 3 wk old, weighing 32-38 g. Under anesthesia, the animals were injected in the external jugular vein with 0.1-0.3 ml of an aqueous solution of carrier-free $^{35}$S-labeled inorganic sulfate ($\text{H}_2^{35}\text{SO}_4$), obtained from New England Nuclear Corp., Boston, Mass., or Schwarz/Mann, Orangeburg, N. Y. The dosage administered ranged from 2 to 20 mCi/g body weight.

Three factors necessitated the use of such high dosages: (a) the animals were killed very soon after injection, allowing little time for radioisotope to accumulate in the tissues; (b) the half-life of $^{35}$S is relatively short (87 days), so that low dosages could not be compensated for effectively by long exposure of the autoradiograms; (c) high concentrations of radioisotope must be present to yield enough for analysis in the extremely thin tissue sections required for electron microscopy autoradiography.

The animals were killed at 5, 10, 15, or 30 min after injection. A solution containing 1% formaldehyde and 1% glutaraldehyde in phosphate buffer, pH 7.1, was perfused into the heart at 70-80 mm Hg pressure for about 10 min. Selected tissues, including ovary, bone marrow, cornea, intercostal nerve, and sympathetic ganglia, were then removed and placed in the same fixative for at least 2 h. The specimens were next cut into smaller pieces, which were post-fixed in 1% osmium tetroxide in the same buffer for 1 h. They were then dehydrated in ethanol and embedded in Araldite.

Sections of silver interference color, cut on an LKB Ultrotome, were deposited on glass microscope slides previously coated with celloidin. The sections were stained with uranyl acetate and lead citrate, coated with carbon, then dipped into Ilford L4 emulsion which was maintained at 40°C and diluted 1:5 with water. The preparations were exposed in the dark under low humidity at 4°C for up to 6 mo. Most of the slides were developed in Phenidon developer (36) for 1 min at 15°C. Alternatively, a few slides were developed in Microdot X for 4 min at 17°C. After fixation in 30% sodium thiosulfate, the celloidin membrane was separated from the slide by floating on water, and grids were placed over the sections. The membrane and grids were then removed from the surface of the water by adhesion to wet filter paper. After drying, the grids were detached from the membrane and the celloidin was removed by incubation in isopropyl acetate for 3 min.

The autoradiograms were examined in a Siemens Elmiskop 1A electron microscope.

**RESULTS**

Many different types of cells in vertebrate animals take up inorganic sulfate, although its utilization is not universal. In the tissues sampled in this study, for example, no incorporation was observed in skeletal muscle cells, plasma cells, and erythrocytes. Among those cells which do use sulfate, there is a wide range in the amount incorporated which depends upon the specialization of the cell. For instance, fibroblasts metabolize more sulfate than do most neurons. Sulfate incorporation is also influenced by the state of cellular activity. Fibroblasts actively engaged in secreting extracellular material, for example, use more sulfate than "resting" fibroblasts. Incorporation may also vary depending upon the state of differentiation of the cell. This is particularly apparent in the case of developing granular leukocytes in bone marrow, where immature stages use the most sulfate. However, differences in the amount of incorporation are not accompanied by differences in the site of incorporation within the cell. In addition, no distinctions were recorded between rats and mice regarding the intracellular site of sulfation. Furthermore, there were no apparent shifts in the distribution of labeling during the first 30 min after the injection of $^{35}$S sulfate. Therefore, results obtained from the two species and the four time intervals have been combined.

Incorporation of radioactive sulfate was documented in 14 different types of cells. In bone marrow, neutrophilic, basophilic, and eosinophilic myelocyes were labeled (Figs. 1-3), as were megakaryocytes (Fig. 5), and reticular cells lining the sinusoids (Fig. 6). In intercostal nerve and sympathetic ganglia, endothelial cells lining small blood vessels (Figs. 7, 8) and mast cells (Fig. 4) were observed to take up $^{35}$S sulfate, as were fibroblasts (Fig. 9) and Schwann cells (Figs. 11, 13), including those associated with both myelinated and unmyelinated axons. Also labeled in the ganglia of the sympathetic chain were satellite cells (Fig. 12), and the ganglion cells with which they were associated (Fig. 14). Keratocytes were labeled in the cornea (Fig. 10). In the ovary, inorganic sulfate was taken up by fibroblasts, follicular cells associated with de-
FIGURE 1 Neutrophilic myelocyte in the bone marrow of a rat killed 5 min after injection of $[^{35}S]$sulfate. Radioactivity is highly concentrated in the Golgi complex (G). Relatively few silver grains are scattered over other components of the cell. Electron microscope autoradiogram. $\times$ 9,000.

