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Stereology of Liver Biopsies from Healthy Volunteers* **

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Summary. The stereological model and the base-line data of normal human liver needle biopsy-specimens are presented. Four reference systems were introduced: 1 cm^3 of liver tissue, 1 cm^3 of hepatocyte, 1 cm^3 of hepatocytic cytoplasm and the volume of an average "mononuclear" hepatocyte. The sampling was done at three levels of magnification (1,000 ×, 5,000 × and 10,000 ×). A lobular differentiation was not considered. The baseline data show strikingly small variations (s.e. less than 10%) within the individual biopsy specimen and within the group of four biopsies. There is no principal difference between human beings presented here, rats, mice and dogs. Only the mean individual volume of human hepatocytes is clearly larger than in rodents. The problems and limitations of stereological work on liver biopsy specimens are discussed.

Key words: Human liver — Electron microscopy — Stereology.

The reactions of the human liver to various agents such as drugs and viruses can only be determined in humans, even when experimental screening in animals has been favorable. In order to evaluate the reaction of the liver to a given agent and/or its metabolites the following approaches are usually applied:

1. Determination of liver specific biochemical values in blood.
2. Biochemical analysis of liver tissue homogenates.
3. Qualitative, i.e. descriptive light and/or electron microscopic examination of liver biopsies.

It is apparent from many clinical trials that none of the above methods gives a true reflection of the drug-induced liver reaction. For instance, biochemical data of blood and liver homogenate which are known to depend on many variables are often, but not necessarily, correlated with overt morphological alterations.

In the last years stereology has been demonstrated to be an important complement to biochemical data. A good correlation between biochemical findings and stereological data, e.g. proliferation of smooth endoplasmic reticulum in enzyme induction by phenobarbital (Staeubli et al., 1969) has been found. Comparative stereological and biochemical analyses showed that under different experimental conditions the center-to-center distance of cytochromes changes (Reith et al., 1973). These different enzyme densities are of primordial importance in the interpretation of membrane proliferation processes (Frigg and Rohr, in preparation). Finally, stereology has shown that the liver cell has only a limited spectrum of reaction patterns for a much larger series of biochemically well defined disturbances of the intermediary metabolism (Rohr and Riede, 1973). Therefore, in our study a stereological model of the human liver paranchymal cell and the stereological base-line data of normal human liver needle biopsy-specimens are presented.

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Material and Methods

Two weeks prior to liver biopsy four volunteer medical students (Table 6) were set on a diet consisting of three meals rich in protein and representative for the average caloric intake in Switzerland. Strict abstinence from smoking, alcohol and drugs was observed during this period. One day prior to liver biopsy serum samples were taken and routinely checked for transaminases, bilirubin, alkaline phosphatase, cholesterol and Australia antigen (Table 6). Blind liver biopsy using a Menghini needle were performed at 8 a.m. after a fasting period of 12 h. Part of the biopsy material was fixed in 4% neutral formalin for light microscopy. An equal amount was snap-frozen and studied for the presence of HB_s- and HB_c-antigen, IgG, IgM and complement (C'3) according to procedures described elsewhere (Gudat et al., 1975). For electron microscopy liver tissue samples were processed immediately as follows:

Electron Microscopy

Tissue blocks from the liver biopsy-specimens, 0.5 mm on a side, were fixed in 1.33% osmium s-collidine buffer (220–240 mosm) at pH 7.4 for 2 h at 4° C.

These blocks were dehydrated by passing them through increasing concentrations of cold alcohol followed by propylene oxide, and were finally embedded in Epon (Luft, 1961). Sections (interference color: grey to silver, 70–80 nm) were cut with a diamond knife on a Reichert Ultramicrotome, mounted on 200—mesh grids, and stained with uranyl acetate and lead citrate (Reynolds, 1963). Semithin sections were stained with azure-methylene blue or toluidine blue.

Micrographs were made on 70 mm film plates with a Zeiss EM 9 A electron microscope. The film plates, together with the corresponding test line and point sets, were enlarged approximatively 3.5 times and evaluated.

For morphometric measurements homogeneous areas of the liver lobule lacking portal tracts and central venes were selected from semithin sections. Automatic sampling stage microscope (Wild, Heerbrugg, Switzerland) for lightmicroscopy and a Zeiss electron microscope EM 9 A.

Stereological Procedure

General stereological procedure according to Weibel (1969 and 1973) and Rohr et al. (1975).

Calculations of the mean volume of the single 'mononuclear' hepatocyte (Weibel, 1969) according to the methods of Giger (1967).

