

MORPHOMETRY OF THE GOLGI APPARATUS IN DEVELOPING LIVER

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INTRODUCTION

The Golgi apparatus is an organelle which appears to contribute both to the development and to the normal function of the liver cell. The complex arrangement of membranes in the Golgi apparatus in the rat liver has been revealed by electron microscope examination of tissue sections (1, 2)

and, more recently, of negatively stained preparations from isolated Golgi fractions (3).¹ From

¹Sturgess, J. M., E. Minaker, and M. A. Moscarello. 1972. The Golgi complex. I. Isolation and ultrastructure in normal rat liver. Submitted for publication.

qualitative studies with thin sections, it is difficult to determine the relationship between structure and the many functions of the Golgi apparatus. Packaging and transport of secretory proteins (4) and biosynthesis of glycoproteins (5), important both in secretion and as cell surface determinants of the plasma membrane (6), have been attributed to the Golgi apparatus as well as a role in lipid and lipoprotein secretion (7).

Further understanding of the structural and functional organization of the Golgi apparatus requires quantitative morphometric analysis with biochemical studies, so that variation in structure or arrangement of the membranes may be related to changes in their activity. In the present study, electron microscope stereological techniques have been applied to provide a morphometric model of the Golgi apparatus in normal rat liver at different stages of development. To our knowledge, no quantitative data are available at present on the sequential changes of the Golgi apparatus during the postnatal development period.

MATERIALS AND METHODS

20 male Wistar rats were used in these studies. Groups of four rats were sacrificed at weekly intervals for 5 wk after weaning. After overnight fasting, each rat was anesthetized with ether and the liver was resected. Samples of liver were quickly taken from the right lobe, cut into blocks about 1 mm³ size, and fixed in chromium-osmium fixative, pH 7.4 (8) for 90 min. Tissue blocks were rinsed, dehydrated in graded alcohol solutions, and embedded in Epon-Araldite. Ultrathin sections were cut on a Reichert ultramicrotome using a diamond knife. Contrast was enhanced by staining with uranyl acetate and lead citrate. The sections were examined in a Philips EM 300 at 60 kv and electron micrographs were recorded on 3 × 4 inch plates.

Sampling

From the pooled tissue blocks which included different regions of the liver lobule, four blocks were selected at random for electron microscope examination. At low magnification, two to four electron micrographs were taken from thin sections of each block to give prints of 2500 magnification. Higher magnification was required to resolve cytoplasmic membranes and, for this, 40 electron micrographs were recorded using random sampling methods to provide prints of approximately 50,000 final magnification. For each animal, a total of eight micrographs at 2500 magnification and 160 micrographs

at 50,000 magnification were examined. Magnification was calibrated for each series of micrographs using a 54,000 line per inch diffraction grating replica.

Morphometry

The principles of the morphometric procedures are based on those previously described (9). The number of hepatocytes per unit volume of liver and the average volume of individual hepatocytes were calculated from prints of 2500 magnification, using a point counting system. Nuclear diameters were obtained with a Zeiss particle analyzer.

For quantitation purposes, the Golgi apparatus included those smooth-surfaced membranes arranged in characteristic stacks of parallel cisternae, small vesicles or tubules, and larger secretory vesicles (Fig. 1). Both the volume and membrane surface of the Golgi apparatus were determined from electron micrographs of 50,000 magnification using a multi-purpose counting grid. The grid was ruled with a series of lines of known length: the ends of each line served as points. The volume was calculated from points overlying the Golgi apparatus, including both intercisternal and intracisternal spaces, so that the final volume represented the volume of the Golgi complex rather than the volume of the individual cisternae. The surface area of the membranes was determined from the number of intersections of Golgi membranes with the test lines.

All data were entered in *ad hoc* forms and processed electronically, using programs designed to calculate the individual volume and surface density values. Significance of differences and homogeneity between samples was determined by statistical analysis.

RESULTS

The variation in body weight and liver weight for each group of four rats, aged 3, 4, 5, 6, and 7 wk, is shown in Table I. The coefficient of variation in weight was less than 3% for all groups: the highest variation appeared among the older rats.

Number of Size of Hepatocytes

The number of hepatocyte nuclei per gram of liver is summarized for each group in Table II. At 3 wk, the number of nuclei per gram of liver was 234×10^6 which decreased to 167×10^6 at 5 wk and then remained constant.

The mean volume of the hepatocytes for each group is also shown in Table II. At 3 wk, the mean hepatocyte volume was low, 3940 μm^3 , but increased at 4 wk to an average of 5274 μm^3 . From 4 to 7 wk, the mean hepatocyte volume showed no significant variation.

