CORRELATED MORPHOMETRIC AND BIOCHEMICAL STUDIES ON THE LIVER CELL

I. Morphometric Model, Stereologic Methods, and Normal Morphometric Data for Rat Liver

EWALD R. WEIBEL, WILLY STÄUBLI, HANS RUDOLF GNÄGI, and FELIX A. HESS

From the Department of Anatomy, University of Berne, Switzerland, and the Research Laboratories of the Pharmaceutical Department of CIBA Limited, Basle, Switzerland

ABSTRACT

The basic morphological properties of liver cells are defined in the form of a morphometric model to permit integrated quantitative characterization of functionally important parameters. Stereologic methods which allow efficient and reliable quantitative evaluation of sectioned liver tissue are presented. Material, obtained by a rigorous three-stage sampling procedure from five normal rat livers, is systematically subjected to this analysis at four levels of magnification. This yields quantitative data which are expressed as "densities," i.e. content per 1 ml of tissue, as "specific dimensions" related to 100 g body weight, and as absolute dimensions per average "mononuclear" hepatocyte. Base line data relating to the normal rat liver are presented for the entire spectrum of parameters. As examples, 1 ml of liver tissue contains 169 $\times$ 10^6 hepatocyte nuclei, some 90 $\times$ 10^6 nuclei of other cells, and 280 $\times$ 10^9 mitochondria. Hepatocyte cytoplasm accounts for 77% of liver volume, and the mitochondria for 18%. The surface area of endoplasmic reticulum membranes in 1 ml of liver tissue measures 11 m^2 of which are $\frac{2}{3}$ of the rough form carrying some 2 $\times$ 10^{13} ribosomes. The surface area of mitochondrial cristae in the unit volume is estimated at 6 m^2. The validity and applicability of the method are discussed, and the data are compared with available information from other studies.

INTRODUCTION

The liver cell responds to numerous stimuli by alterations in the structure and content of organelles, which appear to be correlaries to biochemical changes observed in subcellular fractions. In correlated electron microscopic and biochemical studies the biochemical data are usually quantitative, while morphological information is mostly restricted to semiquantitative descriptions based on subjective judgment alone. This approach does not permit statistical testing of the data and, hence, precludes proper correlation of morphological and biochemical data.

In recent years stereologic methods have become available which permit efficient and re-

liable measurement of structures by simple counting or measuring procedures applied to electron micrographs of sectioned tissue (1–7, for extensive references cf. 8). Quantitative data can thus be obtained on cells fixed intact, and can be correlated with information retrieved from subcellular fractions.

This paper presents the morphometric basis for a series of correlated experimental studies on structural and biochemical alterations of the liver cell induced by phenobarbital (9) and other compounds. It relates (*a*) a general morphometric model of the hepatocyte, (*b*) the stereologic methods required for quantitatively defining its parameters, and (*c*) corresponding base line data for the normal rat liver. Loud (7) has recently published a similar study, with particular emphasis on sublobular variations. The present study considers "average" cells, as they are essentially studied in liver homogenates. Disregard of sublobular variations for the present purpose appears justified in view of Loud's findings of extensive homogeneity throughout the liver lobule (7).

MORPHOMETRIC MODEL OF HEPATOCYTE

To define a morphometric model, liver tissue is systematically subdivided into compartments,[1] each of which forms a structural entity. These compartments are schematically represented in Fig. 1, with an indication of their relationship to subcellular fractions. For the present purposes a number of simplifications and conventions are introduced:

(*a*) The extrahepatocytic space is understood to comprise the sinusoids with Kupffer cells and Disse space, the bile canaliculi, as well as extralobular tissue. This compartment is not further subdivided, since our studies will relate to hepatocytes only.

(*b*) To avoid difficulties and arbitrary judgement at transition points the term "endoplasmic reticulum" is here understood to include all membrane-bounded cytoplasmic cisternae, tubules, and vesicles. In particular, the perinuclear cisterna is considered as part of rough ER space, its external ribosome-studded membrane a portion of rough ER membrane. Similarly all Golgi elements are attributed to smooth ER. In the scope of this study a differentiation of perinuclear space and Golgi elements as separate entities appeared unnecessary; they contribute only a small fraction to the respective parameters and are, at least in part, also included in microsomal fractions of liver homogenate.

(*c*) The term "cytoplasmic groundsubstance" is defined as the cytoplasmic space extending from the cell membrane to the surface of membrane-bounded organelles. It thus includes glycogen granules, lipid droplets, and free as well as bound ribosomes. Volume of rough endoplasmic reticulum therefore refers only to cisternal space with membrane, but excludes the attached ribosomal layer.

The second part of Fig. 1 summarizes the morphometric parameters which define the model. They measure the volumes V of the compartments,[1] the surface area S of their bounding membranes, the number N of particles,[1] and their size estimated by their mean volume $\bar{v}$. To set these parameters in relation to the organ and to the organism, two basic measurements are, of course, necessary: the liver volume V_L and the body weight W. The internal parameters of hepatic structure can be expressed in three different ways:

STRUCTURAL DENSITIES or concentrations indicate the amount of a particular structure present in the unit volume of tissue. Accordingly, we would distinguish between: *volume density* (symbol[2]: V_V), representing the volume fraction of tissue occupied by this compartment; *surface density* (S_V) measuring the surface of membranes in the unit tissue volume; *numerical density* (N_V) indicating the number of particles in the unit tissue volume. It should be noted at this point that it may be of occasional interest to express one parameter in relation to another associated parameter. In this sense it may be meaningful or practical to measure, e.g., the surface density of endoplasmic reticulum membranes in relation to hepatocyte cytoplasm (see Methods).

ABSOLUTE DIMENSIONS represent, e.g., total membrane surface of smooth endoplasmic reticulum of all hepatocytes of the entire liver, or the number and volume of all liver mitochondria, etc. These are obtained by multiplying the structural density values with the liver volume V_L.

[1] The term "compartment" is here defined as the fraction of space occupied by the totality of a given constituent, while "particle" refers to individual organelles.

[2] The notation used throughout this study conforms to the principle accepted by the International Society for Stereology (6, 8, 11).

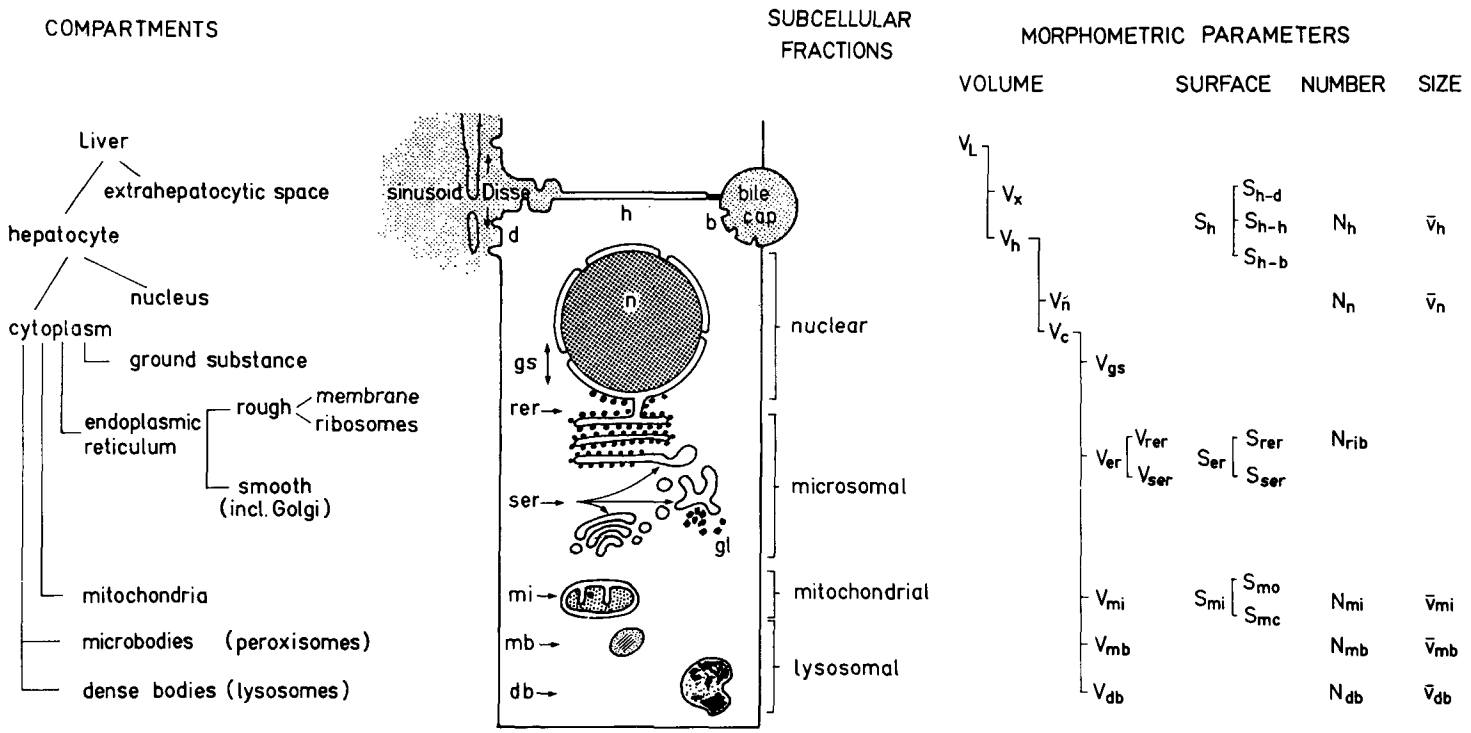

FIGURE 1 Morphometric model of hepatocyte.

SPECIFIC DIMENSIONS[3] are likewise absolute dimensions, but they refer to the liver of an animal of standardized body weight. Normal liver volume V_L is a function of body weight W, which can be assumed to be linear within age groups (10). The quotient V_L/W therefore measures the liver volume of a standardized animal of unit weight. "Specific dimensions" can thus be obtained by multiplying the corresponding structural densities with V_L/W instead of V_L.

Specific dimensions will be used in the present and the accompanying paper (9) to compensate for variations in body size of the experimental animals. *All absolute dimensions will be given in reference to rats of 100 g body weight*, which we have arbitrarily chosen as standard.

METHODS

Preparation of Tissue

Rats, were lightly anesthetized with ether and sacrificed by severing the neck. After immediate laparotomy the liver was resected in toto and weighed. Subsequently, three thin slices were cut from the major left lobe and diced into the usual tissue cubes of about 1 mm diameter. These were fixed for 2 hr in cold phosphate-buffered 1% osmium tetroxide (35), and, after dehydration in graded acetone, embedded in Araldite.

The blocks were sectioned on a Porter-Blum ultramicrotome with a diamond knife. Section thickness was kept as constant as possible; it is estimated from the silver interference color to be of the order of 600–900 A (12). The sections were picked up on 200-mesh copper grids fitted with a carbon-coated parlodion film. Constrast was enhanced by double staining with uranyl-acetate and lead citrate (13). The sections were examined in an Elmiskop I, random electron micrographs being recorded on 35 mm film for stereologic analysis (6).