The primary magnification was checked by including a micrograph of a calibration grating (Zeiss A.G., Oberkochen, Germany) on each film plate batch.

Stereological Model of the Human Liver

The stereological model for the human liver is represented in Figure 1.

The liver was divided into the extrahepatocytic and the hepatocytic space. In the present study, due to small size of the liver biopsy specimens, the extrahepatocytic space was not further analysed into the subdivisions shown in Figure 1. The hepatocytic space was further subdivided into well defined compartments, which were defined as the aggregate of all elements of a given component (Bolender, 1974).

Such a design of the stereological model guarantees the most possible flexibility in including different reference systems according to the type of experimental study in question.

Basically four reference systems were used in the stereological model of the hepatocyte:

1. one cubic centimeter of liver tissue;
2. one cubic centimeter of hepatocyte;
3. one cubic centimeter of hepatocytic cytoplasm and
4. the volume of an average 'mononuclear' hepatocyte.

These four reference systems allow comparisons with biochemical results on certain assumptions (Bolender, 1974). For instance biochemical data are often related to a unit tissue weight, and therefore stereologically determined volumes must be converted to weights. For this purpose the specific gravity of the liver must be determined (Bolender, 1974; Weibel et al., 1969).

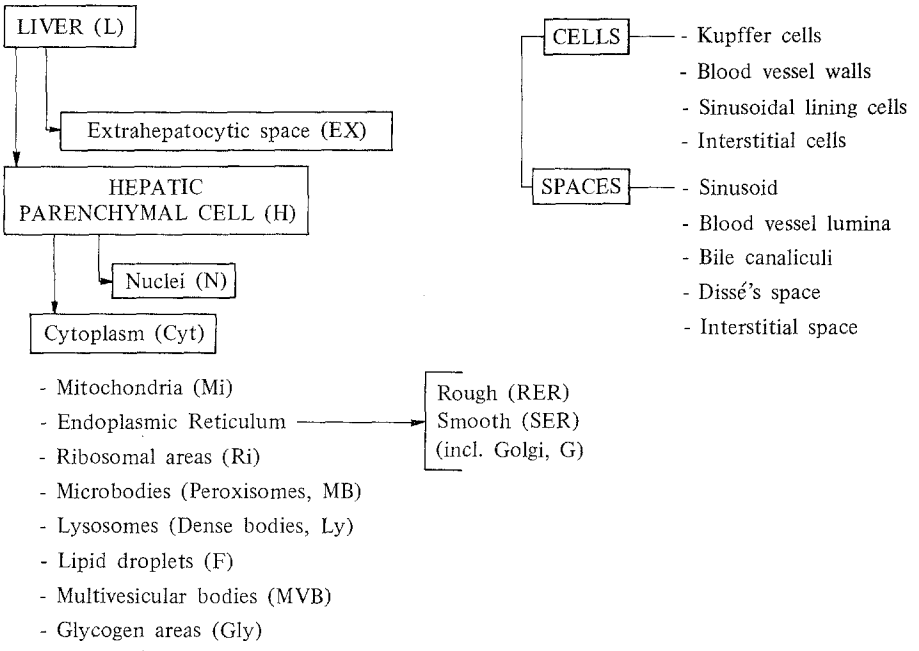

Fig. 1. Stereological model of the human liver

Morphological Criteria

The different tissue and cell compartments were evaluated with the light microscope or on the electron micrographs according to the following conventions:

1. As mentioned above the extrahepatocytic space was not further analysed.
2. The rough endoplasmic reticulum included cell cisternae, tubules and vesicles with membranes which were studded with ribosomes. The perinuclear cisternae and the ribosomal areas were added to the rough endoplasmic reticulum.
3. Cisternae, tubules without ribosomes, including the Golgi apparatus, were included in the smooth endoplasmic reticulum.
4. The term 'lysosomes' comprises autophagic vacuoles, cyto-, phagolysosomes and residual bodies.
5. Glycogen areas were evaluated together with the cytoplasmic ground substance, since glycogen cannot be visualized when s-collidine buffer is used.
6. At level I the extrahepatocytic space (V_{vex}) comprises blood vessels, interstitial space and cells, as well as sinusoids and Kupffer cells, but not the space of Dissé, capillaries and the bile canaliculi, which can be identified only at the higher magnification of level II ($=V_{vex}$).

Multi-Level Sampling (Weibel, 1969)

The different cellular components cannot be evaluated at a single stage of magnification due to the broad differences in shape, size and frequencies of these components.