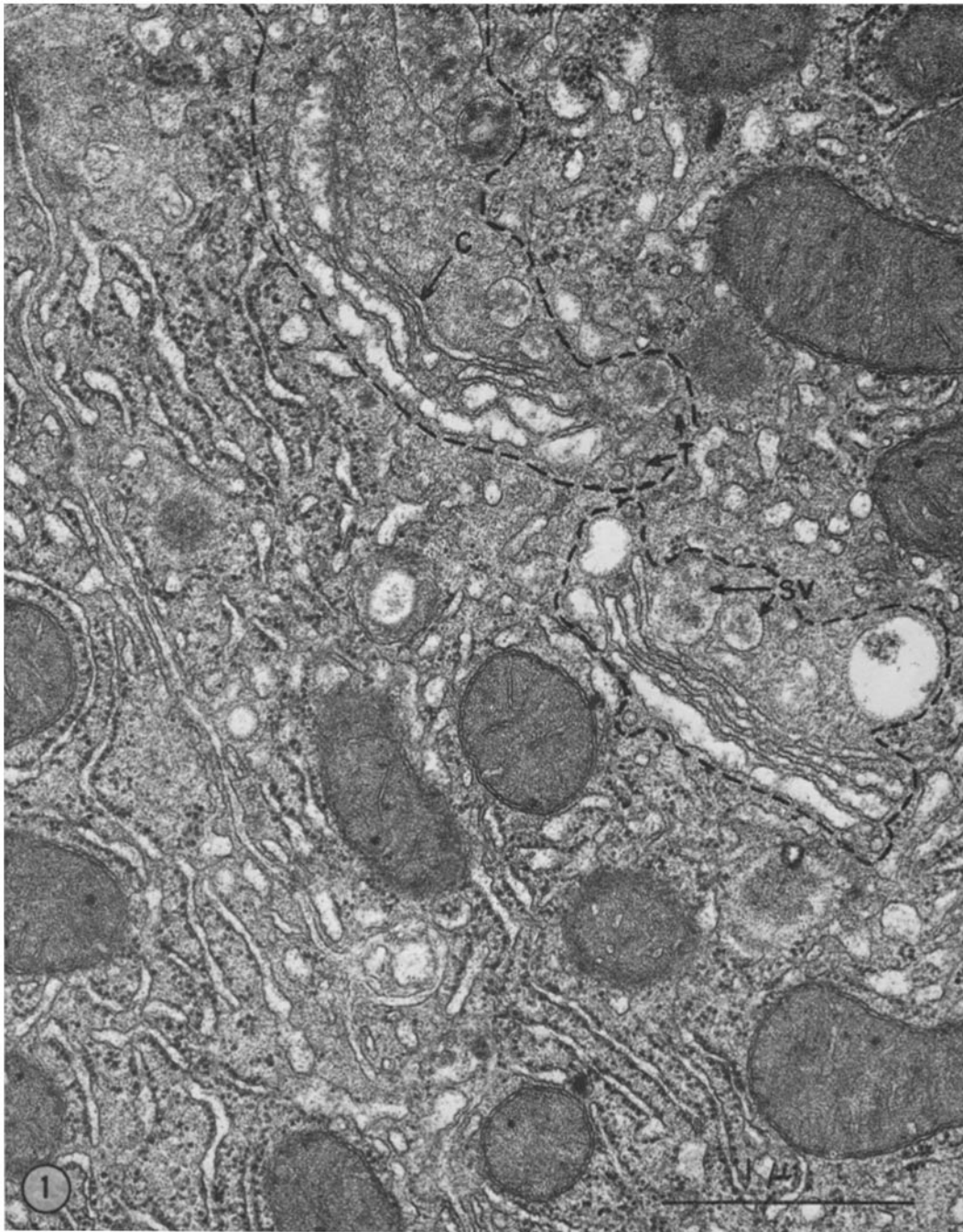


FIG. 1 Electron micrograph of rat hepatocyte cytoplasm showing the Golgi apparatus with parallel flattened sacs or cisternae (*C*), small vesicles (*T*), and larger secretory vesicles (*SV*). Morphometric measurements were made by superimposing a multipurpose counting grid over each micrograph. Those points and linear intercepts within the broken line were recorded as the Golgi complex. $\times 36,200$.

TABLE I
Body and Liver Weight of Rats

| Age | Body weight | Liver weight |
|-----------|----------------|--------------|
| <i>wk</i> | <i>g</i> | <i>g %</i> |
| 3 | 53.25 ± 2.06* | 4.17 ± 0.25 |
| 4 | 70.80 ± 1.32 | 4.24 ± 0.11 |
| 5 | 132.00 ± 4.10 | 4.02 ± 0.10 |
| 6 | 179.00 ± 10.59 | 3.89 ± 0.10 |
| 7 | 223.80 ± 8.49 | 3.72 ± 0.07 |

* Means ± standard error of the mean.