FIGURE 2 Basophilic myelocyte from rat bone marrow, 5 min after injection of $[^{35}S]$sulfate. The cell has taken up the labeled sulfate and concentrated it in the Golgi complex (G). The arrow indicates a centriole. Electron microscope autoradiogram. $\times$ 8,300.
veloping follicles (Fig. 15), and interstitial cells (Fig. 16).

Practically all of the labeling was confined to the Golgi complex in each of the labeled cells. The appearance of this organelle varied among the different types of cells. Usually, it consisted of agranular membranes arranged in layers in association with numerous small vesicles. Frequently, the vesicular component predominated. In granulocytes and megakaryocytes immature granules in process of formation were present within the Golgi complex (Figs. 3, 5). One or more centrioles were often visible lying within or adjacent to the Golgi complex (Figs. 2, 5). Neither the centrioles

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FIGURE 5  Portion of a megakaryocyte from mouse bone marrow, 10 min after injection of [35S]sulfate. Exposed silver grains are associated with the Golgi complex. The small arrows indicate centrioles; the larger arrow points to two condensing vacuoles (immature granules). Electron microscope autoradiogram. X 11,600.
FIGURE 6 Reticular cell lining a sinusoid in the bone marrow of a rat killed 5 min after injection of $^{35}$S sulfate. The radioactive material has been concentrated in the Golgi complex (G). Electron microscope autoradiogram. $\times$ 11,200.

FIGURE 7 Endothelial cell lining a small blood vessel in rat sympathetic ganglion, 30 min after injection of $^{35}$S sulfate. The radioactive sulfate has been preferentially bound in the Golgi complex (G). Electron microscope autoradiogram. $\times$ 10,325.

FIGURE 8 Endothelial cell lining a small blood vessel located in the intercostal nerve of a rat killed 30 min after injection of $^{35}$S sulfate. Labeling is concentrated in the Golgi complex (G). Electron microscope autoradiogram. $\times$ 16,225.
FIGURE 9 Fibroblast located in the intercostal nerve of a rat killed 5 min after injection of $[^35S]$sulfate. The Golgi complex is heavily labeled, whereas other parts of the cell are essentially free of radioactivity. Electron microscope autoradiogram. $\times$ 15,200.

FIGURE 10 Keratocyte from the cornea of a mouse killed 30 min after injection of $[^35S]$sulfate. Labeling is concentrated in the vesicles and smooth membranes of the Golgi complex. Exposed silver grains located over the adjacent edge of the nucleus (N) may have been hit by beta particles emitted from a source located in the Golgi complex. C, bundles of collagen fibers. Electron microscope autoradiogram. $\times$ 19,520.
nor the developing granules showed any tendency for specific labeling, except in eosinophilic myelocytes, in which the earliest stage of granule formation (condensing vacuoles) sometimes showed signs of sulfate uptake (Fig. 3). In contrast, the stacked membranes and vesicles were both significantly labeled at all intervals examined between 5 and 30 minutes.

In addition to the intense labeling of the Golgi complex, a few scattered silver grains, representing a very small proportion of the total cellular radioactivity, occasionally occurred over other cellular components, such as the nucleus, or cytoplasmic granules, mitochondria, and endoplasmic reticulum. None of these structures gave evidence of specific, concentrated labeling. Often, they
showed no labeling at all, even in the presence of an intensely reactive Golgi complex. Exposed grains which did not lie within the general confines of the Golgi complex proper tended to occur with greatest frequency at the edge of the Golgi zone. Because the Golgi complex almost invariably was situated in a nuclear indentation (Figs. 1, 2, 5), or directly next to the nucleus (Figs. 3, 4, 6–16), some of the grains lying over the nucleus apparently were due to “scatter” from a source in the Golgi complex (Figs. 3, 10). Nevertheless, in the majority of cases, labeling of structures other than the Golgi complex did not exceed background levels, and was randomly scattered, without apparent relationship to any specific cell structure. This was not true, however, at intervals after 30 min, when labeling shifted out of the Golgi complex, and was secreted, stored in cytoplasmic granules, or was in other ways redistributed with the cell.

**DISCUSSION**

Inorganic sulfate, labeled with $^{35}$S, and administered intravenously in rats and mice, was rapidly taken up by a wide variety of cell types in the several tissues examined. Perfusion, prolonged aqueous fixation, and subsequent extraction procedures removed unbound sulfate from the tissues. The residual radioactivity must have represented sulfate bound to large molecules which were stabilized by the fixative and retained in the sections. The location of the radioactivity was determined by high resolution autoradiography. Practically all of the silver grains were situated over the vesicles and agranular membranes of the Golgi complex. Although a few exposed silver grains were associated with other cellular structures, these grains tended to occur at the edge of the Golgi complex, and could be attributed to beta particles arising from a source in the Golgi complex. A weak, randomly distributed scattering of silver grains which was unrelated to any particular cell structure was due to background.