Therefore, sampling was done at three different magnifications so as to establish an adequate relationship between the size of the components and the point or line sets.

Level I: (1,000×). For stereological measurements 5 semithin sections for each liver biopsy specimen were selected. For each semithin section at least 10 test areas were analysed (=50 test areas per liver biopsy specimen).

Level II: (5,000×) and III (10,000×). At least 3 tissue blocks for each liver biopsy specimen were sectioned. The areas to be evaluated were selected according to systematic

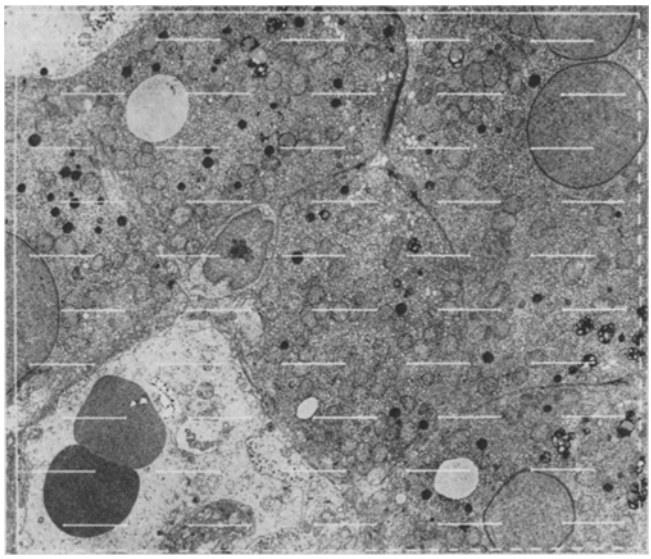

Fig. 2. Human liver cell with superimposed multipurpose test lattice. Level I ($\times$ 1,200)

sampling procedure by photographing in regular steps in predetermined corners of the meshes of the supporting copper grid (Weibel, 1969). For each liver biopsy specimen (3 tissue blocks) a total of 60(3 $\times$ 10 at level II and 3 $\times$ 10 at level III) micrographs were analysed.

Test Systems

The test systems applied to the three sampling levels were as follows:

Level I (Fig. 2). Multipurpose lattice [$P_T = 100$ (Weibel, 1973)]. This was also the level at which the number of nuclear profiles per test area for the calculation of the numerical density of nuclei, was determined (β, shape factor: 1.45, K: 1.07, a factor dependent on the size distribution of the nuclii).

Level II (Fig. 3). Double square lattice (1:9/121:1,089), where 1:9 signifies the ratio of coarse to fine points, and 121:1,089 the number of coarse to fine points.

In addition the number of mitochondrial profiles within the test area was determined at this level. In order to estimate the shape factor (β) of the mitochondria for each liver biopsy specimen the ratio of the axes of about 100 mitochondrial profiles was measured. β was found to be 2.1–2.2.

Level III (Fig. 4). At this stage the number of intersections of the 50 test lines of the multipurpose test lattice (Weibel, 1973) with the membrane traces of the RER and SER, mitochondrial innner- and outer membranes respectively, was determined. The total length of the linear probe (L_T) was equal to one-half of the number of test points ($P_T = 100$) times d, the individual test line length.

Sampling Size

According to Weibel (1969) the number of test points per representative sample unit should be approximately:

$$PT = 0.453 \times \frac{(1 - V_V)}{V_V \times E^2 (V_V)}, \text{ where E,}$$

a function of V_V, E (V_V), is the relative error, which we accepted to be 5%. For instance for mitochondria, for which the volume density amounts to 0.2%, 724 test points would be needed for each liver biopsy specimen. However, a total of 30 $\times$ 121 = 3,630 test points were