Sampling

Since only a minute fraction of the tissue could be examined, a rigorous sampling procedure had to be defined.

1. The *primary* sample consisted of some 40 tissue cubes obtained from dicing the thin liver slices. These blocks were derived from three regions of the liver; the remaining liver pieces were used for biochemical analysis. This primary sampling procedure strived for adequate correspondence between the samples subjected to biochemical and to morphometric analysis.

2. From the pooled and mixed blocks of the primary sample prepared for morphometry, five blocks were chosen at random to represent the *secondary* sample. Each of these blocks was sectioned at 1 μ for light microscopy and at 600–900 A for electron microscopy. At low magnification, one technically perfect section from each block was selected for further analysis.

3. The *tertiary* sample consisted of micrographs recorded on the sections of the secondary sample. This sampling stage needed to be further subdivided since micrographs used for stereologic analysis have to meet two requirements: (*a*) they must be large enough to include a representative sample of all structures. (*b*) they must provide sufficient resolution for unambiguous and precise recognition of the finest structures.

Since these two conditions cannot be simultaneously met on one and the same micrograph in a broad study as the present, it was necessary to employ four levels of magnification. Table I summarizes the parameters determined at these levels:

I. Paraffin sections of normal rat liver, derived from similar material, were evaluated at 200 × magnification, by using an automatic sampling stage microscope of Wild Heerbrugg Instruments, Inc., Heerbrugg, Switzerland (14, 15) for sampling of microscope fields.

II. From 1 μ thick sections, cut from the blocks of sampling stage II, light micrographs were recorded at a magnification of 1000. For each animal 10 micrographs (2 from each block) were recorded.

III. Six electron micrographs were recorded on 35 mm film from each section at primary magnification of 2500. Random sampling of these fields was ensured by positioning the fluorescent viewing screen of the microscope into one specified corner of six consecutive squares of the supporting copper grid, as previously described (6). This yielded a tertiary sample of 30 micrographs per animal at this level of magnification.

IV. For higher resolution of cytoplasmic membranes, six electron micrographs were recorded from the same sections at primary magnification of 10,000. The rules for positioning the screen were the same as at level III; however, fields containing less than 50% cytoplasm were discarded, since, at this level, all measurements were to be estimated in relation to hepatocyte cytoplasm. The tertiary sample of this level comprised again 30 micrographs per animal.

The further analysis of the electron micrographs of

[3] The term "specific" is here adopted to mean "relative to 100 g body weight." The term "relative" is reserved for the description of relationship between parameters inside the organ.

levels III and IV followed essentially the procedures previously described (6). The negative film strips were contact-printed on film. These prints were examined in a table projector unit yielding a ninefold secondary magnification. The final magnifications thus were × 22,500 for level III and × 90,000 for level IV. The size of the fields is shown on Fig. 2 in relation to hepatocyte dimensions.

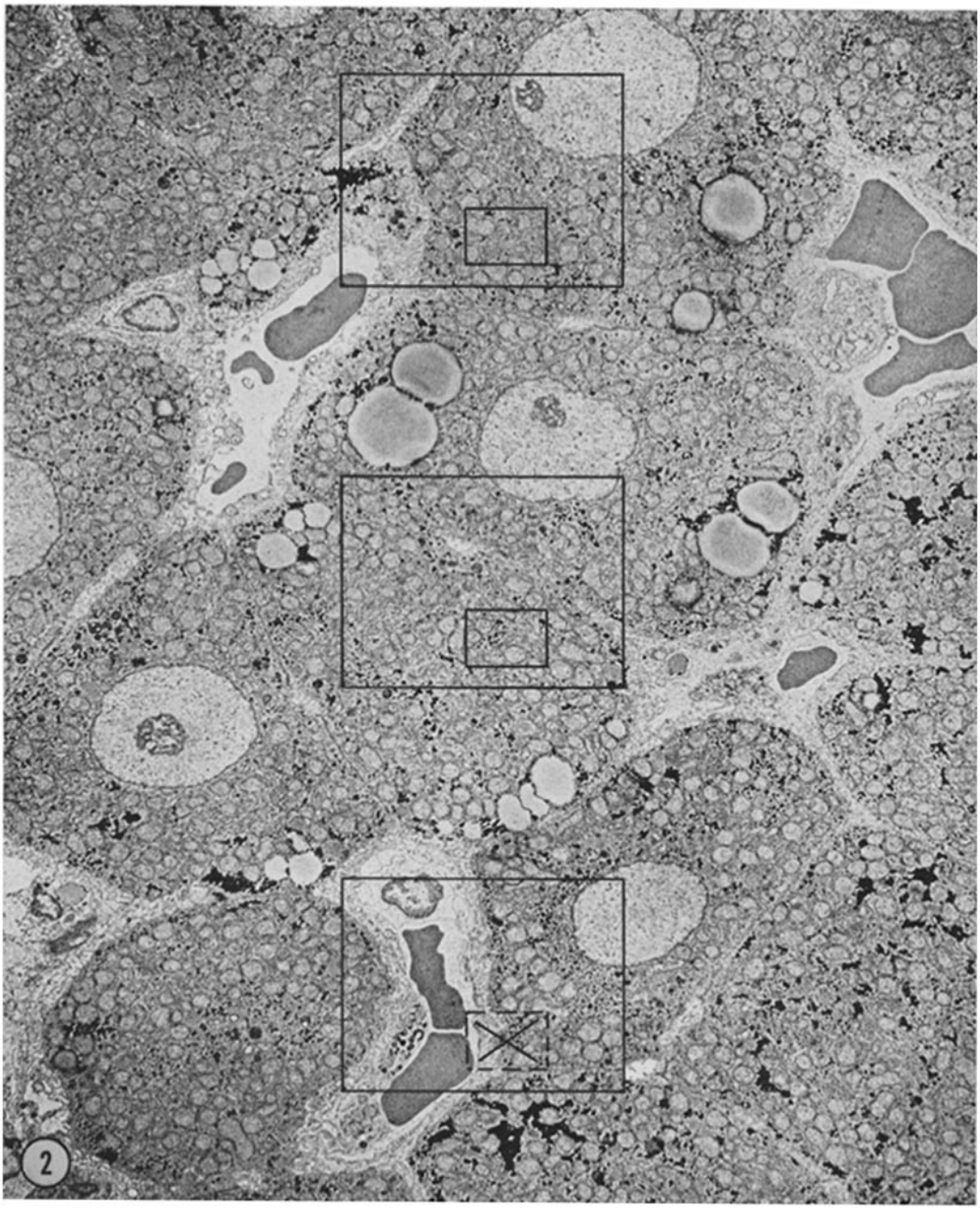

FIGURE 2 Low power electron micrograph of normal rat liver. Three fields of level III and IV (large and small rectangles) are marked; one field of level IV (×) is discarded because it does not contain enough cytoplasm. × 3,000.

TABLE I

Synopsis of Stereologic Procedures

For explanation of symbols see Fig. 1 and Table III.

Level	Test system	Primary counts	Parameters calculated	Equation
I	Point lattice	P_{Pxe}	V_{xe}, extralobular tissue	1
II	Point lattice	P_{Pnh}, P_{Pnx}	N_{Vnh}, N_{Vnx}	3,4
	Frame	N_{Anh}, N_{Anx}		
III	Point lattice	P_{Px}	V_{Vx}	1
		P_{Pn}	V_{Vn}	1
		P_{Pmi}	V_{Vmi}	1
		P_{Pmb}	V_{Vmb}	1
		P_{Pdb}	V_{Vdb}	1
		P_{Poc}	V_V other cytopl. c.	1
	Frame	N_{Ami}	N_{Vmi}	3
		N_{Amb}	N_{Vmb}	3
	Line grid	$I_{Lh(-d,\ -h,\ -b)}$	$S_{Vh(-d,\ -h,\ b)}$	2
IV	Multipurpose	P_{Prer}	V_{Vrer}*	1
		P_{Pser}	V_{Vser}*	1
		I_{Lrer}	S_{Vrer}*	2
		I_{Lser}	S_{Vser}*	2
		P_{Pmi}	V_{Vmi}*	1
		I_{Lmo}	S_{Vmo}*	2
		I_{Lmc}	S_{Vmc}*	5
		P_{Pgs}	V_{Vgs}*	1

* Asterisk marks densities in cytoplasm.

Stereologic Procedures

Principles

The choice of an appropriate measuring technique for morphometric studies is essentially determined by two requirements: (*a*) the precision required for each individual measurement and for the total estimate, (*b*) the time necessary and available for evaluation of the total sample, i.e., the efficiency of the method in relation to the cost of work (6).

In this study, it appeared useless to measure individual profiles of structures with high precision. However, it was important to use a highly efficient method, in order to survey a larger tissue sample. This was achieved by application of stereologic point-counting procedures (6, 16), as follows.

The volumetric density V_{Vi} is estimated by placing a lattice of P_T test points on a section and determining the fraction $P_{Pi} = P_i/P_T$ of these points enclosed within profiles of the structure:

$$V_{Vi} = P_{mi} \qquad (1)$$

The surface density S_{Vi} is directly derived from counts of the intersection points[4] I_i of the surface contour of profiles with test lines of known length L_T (17, 8):

$$S_{Vi} = 2 \times I_{Li} = 2\ I_i/L_T \qquad (2)$$

The numerical density N_V of particulate structures can be estimated from counts of particle profiles in a test area of known size (18. 19):

$$N_{Vi} = \frac{1}{\beta_i} \times \frac{N_{Ai}^{3/2}}{V_{Vi}^{1/2}} \times K \qquad (3)$$

where N_{Ai} is the number of profiles per unit test area[5] or the actually counted number divided by the test area. the coefficient β is shape dependent and has been discussed elsewhere (18). The factor $K > 1$

[4] In previous papers (5, 6, 8) the symbol N was used used for intersection points. To avoid confusion it is replaced by I.

[5] In this paper "unit length" is taken as 1 cm; accordingly, unit area = 1 cm^2, unit volume = 1 cm^3 = 1 ml.

depends on the size distribution of the particles (19); for lack of adequate information it will be assumed to be close to unity in this study, which causes an underestimation by 5–10% of the particle counts (3, 19, 20). In the case of hepatocyte nuclei the size distribution was found to have a coefficient of variation of 14%, so that the coefficient K could be estimated at 1.03 (19).

The size distribution of hepatocyte nuclei was derived from the size distribution of section profiles by applying the transformation of Wicksell for spheres (26, 37), adapting the computer program recently published by Baudhuin (38). This provided a second independent approach for estimating the number of hepatocyte nuclei by the method of De Hoff and Rhines (39):

$$N_{Vn} = N_{An}/\overline{D} \qquad (4)$$

where N_{An} is the number of nuclear profiles counted per unit section area corrected for the loss of small profiles and the effect of section thickness (cf. Fig. 8 *a*), and $\overline{D}$ is the mean nuclear diameter.

Application to Level I

The viewing screen of the automatic sampling stage microscope (14) was fitted with a square lattice containing 100 test points. This allowed estimation of the relative volumes of lobular and extralobular tissue.