TABLE II
Number of Nuclei and Cytoplasmic Volume of Hepatocytes

| Age | Nuclei (cells) | Volume |
|-----------|----------------------|-----------------|
| <i>wk</i> | × 10 ⁶ /g | μm ³ |
| 3 | 243 ± 8* | 3940 ± 140 |
| 4 | 181 ± 7 | 5274 ± 211 |
| 5 | 167 ± 7 | 5583 ± 171 |
| 6 | 161 ± 10 | 5260 ± 302 |
| 7 | 167 ± 7 | 5337 ± 175 |

* Means ± standard error.

Volume of Golgi Apparatus

The variation in volume of the Golgi apparatus expressed as μm³ per cell is shown in Fig. 2 and as cubic centimeters per gram of liver in Fig. 3. Each point on the curves represents the mean value for four random tissue sections studied for each of four animals and includes quantitation of at least 640 micrographs. Between 3 and 5 wk of age, the volume of Golgi apparatus increased twofold, expressed in terms of cell volume and of liver weight. The maximum volume was found at 5 wk when the Golgi apparatus occupied 244 μm³ in the cell, approximately 4% of the total cell volume. Expressed as volume per gram of liver, the maximum Golgi volume found was 0.041 cm³ per g. From 6 to 7 wk, the volume of the Golgi apparatus was constant: 130–150 μm³ per cell or 0.022–0.024 cm³ per g of liver; these values were lower than at 5 wk.

Surface Density of Golgi Apparatus

The variation in membrane surface of the Golgi apparatus, expressed as μm² per cell is shown in Fig. 4 and as m² per gram liver in Fig. 5. The variation in membrane surface during development followed a pattern similar to that described

for volume. The membrane surface of the Golgi apparatus increased from 7.49 to 10³ μm² per cell or 1.82 m² per g liver at 3 wk to a maximum of 12.9 × 10³ μm² per cell or 2.15 m² per g liver at

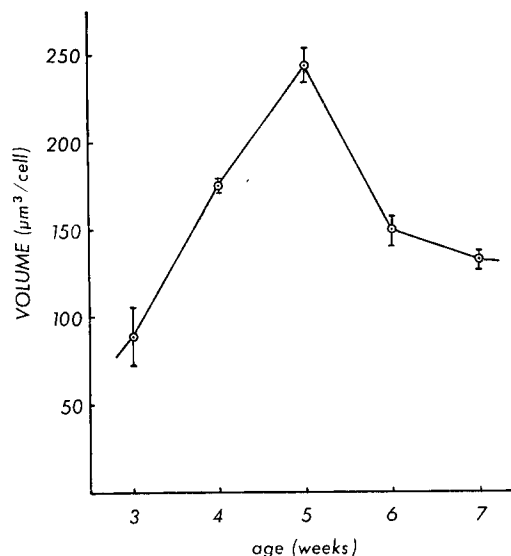


FIGURE 2 Variation in volume of the Golgi apparatus (μm³/cell) in rat liver at different stages of development. Each point represents the mean of four random tissue sections for each of four animals; vertical lines represent the standard error.

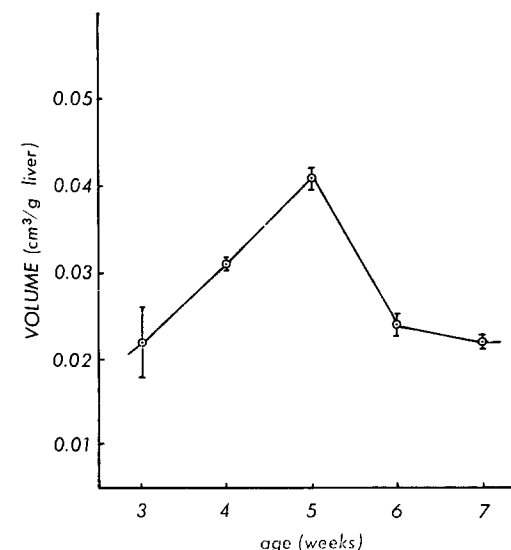


FIGURE 3 Variation in volume of the Golgi apparatus (cm³/liver) in rat liver at different stages of development.

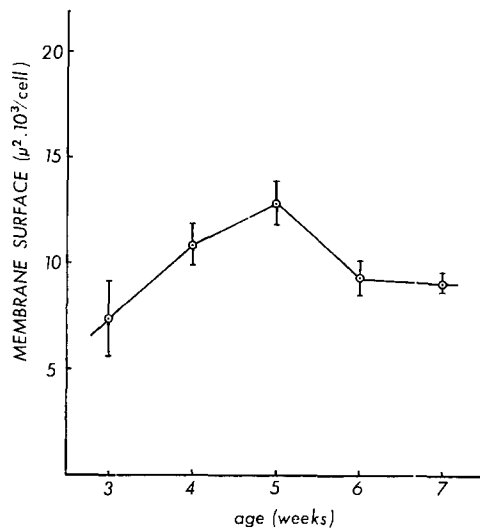


FIGURE 4 Variation in membrane surface of the Golgi apparatus ($\mu\text{m}^2/\text{cell}$) in rat liver at different stages of development.