There have been no previous reports of sulfate incorporation into the Golgi complex in any of the 14 types of cells described above, although many of the cells are known from earlier studies to be sulfate users.

There are previous accounts of the labeling of neutrophilic myelocytes after exposure to $[^35S]$-sulfate (28, 34). Staining for acid MPS is positive in neutrophils (15, 24, 28). The azurophil granules are particularly reactive, and with one method the Golgi complex is also stained (24). There is one previous report which specifically mentions labeling of basophilic myelocytes after exposure to $[^35S]$-sulfate (28). Basophil granules stain positively with various procedures for acid MPS (15, 24, 28), including, possibly, the sulfated MPS, heparin (2). Uptake of $[^35S]$-sulfate by eosinophilic myelocytes has been noted before (22, 28, 34). After incorporation into the Golgi complex, labeled material subsequently appears in azurophil granules (59). The staining characteristics of these granules indicate that they contain sulfated MPS (15, 24, 28).

An acid MPS which appears to be chondroitin sulfate has been extracted from leukocytes (11, 17). Olson and Gardell (48) specify that it is chondroitin sulfate A. The greater part of the enzyme activities involved in chondroitin sulfate synthesis in marrow myeloid cells resides in the smooth membrane fraction of the microsomes (47). The MPS formed in this cell fraction is subsequently transported to granules (46). Electron microscopy has shown that the granules are assembled in the Golgi complex (3, 4). These findings fit well with the results of the present study, in which sulfation was shown to occur in the Golgi complex in the marrow granular myelocytes of rats and mice.

Incorporation of $[^35S]$-sulfate by megakaryocytes has been documented by autoradiography (5, 13, 28). Jones (30) reported evidence that the Golgi complex in these cells produces the specific granules which subsequently occur in the platelets released by the cell into the blood. The platelets stain for sulfated MPS (15), which are concentrated in the nucleol of the granules (55), and are radioactive several days after exposure of the megakaryocytes to $[^35S]$-sulfate (28). A sulfated acid MPS, apparently chondroitin sulfate A, has been isolated from platelets (48).

The present observations represent the first report of sulfate utilization by the reticular cells which line the sinusoidal vessels in bone marrow. Weiss (57) concluded that these cells are in a class which includes fibroblasts and other cells which elaborate and secrete an extracellular matrix. No evidence is available regarding the nature of the sulfated material produced by the reticular lining cells.

There are two previous reports which specifically state that endothelial cells take up inorganic sulfate (12, 26). The use of stains in conjunction with autoradiography led Curran (12)
to conclude that the cells were elaborating sulfated acid MPS.

Numerous investigators have reported autoradiographic evidence for the utilization of $^{35}$S-sulfate by mast cells (5, 13, 31). The sulfated product synthesized in mast cells is heparin (33), a MPS containing both O-sulfate and N-sulfate bonds. Several authors have suggested that the site of origin of mast cell granules is the Golgi complex (32).

Early autoradiographic studies of connective tissue revealed an uptake of $^{35}$S-sulfate in areas rich in collagen (5, 19). The labeled cells proved to be fibroblasts (14). A localization of labeling in the cytoplasm around the nucleus 1 h after exposure to radioactive sulfate was observed (38). The sulfated compounds produced by fibroblasts are chondroitin sulfates B and C (42).

Keratocytes are fibroblast variants which produce the transparent stroma of the cornea. An appreciable uptake of $^{35}$S-sulfate was observed in early autoradiographic studies of this tissue (5, 13). The uptake was attributed to the keratocytes (16), in which the labeling was mainly cytoplasmic (19), and particularly concentrated around the nucleus (8). The sulfated products of these cells are the MPS, chondroitin sulfate A, and keratosulfate (42), and a sulfated glycoprotein (50).