Application to Level II

The light micrographs were examined with a test screen containing a frame of known area (19,700 μ^2) and a quadratic lattice of 841 points. This served to determine volume and number of nuclei of hepatocytes and other cells. Hepatocyte nuclei were assumed to be spherical with a shape coefficient $\beta = 1.38$. On the same micrographs the size distribution of profiles of hepatocyte nuclei contained in an area of 28,500 μ^2 was determined by using a Zeiss Particle Size Analyser.

Application to Level III

At the low magnifications of level III the volumetric composition of lobular liver tissue was to be tested. Because of the wide range in the size of structures, a double-lattice grid with a 1:9 ratio (6) was chosen (Fig. 3). The rectangular frame of the electron micrograph enclosed 891 test points, of which 99 were set off as "heavy points." The coarse grid was used for estimating the volume of the gross compartments: extrahepatocytic space, hepatocyte cytoplasm, and nuclei. The volume of mitochondria, which constitute a large fraction of cytoplasm ($\sim$ 25%), could also be estimated with the coarse grid; a preliminary test had shown that use of the fine grid improved accuracy only by about a factor of 2, while the labor involved was ninefold. The fine grid was employed for estimating the volume of the scarcer organelles recognizable at this magnification: microbodies and dense bodies. A frame of known area (103 μ^2) was used for counting profiles of mitochondria and peroxisomes. For calculation of the number of these organelles per unit volume of tissue the shape coefficient β was assumed to be 2.25 for mitochondria, and 1.45 for peroxisomes. This followed from an estimation of mean axial ratios of elliptic profiles which was 1.8 for mitochondria and 1.2 for peroxisomes. Assuming, in first approximation, an ellipsoidal shape for both organelles, it could be roughly estimated that mitochondria should be about 4 times as long as thick, while the long axis of peroxisomes is estimated at about 1.5 times their diameter. It is recognized that this is a rough estimation which nevertheless can give a reasonable approximation of the number of organelles.

In a separate step, a grid of equidistant lines of known length L_T was used to estimate the surface area of hepatocyte cell membrane. Intersections with parts of the membrane adjoining sinusoids ($I_{h\text{-}d}$), biliary capillaries ($I_{h\text{-}b}$), and neighboring hepatocytes ($I_{h\text{-}h}$) were recorded separately to allow an appreciation of hepatocyte polarity.

Application to Level IV

Since the high power electron micrographs of level IV had been centered on cytoplasm (cf. Fig. 2), all measurements were referred to cytoplasmic volume, whose contribution to total liver volume was determined in levels I and III. Three types of measurement were performed: (*a*) volume of smooth and rough endoplasmic reticulum; volume of mitochondria; volume of cytoplasmic ground substance. (*b*) membrane surface of smooth and rough endoplasmic reticulum; surface of outer mitochondrial membrane and of mitochondrial cristae. (*c*) number of membrane-bound ribosomes.

In order to perform measurements (*a*) and (*b*) in a single step, a multipurpose grid with 18 lines of length z (6) was drawn on the screen (Fig. 4).

In order to express all data relative to cytoplasmic volume, L_T had to be defined as test-line length enclosed in cytoplasm (L_{Tc}), which was estimated from the number of end points P_c enclosed in cytoplasm:

$$L_{Tc} = \tfrac{1}{2}\, P_c \cdot z$$

To facilitate measurement of mitochondrial mem-

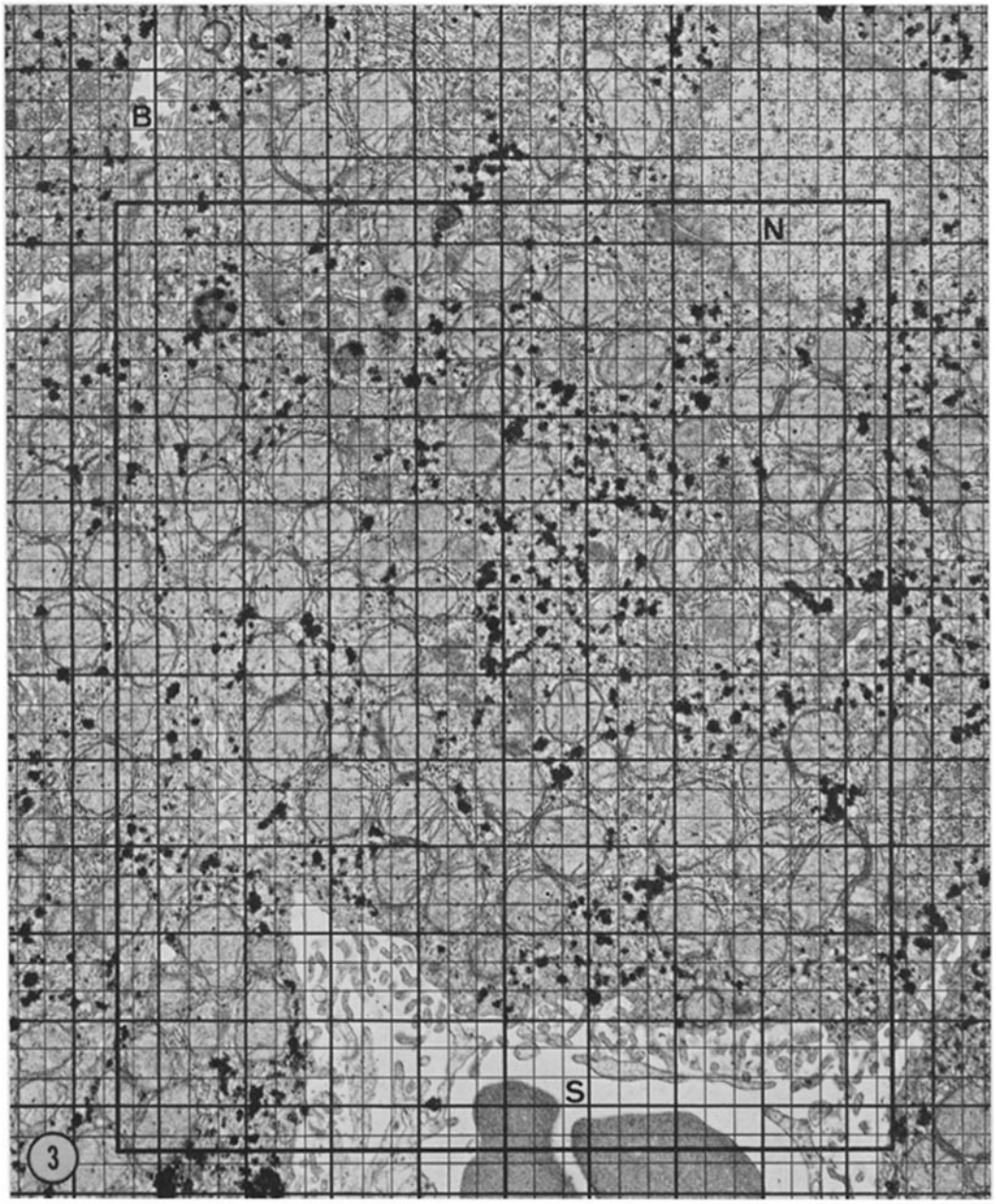

FIGURE 3 Typical micrograph characterizing the sample unit at level III, superimposed with test screen in form of double-period square lattice. Heavy frame delimits actually analyzed area which includes 891 points, of which 99 are set off as "heavy points." Note that a portion of a sinusoid (*S*) and of a nucleus (*N*) is included, while the biliary capillary (*B*) is, here, outside the field. × 12,500.

branes intersections with cristae were counted as a single point (I_{mc}), although two membranes were involved (Fig. 5); similarly, intersections of the test lines with envelope and adjoining parts of the inner membrane were recorded as a single intersection (I_{mo}).

This necessitated appropriate modification of the formula for calculation of surface density of inner

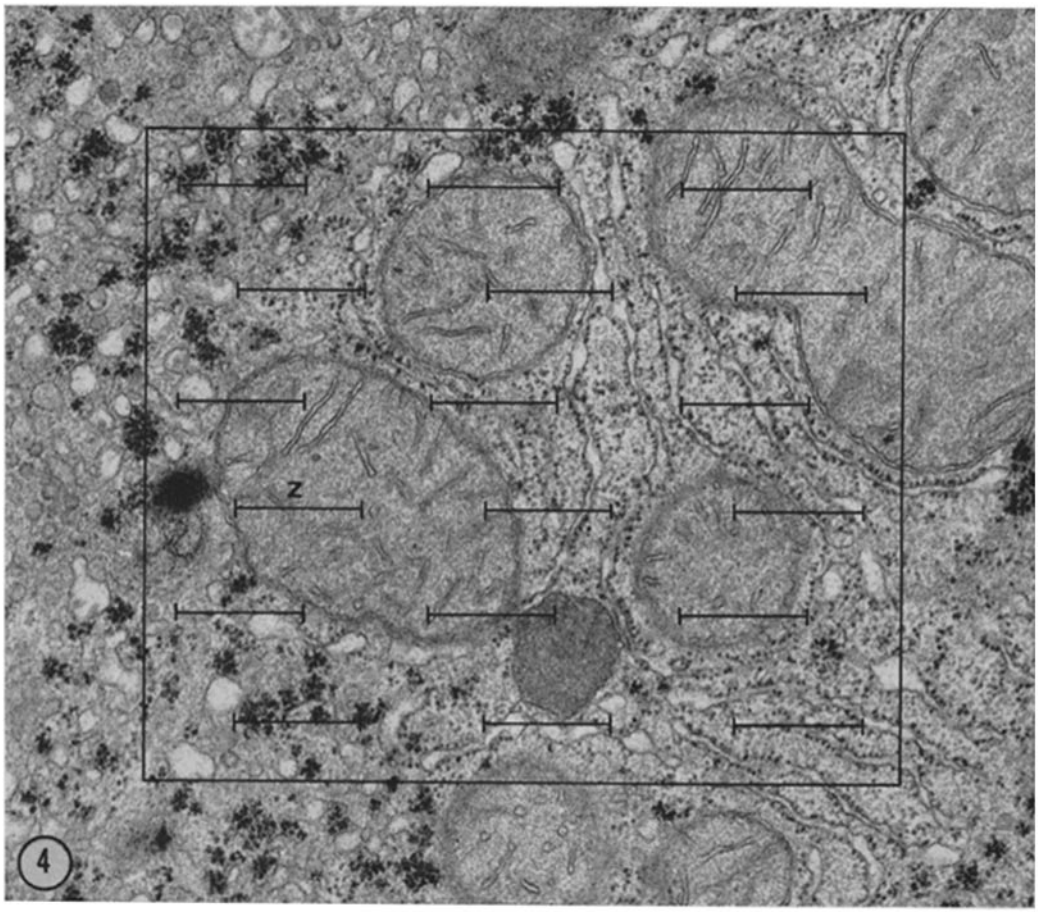

FIGURE 4 Electron micrograph of level IV with multipurpose test screen with 18 test lines of length z enclosed iin frame. Their 36 end points serve as markers for volume estimations. × 37,500.

membrane with cristae (S_{Vmc}):

$$S_{Vmc} = \frac{2 \cdot I_{mo} + 4 \cdot I_{mc}}{L_T} \qquad (5)$$

The number of bound ribosomes was determined by an indirect method: on the electron micrographs of level IV, profiles of rough endoplasmic reticulum with clearly discernible membrane were randomly selected. The contour length of the membrane was measured and the number of ribosomes attached was counted. It must be assumed that the ribosomes counted were attached to a strip of membrane extending through the section thickness of 600–900 A. On the basis of our mode of selection the width of this strip was estimated to be approximately 850 A. This allowed estimation of the number of ribosomes attached to 1 μ^2 of rough ER membrane.