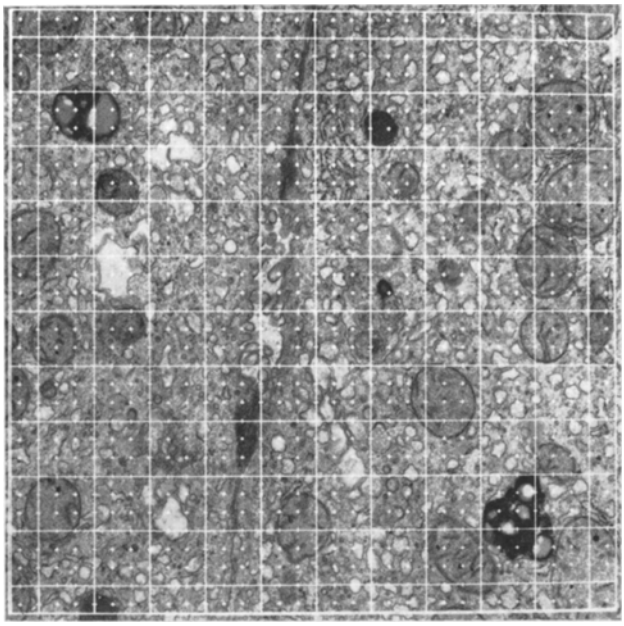

Fig. 3. Detail of human liver cell with superimposed double quadratic test lattice allowing determination of N_V by counting number of profiles and V_V by counting test points over profiles at level II ($\times$ 5,000)

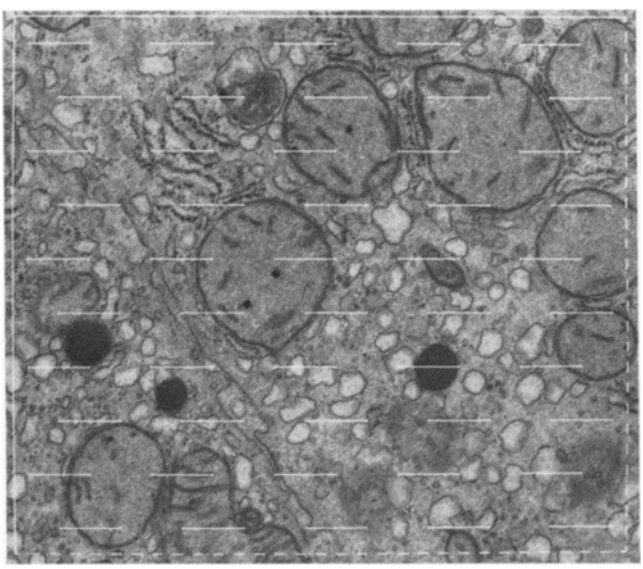

Fig. 4. Detail of human liver cell with superimposed multipurpose test lattice allowing determination of V_V by counting end points of test lines over profiles and S_V by counting intersections of test lines with membrane traces at level III ($\times$ 10,000)

counted. For smaller cell compartments (smooth endoplasmic reticulum and fat droplets, volume = 0.07) the corresponding minimal number of test points should be 2,407 ($V_V = 0.01$, $P_T = 17{,}938$, whereas $30 \times 1{,}089 = 32{,}670$ test points per liver biopsy specimen were evaluated).

Principles of Stereology

Stereological data as relative measurements are expressed as densities, which relate a volume, a surface area or a number to a unit volume (Weibel, 1969; Weibel, 1973).

In the stereological model of the human liver presented here we used the following reference volumes (= unit volume):

Liver,
Hepatocyte,
Hepatocytic cytoplasm.

Finally instead of relating to a unit volume, the determined densities of cellular components can be related, as absolute values, to an average 'mononuclear' hepatocyte (Weibel, 1969): The average volume of a hepatocyte was calculated by dividing the volume density of the hepatocytes (V_{VH}) by the numerical particle density of the hepatocytic nuclei (N_{VNH}).

For a better understanding of the structure to function relationships the following additional reference systems were introduced:

Unit volume of mitochondria,
Unit volume of RER,
Unit volume of SER.

Thus, for example, the surface of the inner or outer mitochondrial membranes was calculated per unit volume of mitochondria. When calculating such 'secondary parameters' the different densities (volume, surface, number) must be related to the same unit of reference volume.

The numerical density (N_V) of nuclei was calculated by the formula developed by Weibel and Gomez (1969).

The calculation of the average volume of a 'mononuclear' hepatocyte can be performed by the approach proposed by Giger (1967).

Volume densities and surface densities were determined according to Glagoleff (1933), Weibel et al. (1966), Weibel (1973), and Mayhew and Cruz Orive (1974).

Data Processing

The calculation of data was performed on an Olivetti Programma 602 desk computer connected with a magnetic tape unit MLU 600. The program permitted fully automated evaluation following initial manual input of data.

Results

In Table 1–5 the different components of the human liver biopsy specimens are shown as volume and surface areas. The values of this parameters are related to the reference spaces 'liver tissue' (Table 1), 'hepatocyte' (Table 2), 'hepatocytic cytoplasm' (Table 3) and as absolute values to the average 'mononuclear' hepatocyte (Table 5).

It can be seen from Figure 5 that the hepatocytes account for 79.3% and the extrahepatocytic space for 20.7% of the liver tissue.