It has been shown for the first time in this report that in the peripheral nervous system of rats and mice, inorganic sulfate is incorporated into the Golgi complex of sympathetic ganglion cells, satellite cells, and Schwann cells. Previous work dealing with sulfate utilization in the peripheral nervous system is extremely limited. There is one report, citing a low uptake of $^{35}$S-sulfate in the peripheral nerves of mice (13). Histochemistry revealed no evidence of sulfated MPS in peripheral nerve, except for a very thin layer between axons and their myelin sheaths (1). However, extracts of peripheral nerve have yielded chondroitin sulfates A and C, and heparan sulfate (9). More attention has been paid to the central nervous system, where several autoradiographic studies have recorded incorporation of $^{35}$S-sulfate, which is taken up by both neurons and glial cells (26). The two major types of sulfated materials in the central nervous system are sulfated lipids (sulfatides), which are concentrated in myelin, and sulfated MPS, predominantly chondroitin sulfates A and C, whose location is uncertain (53, 54). Myelin appears to be free of MPS (39). Evidence for the presence of sulfated glycoprotein has been presented (40).

These findings suggest that incorporation of inorganic sulfate into Schwann cells associated with peripheral myelinated nerves is due to the formation of sulfated lipid destined for the myelin. (Transfer of $^{35}$S-labeled lipid to myelin has been documented in the central nervous system [26]). The sulfated material produced by Schwann cells associated with unmyelinated nerves, by satellite cells, and by sympathetic ganglion cells is presumably MPS, with chondroitin sulfates A and C predominating, or possibly sulfated glycoprotein.

Sulfated lipid synthesis has been localized in the microsomal fraction of brain tissue. Subfractionation of the microsomes indicates that the sulfatides are produced in association with the smooth membrane subfraction (25), a finding which is consistent with the autoradiographic evidence that sulfate is specifically incorporated into the Golgi complex of myelinating Schwann cells.

The first autoradiographic studies of $^{35}$S-sulfate incorporation in the ovary showed concentration of labeling in the follicular fluid, hours after exposure to the radioisotope (7, 27). Examination of earlier intervals revealed that uptake occurred in the follicular cells, which then released the sulfated material into the fluid (13, 43, 45). Hadek (23) reported evidence, based on electron microscopy, that follicular fluid secretion involves the Golgi complex. The sulfated component in the follicular fluid is a MPS of the chondroitin sulfate type (29).

Utilization of $^{35}$S-sulfate by ovarian interstitial cells has not been reported previously. The func-

Figure 13 Schwann cell surrounding a myelinated axon in the intercostal nerve of a rat killed 30 min after injection of $^{35}$S-sulfate. The cell has incorporated the radioactive sulfate and concentrated it in the Golgi complex (G). A, axon; M, myelin. Electron microscope autoradiogram. X 18,800.

Figure 14 Portion of a ganglion cell from the sympathetic chain of a rat killed 5 min after injection of $^{35}$S-sulfate. The radioactive sulfate has been concentrated in the smooth membranes and vesicles of the Golgi complex (G). Other parts of the cell remain unlabeled. Electron microscope autoradiogram. X 18,800.
tion of these cells is the production of steroid hormones, particularly estrogens (10, 41, 49). Sulfate is known to play a role in the biosynthesis and metabolism of steroids (52), although the precise role of the sulfated steroid intermediates is still uncertain (51).

In summary, published work has shown that [35S]sulfate is incorporated into the Golgi complex in chondrocytes, goblet cells, and pancreatic acinar cells. In the present report, 14 additional specialized types of cells have been shown to concentrate inorganic sulfate in the Golgi complex. A similar finding has been recorded elsewhere in two other cell types (56). Although the sulfated cell product cannot be identified with certainty in every case, available evidence indicates that the examples studied include several different sulfated MPS, sulfated glycoproteins, sulfated lipids, and sulfated steroids.

These findings indicate that the Golgi complex plays a major role in sulfate metabolism. Because APS and PAPS are water soluble, they might not have been retained in the cells by the aqueous aldehyde fixative. Thus, it is not definitively established by these experiments that sulfate activation occurs in the Golgi membranes. However, at the very least, the final sulfated products must have been retained in place by the fixative solution. Because the site at which the radioactive sulfate was initially detected was without exception the Golgi complex, it may be concluded that the sulfotransferases are associated with the Golgi membranes. These results therefore suggest the generalization that the enzymes required for the transfer of active sulfate to a variety of acceptor molecules, and possibly the enzymes involved in the previous step of activating the sulfate by ATP, are located in the agranular membranes and vesicles of the Golgi complex.

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Figure 15 Follicular cell from the ovary of a mouse killed 10 min after injection of [35S]sulfate. The exposed silver grains are restricted to the region of the Golgi complex. Electron microscope autoradiogram. Microdol X developer. × 13,750.

Figure 16 Interstitial cell from the ovary of a mouse killed 10 min after injection of [35S]sulfate. The radioactive sulfate has been concentrated in the Golgi complex (G). Electron microscope autoradiogram. Microdol X developer. × 10,550.

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