Determination of Number and Size of Hepatocytes

The number of hepatocytes in the unit volume of liver N_{Vh} was estimated from the numerical density of hepatocyte nuclei N_{Vn} obtained in level II. This indirect approach was adopted because nuclei are more easily counted than whole cells.

The average volume of an individual hepatocyte $\bar{v}_h$ was estimated by dividing V_{Vh} by N_{Vn}; hence, $\bar{v}_h$ represents the volume of cell attributable to one nucleus and does not consider binucleated cells. As a further estimate of cell size the measurements of surface area of the hepatocyte membranes in the unit tissue volume S_{Vh} was used to calculate the average diameter $\bar{d}$ of hepatocytes from the volume-to-surface ratio

$$v/s = V_V/S_V \qquad (6)$$

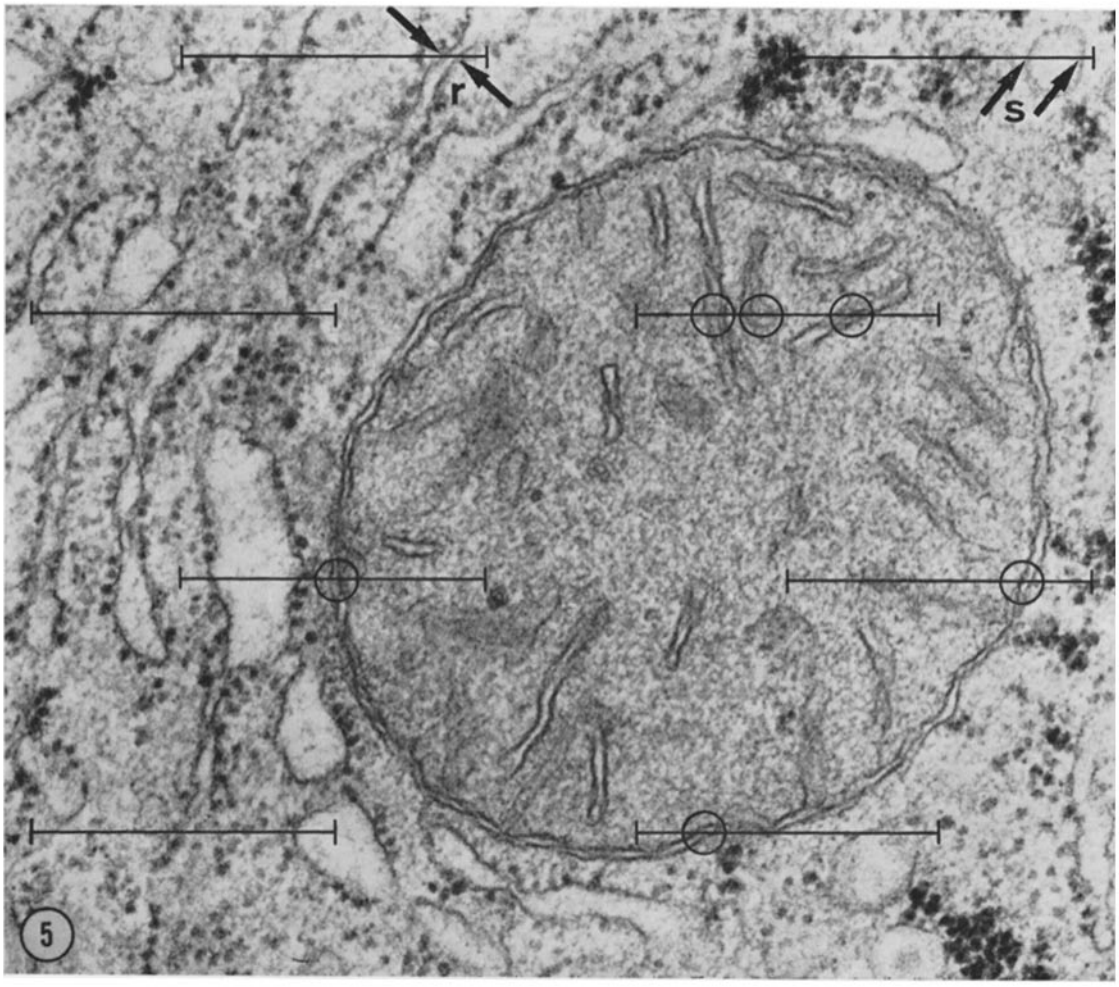

FIGURE 5 Actual size of projected micrograph of level IV with mitochondrion and ER cisternae; 8 test lines are indicated. Intersections of test lines with mitochondrial membranes are encircled; note that double intersection of cristae and "outer" membranes are counted as one point (compare text). Arrows indicate intersections of test lines with rough (r) and smooth (s) ER membranes.

Assuming the shape of the hepatocyte to be similar to either a cube or a sphere it can easily be shown that $\bar{d} = 6 \cdot v/s$ (21); $\bar{d}$ designates mean diameter, with respect to a sphere, and mean side length for a cubic model.

In addition, the membrane surface of the individual hepatocyte is estimated by division of S_{vh} by N_{vh}.

CALIBRATION

The magnification of electron micrographs and the size of test line and of test area were calibrated by means of a carbon grating replica having 28,700 lines per inch. Extreme variations were less than $\pm$ 5% of the mean.

DATA RECORDING AND COMPUTATION

The primary data were recorded as differential counts in ten electrical counters fed from a keyboard. The original device (6) has been considerably improved (22), in that transfer of data onto tables and onto punch cards is automatic. The counts were tallied over one micrograph (unit of sampling stage 3); before advancing the film by one frame, a push-button triggered the transfer of data onto table and punch cards. This greatly improved efficiency: analysis of ten parameters on one film with 30 electron micrographs, corresponding to one animal, was performed in less than 2 hr. With the primary data on punch cards, the subsequent calculation of param-

eters, including statistical analysis, was performed by computer.

The computer program used for this study and for the accompanying paper (9) formed part of a general-purpose morphometric computer program the details of which will be presented elsewhere.[6] It is composed of three parts:

1. Computation of the characteristic parameters (Table I) from the primary data averaged over the 30 micrographs of the tertiary sample per animal.

2. For 14 basic parameters specific values, related to 100 g body weight, are calculated. The values for individual animals are averaged per experimental group, and the standard deviation of the group mean (standard error, ± SE) is calculated.

3. The last part of the program performs a statistical comparison of group averages of the specific parameters by means of Student's t test; this is used for evaluation of experimental data as in the accompanying paper (9).

Systematic Errors of Measurement

Two basic sources of systematic error must be expected to affect these data. Compression of the tissue by sectioning caused a reduction of section length by about 10%, while the width remained unchanged. Since Loud (23) had shown that this effect is, at least in part, compensated by optical distortion of the electron micrograph, no correction was introduced. For comparative work (9) such a correction is unnecessary since it can be assumed that all experimental groups are affected by the same degree of systematic errors.

In several instances the effect of section thickness on the primary data had to be taken into account.

(*a*) Nuclear profiles were counted and measured on sections 1 μ thick. The errors introduced by the ratio of section thickness to mean diameter of about 1:8 had to be corrected since a systematic error in N_{Vn} would have seriously affected the computation of "dimensions per hepatocyte." Section thickness was estimated to cause an overestimation of N_{An} by 11%; on the other hand, nuclear profiles with a diameter $d < 3.4$ μ were not recognizable which resulted in a deficit of 10% in the profile counts; the two errors in N_{An} canceled so that no correction was necessary. The error affecting V_{Vn} due to section thickness amounted to an overestimation by some 16% which was partially compensated for by failure of recognizing small profiles; it was estimated that the residual error could be corrected by reducing the observed V_{Vn} by a factor of 0.9. The Wicksell transformation used in this study (37, 38) includes a correction for both section thickness and loss of small profiles (Fig. 8 *a*).

(*b*) The diameter of ribosomes (~ 150 A) is about 1/5 of the average section thickness of 750 A. The observed counts were overestimates and were corrected, according to Abercrombie, by a factor of 0.83 (cf. reference 28).

(*c*) Membrane structures such as cisternae of endoplasmic reticulum and mitochondrial cristae are difficult to recognize when they are sectioned at an angle of less than 45°. This is essentially due to Holmes effect (cf. references 24, and 8 p. 96), since the translucent cisternal and cristal spaces are obscured by the denser cytoplasmic matrix. A recent study by Loud (36) has shown that up to 1/3 of membrane profiles may be "lost" by Holmes effect. The data on membranes presented in tables are uncorrected because of uncertainty in the degree of error; estimated correction factors are indicated where they are relevant.

MATERIAL

The five animals used in this part of the study served, at the same time, as controls for the investigation of changes induced by phenobarbital (9) and were hence treated in the same fashion. They were male albino rats (Wistar-derived) from a closed breeding colony, 8–9 wk old, each from a different litter, fed *ad libitum* with standardized laboratory chow, and *fasted* for 24 hr before sacrifice. The weight of these animals is given in Table II.

RESULTS

Size of Liver

With an average body weight of 173.6 g the liver volume of these animals amounted to 5.84 ml (Table II). The specific liver volume, de-

TABLE II

Basic Data on Control Rats, 8–9 wk Old

Animal No.	Body wt	Liver wt	Liver volume	Specific liver volume
	g	*g*	*ml*	*ml/100 g body wt*
1	163	5.93	5.84	3.41
2	174	6.14	5.76	3.30
3	178	6.49	6.11	3.42
4	179	6.16	5.77	3.22
5	174	6.39	5.99	3.44
Mean	173.6	6.22	5.84	3.36
Coefficient of variation, %	3.7	3.6	3.7	2.8

[6] Gnägi, H. R., H. Giger, and E. R. Weibel. General-purpose stereologic computing system. In preparation.

fined as volume per 100 g body weight, was 3.36 ml on the average. The coefficient of variation of this basic measurement is 2.8%, and thus smaller than variation in total liver volume even in this quite homogeneous group of animals.

Composition of Liver Tissue and Extrahepatocytic Space

Table III presents a synopsis of all the data obtained in this study. Light microscopic studies (level I) revealed that 96% of rat liver volume was lobular parenchyma, i.e., hepatocytes, sinusoids, Disse space, and biliary capillaries; the remaining 4% included portal triads, hepatic and central veins.

The sample investigated morphometrically by electron microscopy included only lobular tissue. Of this, 87% was made up of hepatocytes, while sinusoids, perisinusoidal space, and biliary capillaries amounted to 13%. In relation to total liver volume, it must thus be derived from these data that hepatocytes occupy 83% of the volume (Fig. 6).

No further subdivision of extrahepatocytic space was attempted in this study; however, it was estimated to contain some 90×10^6 cell nuclei per 1 ml of tissue.