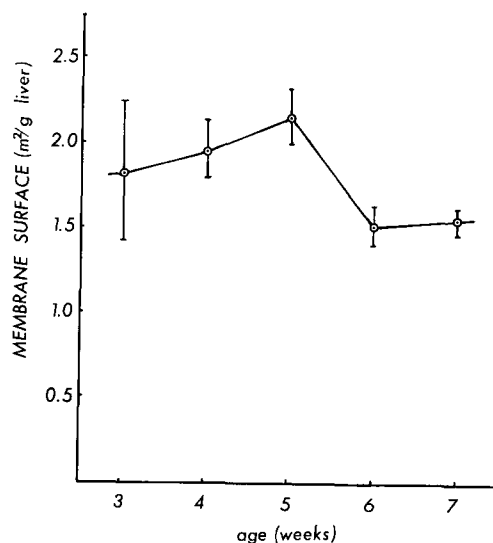


FIGURE 5 Variation in membrane surface of the Golgi apparatus (m^2/cell) in rat liver at different stages of development.

5 wk. Between 6 and 7 weeks, the membrane surface reached a steady value of $9.2 \times 10^3 \mu\text{m}^2$ per cell or 1.5 m^2 per g liver.

DISCUSSION

Both volume and surface of membranes in the Golgi apparatus have been measured quantitatively in normal rat liver for the first time. Rat liver

provided a useful model since it has a relatively homogeneous cell population and has been used for previous morphometric studies with other cell membranes including rough and smooth endoplasmic reticulum, mitochondria, and microbodies (9, 10, 11). In addition, correlation of structural and functional variation in other intracellular membranes has been demonstrated using drugs such as phenobarbital (12, 13, 14) and cortisone (15), and under different dietary conditions (16, 17). In previous studies, the Golgi apparatus has been included with the smooth endoplasmic reticulum. Since both the functions and the structural arrangement of the Golgi apparatus are distinct from those of the endoplasmic reticulum, it seemed important to study these membranes separately. In addition, separate evaluation of variation in the Golgi apparatus, the rough, and the smooth endoplasmic reticulum may reveal possible further details regarding the development and possible differentiation of these membrane types (18, 19).

The values of cell size and number calculated for mature rat liver in this study were comparable to those previously reported (10, 11, 12). During the earliest stages of development of the rat liver, both number and volume of hepatocytes varied and achieved a constant level only after 5 wk of age. Significant changes were found in the volume and surface of Golgi membranes in rat liver between 3 and 7 wk of age. This suggested that the proportion of each type of cytoplasmic membrane varied during development. The Golgi apparatus was most prominent at 5 wk when it occupied more than 4.5% of the cytoplasmic volume, compared with 2.5–3%, both at 3 wk and 6–7 wk of age. A similar pattern was found in the organelle whether expressed per cell or per gram liver, so that this reflects a true conformational change, free of influences inherent in the method of calculation. Since the variation in volume was greater than that of membrane surface, change in the form or shape of the Golgi apparatus occurred, as well as an absolute increase in the proportion of Golgi membranes.

In a quantitative ultrastructural study by Rohr et al. (20), variation of cytoplasmic membranes in rat liver during the perinatal period is reported. The volume of the Golgi apparatus was measured separately from that of the endoplasmic reticulum, but no measurements were given for membrane surface and the data were not discussed. The values established for Golgi volume at 8 days after birth

of 92 μm^2 per cell compared well with the values described here of 89.4 μm^2 membrane per cell at 3 wk of age.

Morphometric measurements of rough and smooth endoplasmic reticulum have been made on the material used in this study (21). In the mature rat liver, the volume and surface area of these membranes were similar to those previously reported (10, 12). The Golgi apparatus represented about 12–15% of the volume and 12% of the membrane surface of total endoplasmic reticulum in the mature rat liver. At certain stages of development, i.e., at 5 wk of age, the Golgi apparatus contributed almost 25% of the endoplasmic reticulum volume and up to 14% of the total membrane surface.

Significant variation in the rough and smooth endoplasmic reticulum also appeared at different stages of development (21). The proportion of rough endoplasmic reticulum was highest at 3 wk, whereas the smooth endoplasmic reticulum reached maximum values at 7 wk or later. The appearance and the maximum proportion of Golgi membrane at 5 wk suggested that there was some rearrangement or differentiation of membrane populations in the hepatocyte during maturation of the rat liver.

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