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). From

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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 examina-
tion. 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 magni-
fication. For each animal, a total of eight micro-
graphs 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 ar-
ranged 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 × 10⁶ which decreased to 167 × 10⁶ 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 µm³, but
increased at 4 wk to an average of 5274 µm³. 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. × 36,800.
TABLE I

| Age (wk) | Body weight (g) | Liver weight (g) |
|----------|-----------------|------------------|
| 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

| Age (wk) | Nuclei (cells) | Volume (µm³) |
|----------|----------------|--------------|
| 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
BRIEF NOTES

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 differentiations 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

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**Figure 4** Variation in membrane surface of the Golgi apparatus (µm²/cell) in rat liver at different stages of development.

**Figure 5** Variation in membrane surface of the Golgi apparatus (m²/cell) in rat liver at different stages of development.
of 92 µm² per cell compared well with the values described here of 89.4 µm³ 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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