Number and Size of Hepatocytes

The number of hepatocyte nuclei per 1 ml of liver tissue, computed according to eq 3, amounted to 169×10^6 (SE $\pm 9 \times 10^6$). The specific volume of liver was thus found to contain 568×10^6 ($\pm 28 \times 10^6$) hepatocyte nuclei, on the average (Table III). The variation among individual animals was rather small (Fig. 7).

The number of hepatocyte nuclei derived by equation 4 was 164×10^6 for N_{Vn} and 551×10^6 for the specific number when computed from the pooled data of all five animals (Fig. 8). When the number of nuclei was computed for the individual animals this method yielded considerably larger variations, which is probably due to a high sensitivity of the Wicksell transformation to statistical errors in the observed profile size distribution.

Fig. 8 shows the size distribution of nuclei as derived by Wicksell transformation from the pooled data. With a mean $\bar{D} = 7.93\ \mu$ the nuclear diameters vary between 5 and 11 μ, the variance being $\pm 1.12\ \mu$. The mean nuclear volume is here estimated at 277 μ^3. Estimation of mean nuclear volume from V_{Vn} obtained by point counting and N_{Vn} yielded a nuclear volume of 299 μ^3 (SE $\pm$ 16 μ^3).

Since it has not been possible in this study to determine the relative number of binucleated cells, the term "hepatocyte" will henceforth be understood to mean "cell portion related to one nucleus," or "mononuclear hepatocyte." This appears acceptable since binucleated hepatocytes are generally larger than the mononucleated cells, because of the constancy of nucleo-cytoplasmic volume ratio (10, 25).

The mean volume of the individual hepatocyte was found to be 4940 μ^3 (Fig. 7). This forms the basis for computation of the absolute amount of organelles in the individual hepatocyte, as given in the last column of Table III. The surface area of all hepatocytes in the unit volume of liver tissue measures 0.28 m^2; hence, that of the individual hepatocyte (Fig. 9) averages 1680 μ^2 ($\pm 70\ \mu^2$).

The average diameter of hepatocytes, as derived from the volume-to-surface ratio, was found to be 17.7 (SE $\pm$ 0.8) μ. This fits well with the diameter of 17 μ for a cube of volume 4940 μ^3. These two measurements are largely independent and hence provide a check on the consistency of the data.

Polarity of Hepatocyte

The hepatocyte membrane is in contact with three different compartments: perisinusoidal space, biliary capillary, and neighboring hepatocytes. The fraction of membrane surface abutting on these compartments provides a measure of polarity of the hepatocyte. As shown in Fig. 9, 50% of hepatocyte surface was found to be adjoining on other hepatocytes, while the sinusoidal side occupied 37%, and the biliary capillary wall 13% of the membrane area. The two functional surfaces of the hepatocyte have thus a ratio of 3:1 and comprise half the cell surface. It must be noted though that the method of measurement employed did not account for the surface enlargement by formation of microvilli in biliary capillary and perisinusoidal space. In absolute terms the actual membrane surface present in these locations is estimated to be approximately three times larger than here indicated. It can thus be estimated that about 2000 μ^2 of the actual hepatocyte membrane is in contact with the Disse space, and 700 μ^2 with the biliary capillary.

TABLE III

Normal Values of Morphometric Parameters for Rat Liver Uncorrected for Systematic Errors

Component	Parameter	Symbol	Density per 1 ml of tissue: Mean	Density per 1 ml of tissue: SE	SE in % of mean	Dimension	Specific value per 100 g body wt: Mean	Specific value per 100 g body wt: Dimension	Value per "hepatocyte" (mononuclear)
Liver	Volume	V_L					3.36	cm^3	
Lobular parenchyma	Volume	V_P	0.96	0.005	0.5	cm^3/cm^3	3.22	cm^3	
Extrahepatocytic space	Volume	V_x	0.169	0.005		cm^3/cm^3	0.57	cm^3	
Extralobular	Volume	V_{xe}	0.04		10	cm^3/cm^3	0.14	cm^3	
Intralobular	Volume	V_{xi}	0.129	0.012	10	cm^3/cm^3	0.43	cm^3	
Nuclei	Number	N_{nx}	92×10^6	9×10^6	10	cm^{-3}	309×10^6		
Hepatocytes	Volume	V_h	0.831	0.012	1.5	cm^3/cm^3	2.79	cm^3	4940 μ^3
	Surface	S_h	0.284	0.014	5	m^2/cm^3	0.96	m^2	1680 μ^2
Nuclei	Volume	V_n	0.050	0.0025	5	cm^3/cm^3	0.17	cm^3	300 μ^3
	Number	N_n	169×10^6	9×10^6	5	cm^{-3}	568×10^6		1
Cytoplasm	Volume	V_c	0.771	0.013	1.7	cm^3/cm^3	2.59	cm^3	4640 μ^3
Cytoplasmic ground substance	Volume	V_{gs}	0.444	0.017	4	cm^3/cm^3	1.49	cm^3	2630 μ^3
Endoplasmic reticulum	Volume*	V_{er}	0.128	0.0088	7	cm^3/cm^3	0.427	cm^3	756 μ^3
	Surface*	S_{er}	10.90	0.70	6	m^2/cm^3	36.5	m^2	63000 μ^2
Rough ER	Volume*	V_{rer}	0.0785	0.0069	9	cm^3/cm^3	0.264	cm^3	467 μ^3
	Surface*	S_{rer}	6.25	0.52	8	m^2/cm^3	20.93	m^2	37900 μ^2
Bound ribosomes	Number*	N_{rib}	20.9×10^{14}	2.1×10^{14}	10	cm^{-3}	70×10^{14}		12.7×10^6
Smooth ER	Volume*	V_{ser}	0.049	0.0050	10	cm^3/cm^3	0.163	cm^3	289 μ^3
	Surface*	S_{ser}	4.65	0.55	12	m^2/cm^3	15.61	m^2	25100 μ^2

Mitochondria	Volume	V_{mi}	0.181	0.0045	2	cm^3/cm^3	0.66	cm^3	1170 μ^3
	Number	N_{mi}	280×10^9	10×10^9	3	cm^{-3}	941×10^9		1665
Envelope	Surface	S_{mo}	1.46	0.04	3	m^2/cm^3	4.91	m^2	7450 μ^2
Inner membrane + cristae	Surface‡	S_{mc}	5.88	0.51	9	m^2/cm^3	19.74	m^2	34800 μ^2
Microbodies	Volume	V_{mb}	0.0116	0.0006	5	cm^3/cm^3	0.038	cm^3	67 μ^3
	Number	N_{mb}	62×10^9	9×10^9	14	cm^{-3}	209×10^9		370
Dense bodies	Volume	V_{db}	0.0068	0.00044	6	cm^3/cm^3	0.023	cm^3	41 μ^3

* Underestimates by 20–30%; approximate correction factor 1.25.

‡ Underestimate by 50%; approximate correction factor 1.5.

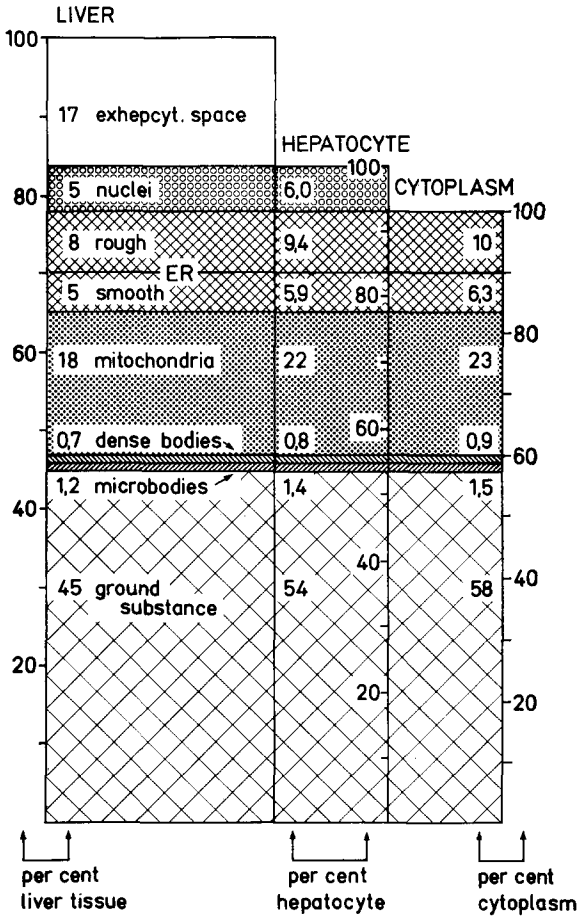

FIGURE 6 Volumetric subdivision of liver tissue, hepatocyte, and hepatocytic cytoplasm.

With regard to the specific rat liver volume of 3.36 ml the sinusoidal surface is of the order of 0.34 m^2, with over 1 m^2 of hepatocyte membrane adjoining. The totality of biliary capillaries in the specific liver volume have a bounding membrane surface of the order of 0.3 m^2.

Subdivision of Hepatocyte

In Fig. 6 the finer subdivision of liver space, hepatocyte, and hepatocyte cytoplasm[7] is graphically represented. More than 50% of the hepatocyte is made up of cytoplasmic ground substance, while nuclei occupy only 6%. The mitochondrial space amounts to 22%, while dense bodies and microbodies jointly contribute about 2%. Some 16% of cytoplasm is made up of cisternae of the endoplasmic reticulum (including Golgi elements), of which about 2/3 appear in the rough form. Table III presents the concentration of these components in total liver tissue, as well as their absolute dimensions per specific liver volume and per individual hepatocyte.

Endoplasmic Reticulum

Figs. 10 and 11 show the individual measurements of volume and surface density of endoplasmic reticulum in cytoplasm, as they were obtained directly from stereologic analysis at magnification level IV (Table I). Each point refers to one animal, i.e., to an analysis of 30 electron micrographs from five tissue blocks. The scatter of the data is due to individual variations and to sampling error. As indicated by the vertical bar the standard error is less than 10% of the mean for all parameters, except for the surface density of smooth membranes, where it amounts to 12%.

In every case the volume of rough ER ex-

[7] For convenience, "cytoplasm" will henceforth be used to mean "hepatocyte cytoplasm."

ceeded that of smooth ER (Fig. 10). The same could be said for the membrane surface, except for one case in which smooth ER membranes were comparatively high (Fig. 11). The mean volumetric density of rough ER cisternae in cytoplasm was 0.10, that of smooth ER 0.061; this difference is statistically significant with an error probability of $P < 0.05$. The mean surface density of membranes in cytoplasm is 8.1 μ^2/μ^3 and 6.0 μ^2/μ^3 for rough and smooth ER, respectively; this difference does not reach a satisfactory level of significance ($P < 0.1$).

The volume-to-surface ratio (v/s) allows estimation of the average width of ER cisternae: for broad flat bags, as rough ER cisternae, it measures the mean half-distance between the membranes ($v/s = d/2$); if the structure is tubular, as is the case for smooth ER, it measures half the radius ($v/s = d/4$). For rough ER, v/s was found to be 131 A on the average, yielding a mean cisternal width of 260 A. For smooth ER, v/s = 107 A; the tubules have thus an average diameter of 430 A. These two values agree quite well with direct measurements performed on electron micrographs, where rough ER cisternae appear 200–300 A wide and smooth ER profiles have a diameter of the order of 300–600 A.

The average number of ribosomes found attached to 1 μ^2 of rough ER membrane is given in Fig. 11 for the individual animals; the group mean was 334 μ^{-2} (SE ± 18).

The total surface of endoplasmic reticulum in the specific liver volume (relating to 100 g body weight) is 37 m^2 (Table III), of which 21 m^2 represents ribosome-studded rough membranes and 16 m^2 represents smooth membranes. The space bounded by these membranes amounts to 0.43 ml for total ER, and the number of attached ribosomes is estimated at 70 × 10^{14} in the specific liver volume. The individual mononuclear hepatocyte is estimated to contain some 63,000 μ^2 of ER membranes to which 12.7 million ribosomes are attached.

As mentioned under Methods, the measurements here obtained for endoplasmic reticulum are systematically underestimated due to Holmes effect. It is roughly estimated that the values of ER membrane surface and ribosomal number

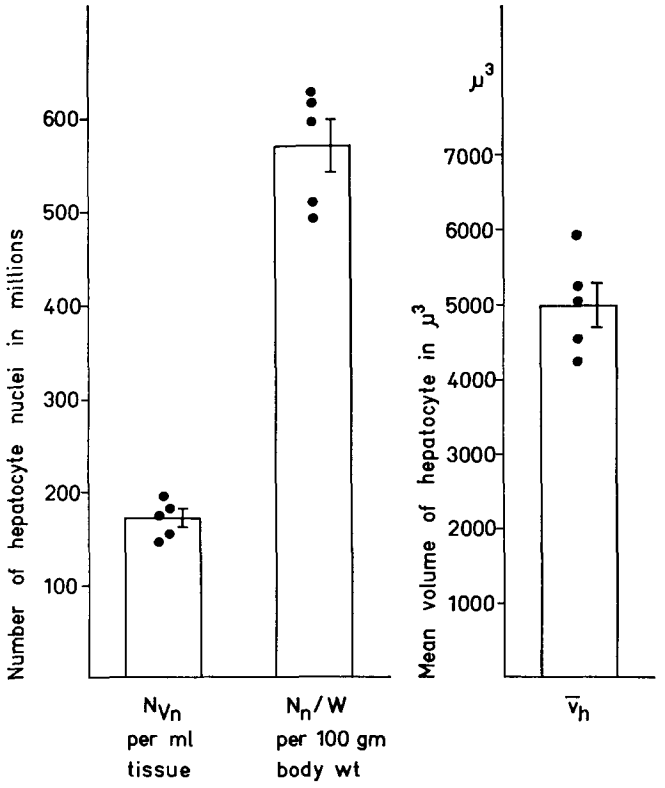

FIGURE 7 Number of hepatocyte nuclei per unit tissue volume and per specific liver volume, and mean volume of hepatocyte. Dots represent individual measurements, columns the group mean, and brackets ± 1 standard error.

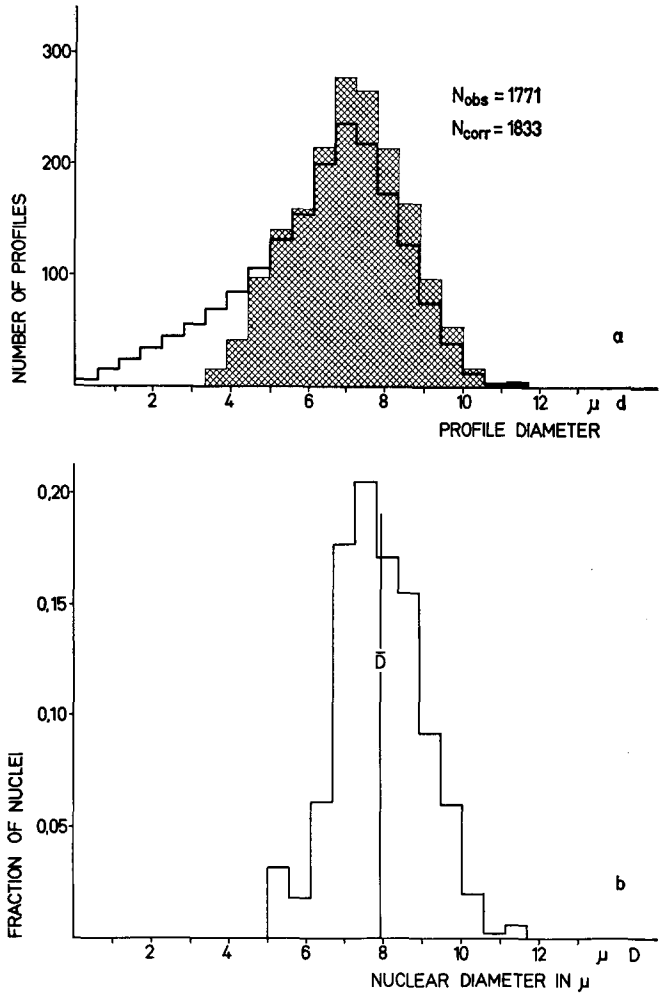

FIGURE 8 Size distribution of nuclear profiles and nuclei of hepatocytes. Data pooled for all five rats. (*a*) Distribution of nuclear profile diameters; shaded area represents observed sizes, heavy line the profile distribution corrected for effect of section thickness and loss of small profiles ($d < 3.4\ \mu$). (*b*) Distribution of nuclear diameters obtained by Wicksell transformation (38, 39).

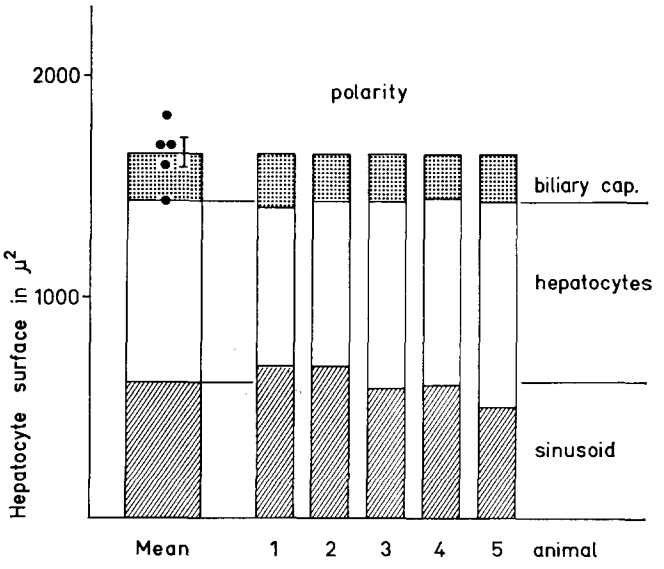

FIGURE 9 Surface area of average hepatocyte with estimate of polarity in terms of fractional sinusoidal and biliary surfaces, as well as contact area with neighboring hepatocytes. Note that surface enlargement by microvilli etc. is not considered.

must thus be increased by a factor of about 1.25 to yield "true" dimensions; but it should be emphasized that the estimated ribosomal number has to be taken with caution, since a number of assumptions were necessary to arrive at this value.

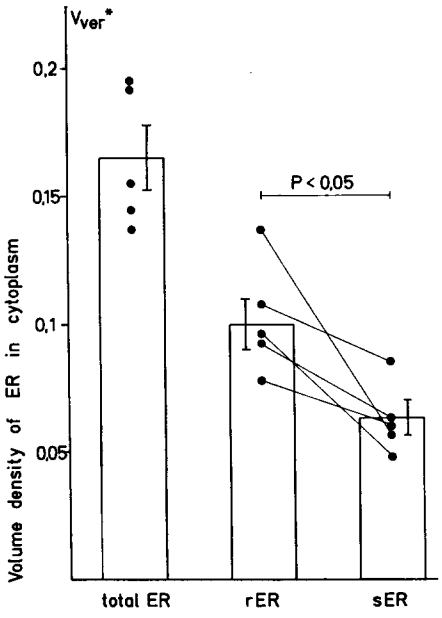

FIGURE 10 Volume density of endoplasmic reticulum in hepatocyte cytoplasm.

Mitochondria

The volume density of hepatocyte mitochondria in liver tissue amounts to 18% (Figs. 6 and 12). The scatter of individual measurements is small (Fig. 12), as expressed in the standard error of only 2% of the mean. The numerical density $N_{V_{mi}}$ was found to be 280 × 10^9 per ml of liver, or 940 × 10^9 per specific liver volume. The membrane surface of mitochondrial envelope measured 1.5 m^2 in the unit volume of tissue, and some 6 m^2 for the inner membrane with cristae; the latter value is probably underestimated by at least 30% (see Methods).

The individual hepatocyte was estimated to contain 1665 mitochondria on the average (Table III); their total volume amounts to 1070 μ^3, which corresponds to 22% of hepatocyte volume (Fig. 6).

The mean mitochondrial volume is estimated at 0.65 μ^3 (Fig. 12), with a standard error of 3% of the mean. Assuming a cylindrical shape of these mitochondria and an average diameter of 0.6 μ, as estimated from direct measurements of the short axis of elliptic profiles on section, the average mitochondrion is about 2.4 μ long. The surface-to-volume ratio of such a cylindrical structure would be 7.5 μ^{-1}, which fits well with the measured ratio of envelope surface to mitochondrial volume of 8.1 μ^{-1}.

The surface area of the inner membrane was

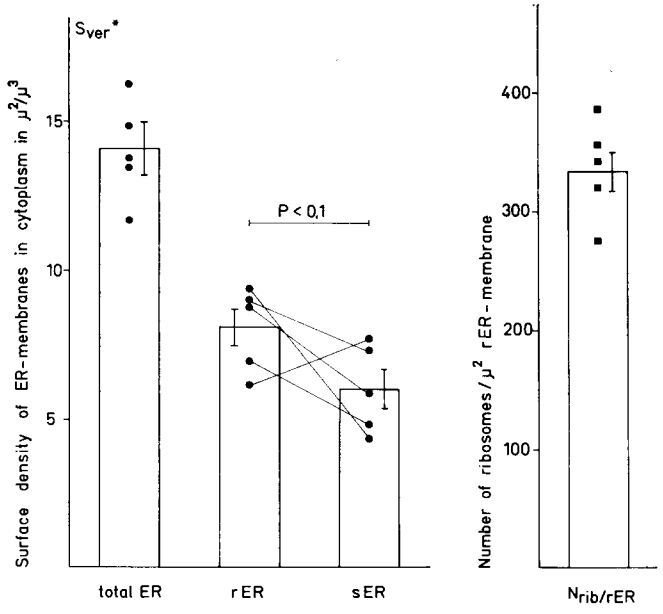

FIGURE 11 Surface density of endoplasmic reticulum membranes in hepatocyte cytoplasm, and number of bound ribosomes per 1 μ^2 of rER membrane.

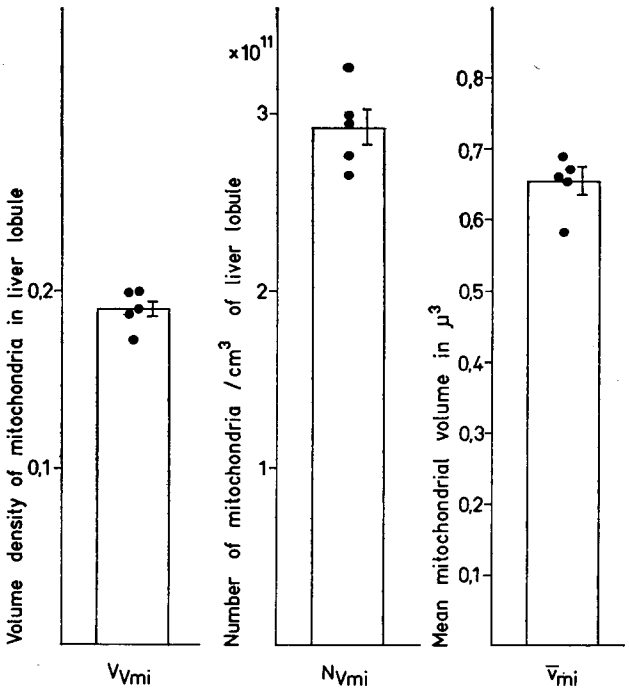

FIGURE 12 Volumetric and numerical density of mitochondria in lobular liver tissue, and mean mitochondrial volume.

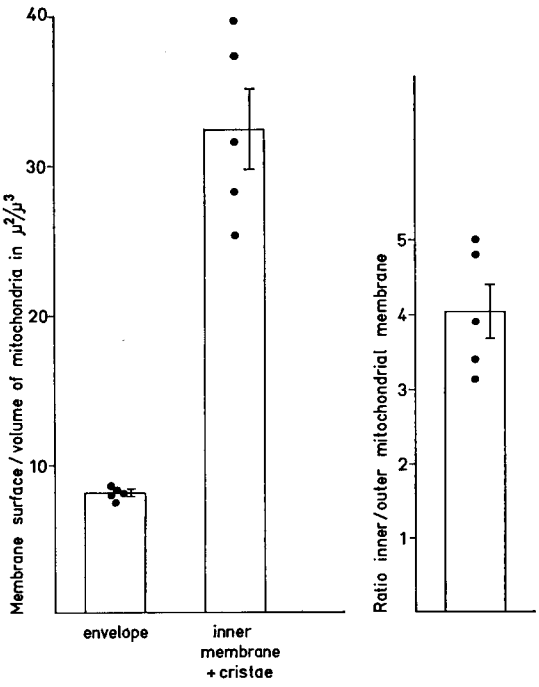

FIGURE 13 Estimates of mitochondrial membrane surface.

found to be, on the average, four times larger than that of the envelope (Fig. 13). The wide scatter of these data is due to the difficulty of measurement of cristae on comparatively thick sections. It is thus likely that the actual surface of inner membrane with cristae is at least 1.5 times larger than here estimated. If this correction is applied to the value of 35,000 μ^2 of inner membrane found per hepatocyte (Table III), it is observed that the total surface areas of inner mitochondrial and endoplasmic reticulum membranes are of the same order of magnitude.

Microbodies and Lysosomes

It is found that one hepatocyte contains about 370 microbodies which contribute some 1.5% to its cytoplasmic volume (Fig. 6 and Table III). Dense bodies make up less than 1% of cytoplasm. Because of large variation in shape and size of dense bodies, their number was not counted. By comparison with microbodies it can, however, be estimated that one hepatocyte may contain 200–300 dense bodies, so that 1 ml of liver tissue would hold approximately 40 × 10^9 of these granules.

DISCUSSION

Appraisal of the Method

The quantitative analysis of tissue sections has the advantage of revealing the topographical relationship between components in its integrity. A properly prepared section obtained through a rigorous sampling procedure will therefore represent a reliable unbiased sample of tissue composition, so that application of stereological techniques can be expected to yield results representative of actual tissue composition, at least within the range of methodical errors discussed below. Baudhuin and Berthet (26) have recently proposed to use sectioned pellets of subcellular fractions, e.g. mitochondria, instead of sections of whole tissue for quantitative studies by methods similar to those used here. It is true that the population of particles studied was more homogeneous than in native liver tissue and probably represented a good random sample. However, it still remains to be established how well a subcellular fraction relates to whole tissue; part of the discrepancies to our results may be explained by a considerable loss of mitochondria in the course of preparation, as will be discussed. Furthermore, homogenization appears to lead to a fragmentation or distortion of these organelles, artifacts which can be avoided to a large extent in careful preparation of intact tissue blocks.

One of the major problems encountered in this type of study relates to the choice of proper sample size. In order to be representative of tissue composition in all its heterogeneity, the section area investigated must be large enough to include the entire distribution of structures; precise recognition and measurement of cytoplasmic organelles, on the other hand, demands high magnification which drastically limits the field of observation. The stepwise sampling procedure employed in this study ensured that the total sample investigated was representative for all phases of liver tissue, while a wide range of magnifications provided adequate resolution for precise measurement of structures. Admittedly, higher precision could have been obtained by using still larger sample sizes, but it was judged preferable at this stage to obtain comprehensive data on a large number of parameters and to accept a certain degree of statistical variation.

Efficiency of the analysis was achieved by three measures. Firstly, the size of the sample was kept small at each level. Nevertheless, the data obtained on five animals were consistent, with standard errors ranging between 1 and 10% for most parameters.

Secondly, point-counting methods of analysis were adopted for measuring. Compared to lineal integration methods, as used by Loud (1, 23, 27), the individual determination has smaller accuracy; however, it is performed much faster so that a larger sample can be evaluated in the same course of time. The ratio of reliability of measurement to effort is therefore more favorable in point-counting analysis.

Thirdly, the analytic procedure was partially automated (22). Analysis of the micrographs is left to the experienced investigator. However, with proper choice of test systems (6), he has merely to decide on the classification of "point traces" generated by confrontation of micrograph and test system. After recording his decisions by means of a simple keyboard-operated data collector, the investigator can rely on fully automated tabulation of data and computation. It should be noted that this procedure, once established, allowed stereologic analysis of the entire range of parameters here presented to be performed in approximately 10 hr per animal. This does not include time for preparation of tissue and recording of micrographs.

The parameters determined in this study were limited to average contents of the liver in organelles etc., and to average sizes of particles. The aim of this study was to provide a broad base line for comparative biochemical and morphometric studies of livers under experimental conditions (9).

Particle size distributions appeared of secondary interest in the context of this study, except for hepatocyte nuclei in which case it was hoped that changes in nuclear size distribution would reflect shifts in the degree of ploidy under the effect of phenobarbital (9). In extension of this project it would also be of interest to determine size distributions of cytoplasmic organelles, although serious difficulties may be encountered particularly with mitochondria; this would help to eliminate part of the uncertainties in some assumptions made in determining particle numbers and sizes.

This type of study is affected by a number of systematic errors which have been extensively treated elsewhere (3, 5, 6, 8, 24). Insofar as they

affect the present investigation, these errors have been given proper consideration in the course of presentation of the methods; where needed, appropriate corrections of the results were explicitly introduced. Some results presented in table form are, however, uncorrected since corrections were based on crude estimations only; rough correction factors are indicated. The most important systematic error related to a loss of endoplasmic reticulum membranes and of mitochondrial cristae of about 30% due to finite section thickness. Because of section thickness some tangential sections of particles may have been missed; this is, however, at least in part, compensated for by the opposite effect of systematic overestimation of nonequatorial sections due to Holmes effect. Nevertheless, the numbers of mitochondria and microbodies may be somewhat underestimated.

Discussion of the Model

In order to allow synthesis of the detailed results a simplified hierarchic model of liver parenchyma with a hepatocyte situated between sinusoid and biliary capillary was introduced. No attempt was made in this study to further localize the hepatocytes within the lobule or within the entire liver. We have purposely sampled the tissue at random, since averages derived from a true random sample will best compare to biochemical data obtained on liver homogenates. Loud (7) has recently estimated that the liver is at least 80% homogeneous with respect to subcellular composition in spite of sublobular variations.

Form of Presentation of Results

The stereologic analysis performed in this study yields, as basic data, concentration parameters: e.g., the volume of a compartment or the number of particles in the unit tissue volume. These data are quite useful since they allow easy calculation of the amount of a given parameter in any aliquot of liver tissue. For presentation of the data we have chosen 1 ml as the standard unit volume throughout this study.

In the subsequent experimental study (9) we have found it useful to express the data as "specific dimensions," i.e., in relation to a standardised body weight of 100 g. This eliminates variations in body size of the experimental animals and is justified because, in the normal animal, the ratio of liver to body weight is constant, at least within age groups (10).

Discussion of Results

Among the few morphometric studies on the liver cell the articles by Loud and co-workers (1, 7, 23, 27–29) are most directly comparable since they are based on an essentially similar analytic method. The main differences between these studies and the present ones are (*a*) that Loud used the method of lineal analysis, while we relied essentially on point-counting procedures; (*b*) that Loud obtained his sample by a combined procedure of random sampling and selection, whereas here a strictly systematized random sampling in hierarchic stages is considered essential. These approaches will yield results differing in some detail but still comparable on a broad basis.

If the data in Loud's studies are converted to units comparable with those used here, good agreement is observed for most parameters. The number of hepatocyte nuclei per unit tissue volume is estimated at 154×10^6 as compared to our 169×10^6, their mean volume at 280 and 298 μ^3, respectively.

Loud (7) estimates that the volume of one parenchymal cell is 5,400 μ^3 as compared to our 4940 μ^3; this difference is related to the difference in nuclear numbers.

In this study the mitochondrial volume was found to be 18.6% of lobular tissue volume, which compares well with Loud's earlier data (1, 23). This value appears to be somewhat higher than that in Loud's recent paper (7) in which he estimated that the volume density of mitochondria in cytoplasm varies from 13 to 20% between the three sublobular zones considered. If weighing factors for these zones, as given by Loud, are applied, the mitochondria are found to make up 18% of cytoplasm volume, as compared to 21% in this study. Comparison of the mitochondrial numbers also shows good agreement with Loud's earlier data, while his newest estimate appears smaller by some 20%. It is hard to explain these differences but they may well be founded in the different sampling procedures employed, or in Loud's choice of a highly hypertonic fixation medium; or they may be due to differences in species, age, and sex of the animals. It is, however, noteworthy that the average dimensions of the individual mitochondrion agree rather well in most respects, except perhaps for their axial ratios; this needs further investigation.

Loud (7) found the numerical ratio of micro-

bodies to mitochondria to be 1:2, while in the present study it was 1:4. It should be noted that our data on mitochondria and microbodies agree well with those obtained earlier in another study (30), and that the ratio of 1:4 is identical with that derived by de Duve and Baudhuin from biochemical data (32).

The only data in our study that differ significantly from Loud's (7) data are the surface densities of endoplasmic reticulum membranes, Loud's values being less than half of ours. This difference is not readily explained. It appears possible that strain or age differences are involved; or it may be that fasting the animals prior to sacrifice had some effect. On the other hand, we have found it necessary to use a magnification considerably higher than that used by Loud for unambiguous identification of intersections between test lines and ER membranes; underestimation due to lower magnification could thus be possible.

If the present data on mitochondria are compared with those recently determined by Baudhuin and Berthet (26) on subcellular fractions, a striking difference is noted: the mitochondrial number per unit volume of liver is estimated by these authors at 490×10^9, and the mean mitochondrial volume at 0.287 μ^3, i.e., less than half of ours. These mitochondria were estimated to make up only 14% of liver tissue, as compared to our value of 18.6%. Similarly, they found significantly smaller values for the inner and the outer membrane surfaces.

Two events appear to be reponsible for these differences: (*a*) an appreciable fraction of mitochondria, of the order of 20–30%, may be lost in the course of cell fractionation; (*b*) a good portion of mitochondria apparently has become fragmented during this procedure. In intact tissue preparations, mitochondria appear as elongated organelles. In this study the average axial ratio of the roughly elliptic profiles was 1.8, and it was estimated from the distribution pattern of profiles that the organelle should be about 4 times longer than thick, on the average. Some mitochondria must be considerably longer, as a few profiles had axial ratios of up to 8. Baudhuin and Berthet (26) assume that the mitochondria in their preparations have an almost spherical shape since the axial ratio of profiles was 1.1, on the average. This finding, together with the elevated number of organelles in spite of smaller total volume, suggests fragmentation of mitochondria upon homogenization or fractionation. A correlated study on intact tissue and cell fractions should establish the artefacts introduced by cell fractionation, to clear up these discrepancies.

Glas and Bahr (33) estimated the mean mitochondrial volume to be 0.43 μ^3 by their method of quantitative electron microscopy. This value is larger than Baudhuin's but still smaller than ours; it should be noted, though, that Glas and Bahr have also studied subcellular fractions and that the axial ratio of whole mitochondria illustrated in their article is 1.1–1.4 and hence even smaller than the axial ratio of mitochondrial profiles observed in sections of intact tissue. This may also be due to fragmentation.

The number of ribosomes has been estimated by Blobel and Potter (34) to be about 6×10^6 per average liver cell. Considering the fact that this value was obtained by an entirely different indirect approach, it compares, at least in order of magnitude, satisfactorily with our value of 12.7×10^6. Blobel and Potter had related their ribosomes to all nuclei in the nuclear fraction which were derived, as they mention, not only from hepatocytes but also from other cells as well. Hepatocytes, however, contribute only about 60% to all nuclei in the liver. If this fact is taken into account, the number of ribosomes per hepatocyte, as derived from Blobel and Potter's data, may reach 9–10 million. The present estimation of ribosomal number, on the other hand, is a rather rough estimate since a number of assumptions had to be made which may easily have introduced a considerable systematic error of unknown degree and direction.

Finally, a light microscopic study by Preis and co-workers (31) on mouse liver should be mentioned. These authors determined the ratio of cytoplasm-to-nuclear-volumes to be 4.6, whereas in our study it amounted to 15.3. Besides species differences, this striking discrepancy undoubtedly results from Holmes effect since the nuclear volume must be considerably over-estimated in 7-μ thick sections with nuclei stained dark.

In this study, we have attempted to characterize the morphometric properties of the normal rat liver cell, the specific aim in mind being to provide quantitative base line data for correlation with biochemical information derived from subcellular fractions of homogenized livers. The methods introduced are efficient and can be useful in correlated biochemical and morphological studies on experi-

mentally induced alterations of hepatocytes, as exemplified in the accompanying paper (9).

The authors would like to thank Mr. J. Suter for his very valuable assistance in this work.

This study was in part supported by the Schweizerischer Nationalfounds zur Förderung der wissenschaftlichen Forschung No. 3952, and by a grant from BIGA No. 78.

Recieved for publication 18 June 1968, and in revised form 20 January 1969.

REFERENCES

1. LOUD, A. V. 1962. A method for the quantitative estimation of cytoplasmic structures. *J. Cell Biol.* **15**:481.
2. LAZAROW, A., and A. M. CARPENTER. 1962. Component quantitation of tissue sections. I. Characterization of the instrument. *J. Histochem. Cytochem.* **10**:324.
3. WEIBEL, E. R. 1963. Morphometry of the human lung. Academic Press, Inc., New York.
4. SITTE, H., and M. STEINHAUSEN. 1963. Stereologische Auswertung elektronenoptischer Aufnahmen der Säugerniere. Proc. 1st Int. Congr. on Stereology, Vienna Medical Academy. 24.
5. WEIBEL, E. R., and B. W. KNIGHT. 1964. A morphometric study on the thickness of the pulmonary air-blood barrier. *J. Cell Biol.* **21**: 367.
6. WEIBEL, E. R., G. S. KISTLER, and W. F SCHERLE. 1966. Practical stereological methods for morphometric cytology. *J. Cell Biol.* **30**:23.
7. LOUD, A. V. 1968. A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. *J. Cell Biol.* **37**:27.
8. WEIBEL, E. R., and H. ELIAS. 1967. Quantitative methods in morphology. Springer-Verlag New York Inc.
9. STÄUBLI, W., E. R. WEIBEL, and R. HESS. 1969. Correlated morphometric and biochemical studies on the liver cell. II. Effect of phenobarbital on rat hepatocytes. *J. Cell Biol.* **41**:92.
10. DOLJANSKI, F. 1960. The growth of the liver with special reference to the mammal. *Int. Rev Cytol.* **10**:217.
11. ELIAS, H. 1967. Stereology. Proc. 2nd Int. Congr. for Stereology. Chicago 1967, Springer-Verlag New York Inc.
12. PEACHEY, L. D. 1958. Thin sections. I. A study of section thickness and physical distortion during microtomy. *J. Biophys. Biochem. Cytol* **4**:233.
13. FRASCA, J. M., and V. R. PARKS. 1965. A routine technique for double staining ultrathin sections using uranyl and lead salts. *J. Cell Biol.* **25**:157.
14. FREERE, R. H., and E. R. WEIBEL. 1967. Stereologic techniques in microscopy. *J. Roy. Microsc. Soc.* **87**:25.
15. GANDER, R. 1967. Morphometric microscope with automatic sampling stage. *In* Stereology. Proc. 2nd Int. Congr. Stereology. Springer. Verlag New York Inc. 289.
16. WEIBEL, E. R. 1967. Structure in space and its appearance on sections. *In* Stereology. Proc. 2nd Int. Congr. Stereology. Springer-Verlag New York Inc. 15.
17. TOMKEIEFF, S. I. 1945. Linear intercepts, areas and volumes. *Nature.* **155**:24.
18. WEIBEL, E. R., and D. M. GOMEZ. 1962. A principle for counting tissue structures on random sections. *J. Appl. Physiol.* **17**:343.
19. KNIGHT, B. W., E. R. WEIBEL, and D. M. GOMEZ. 1963. Effect of size distribution on a principle of counting on sections structures contained in a volume. Proc. 1st Int. Congr. on Stereology, Vienna Medical Academy. 18.
20. WEIBEL, E. R. 1963. Principles and methods for the morphometric study of the lung and other organs. *Lab. Invest.* **12**:131.
21. ELZE, C., und A. HENNIG. 1956. Die inspiratorische Vergrösserung von Volumen und innerer Oberfläche der menschlichen Lunge. *Z. Anat. Entwicklungsgesch.* **119**:457.
22. WEIBEL, E. R. 1967. A semi-automatic system for stereologic work in light and electron microscopy. *In* Stereology. Proc. 2nd Int. Congr. Stereology. Springer-Verlag New York Inc. 275 and 330.
23. LOUD, A. V., W. C. BARANY, and B. A. PACK. 1965. Quantitative evaluation of cytoplasmic structures in electron micrographs. *Lab. Invest.* **14**:996.
24. SITTE, H. 1967. Morphometrische Untersuchungen an Zellen. *In* Quantitative Methods in Morphology, E. R. Weibel and H. Elias, editors. Springer-Verlag New York Inc. 167.
25. SIESS, M., and H. STEGMANN. 1950. Messtechnische Untersuchungen über das Wachstum der Leber der weissen Maus als Grundlage für morphologisch-funktionelle Studien. *Virchows Arch. Pathol. Anat. Physiol. Klin. Med.* **318**:534
26. BAUDHUIN, P., and J. BERTHET. 1967. Electron microscopic examination of subcellular fractions. II. Quantitative analysis of the mitochondrial population isolated from rat liver. *J. Cell Biol.* **35**:631.

27. LOUD, A. V. 1967. Ultrastructural equilibria in liver cell cytoplasm. *In* Stereology. Proc. 2nd Int. Congr. Stereology, Springer New York 72.
28. HAUG, H. 1967. Probleme und Methoden der Strukturzählung im Schnittpräparat. *In* Quantitative Methods in Morphology. E. R. Weibel and H. Elias, editors. Springer-Verlag New York Inc. 58.
29. HOLMES, A. H. 1927. Petrographic methods and calculations. Murby (Thomas) & Co., London.
30. STÄUBLI, W., and R. HESS. 1966. Quantitative aspects of hepatomegaly induced by ethylchlorophenoxi-isobutyrate. *In* Proc. 6th Int. Congr. Electron Microscopy (R. Uyeda, editor). Maruzen Co., Ltd., Tokyo, Japan. 625.
31. PREIS, CHR., G. SCHAUDE, and M. SIESS. 1966. Histometrische Analyse der Lebervergrösserung nach chronischer Einwirkung von Barbituraten und Halothan. *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **254**:489.
32. DE DUVE, C., and P. BAUDHUIN. 1966. Peroxisomes (Microbodies and Related Particles). *Physiol. Rev.* **46** 323.
33. GLAS, U., and G. F. BAHR. 1966. Quantitative study of mitochondria in rat liver. *J. Cell Biol.* **29**:507.
34. BLOBEL, G., and V. R. POTTER. 1967. Studies on free and membrane-bound ribosomes in rat liver. *J. Mol. Biol.* **26**:279.
35. MILLONIG, G. 1962. Further observations on a phosphate buffer for osmium solutions. Proc. Fifth Int. Congr. Electron Microscopy, Philadelphia, August 29-September 5. Sydney S. Breese, Jr., editor. Academic Press Inc., New York. **2**: p-8.
36. LOUD, A. V. 1967. Quantitative estimation of the loss of membrane images resulting from oblique sectioning of biological membranes. *In* Proceedings 25th Anniversary Meeting of Electron Microscopy Society of America. C. J. Arceneaux, editor. Claitor's Book Store, Baton Rouge, La. 144.
37. WICKSELL, S. D. 1925: On the size distribution of sections of a mixture of spheres. *Biometrika* **17**: 84.
38. BAUDHUIN, P. 1968. L'analyse morphologique quantitative de fractions subcellularies. Thèse. Université de Louvain, Louvain, Belgium.
39. DEHOFF, R. T., and F. N. RHINES. 1961. Determination of the number of particles per unit volume from measurements made on random plane sections: the general cylinder and the ellipsoid. *Trans. Amer. Inst. Mining, Met. Petrol. Engrs.* **221**:975.