The nuclei contributed 7.3% of the unit volume of the hepatocyte. The ground substance (= cytoplasmic matrix) was the major cytoplasmic compartment (50.9%) (Fig. 5).

Of the remaining components the RER added 12.1, the SER 7.2, the mitochondria 17.6%, while the dense bodies provided 1.8, the fat droplets 1.9 and

Table 1

Component	Parameter	Symbol	Density per cm^3 of liver tissue		Dimension
			Mean	s.e.	
Extrahepatocytic space	volume	V_{VEX}	0.207	0.014	cm^3/cm^3
Hepatocytes	volume	V_{VH}	0.793	0.014	cm^3/cm^3
Nuclei	volume	V_{VN}	0.056	0.003	cm^3/cm^3
	number	N_{VN}	102.5×10^6	11.1×10^6	cm^{-3}
Mitochondria	volume	V_{VM}	0.14	0.0072	cm^3/cm^3
	number	N_{VM}	0.170×10^{12}	0.016×10^{12}	cm^{-3}
outer membrane	surface	S_{VMO}	1.256	0.106	m^2/cm^3
inner membrane	surface	S_{VMC}	2.984	0.223	m^2/cm^3
Microbodies	volume	V_{VMB}	0.009	—	cm^3/cm^3
	number	N_{VMB}	0.074×10^{12}	0.005×10^{12}	cm^{-3}
Rough endoplasmic reticulum	volume	V_{VRER}	0.096	0.007	cm^3/cm^3
Smooth endoplasmic reticulum	volume	V_{VSER}	0.056	0.003	cm^3/cm^3
Fat	volume	V_{VF}	0.004	—	cm^3/cm^3
Lysosomes	volume	V_{VLY}	0.014	0.001	cm^3/cm^3
Ground substance	volume	V_{VGS}	0.418	0.003	cm^3/cm^3

Table 2

Component	Parameter	Symbol	Density per cm^3 hepatocyte		Dimension
			Mean	s.e.	
Nuclei	volume	V_{VN}	0.073	0.003	cm^3/cm^3
Mitochondria	volume	V_{VM}	0.176	0.007	cm^3/cm^3
	number	N_{VM}	0.213×10^{12}	0.018×10^{12}	cm^{-3}
outer membrane	surface	S_{VMO}	1.585	0.141	m^2/cm^3
inner membrane	surface	S_{VMC}	3.764	0.304	m^2/cm^3
Microbodies	volume	V_{VMB}	0.012	0.000	cm^3/cm^3
	number	N_{VMB}	0.093×10^{12}	0.007×10^{12}	cm^{-3}
Rough endoplasmic reticulum	volume	V_{VRER}	0.121	0.007	cm^3/cm^3
	surface	S_{VRER}	1.133	0.033	m^2/cm^3
Smooth endoplasmic reticulum	volume	V_{VSER}	0.072	0.006	cm^3/cm^3
	surface	S_{VSER}	2.804	0.295	m^2/cm^3
Fat	volume	V_{VF}	0.019	0.016	cm^3/cm^3
Lysosomes	volume	V_{VLY}	0.018	0.001	cm^3/cm^3
Ground substance	volume	V_{VGS}	0.509	0.01	cm^3/cm^3

Table 3

Component	Parameter	Symbol	Density per cm^3 of cytoplasm		Dimension
			Mean	s.e.	
Smooth endoplasmic reticulum	volume	V_{VSER}	0.077	0.006	cm^3/cm^3
	surface	S_{VSER}	3.025	0.325	m^2/cm^3
		S_{VSER}/V_{VSER}	39.285	1.311	m^2/cm^3
Rough endoplasmic reticulum	volume	V_{VRER}	0.130	0.007	cm^3/cm^3
	surface	S_{VRER}	1.221	0.04	m^2/cm^3
		S_{VRER}/V_{VRER}	9.392	0.602	m^2/cm^3
Fat	volume	V_{VF}	0.021	0.0176	cm^3/cm^3
Lysosomes	volume	V_{VLY}	0.02	0.0022	cm^3/cm^3
Mitochondria	volume	V_{VM}	0.19	0.0086	cm^3/cm^3
	number	N_{VM}	0.2302×10^{12}	0.0209×10^{12}	cm^{-3}
outer membrane	surface	S_{VMO}	1.708	0.151	m^2/cm^3
inner membrane	surface	S_{VMC}	4.057	0.329	m^2/cm^3
Microbodies	volume	V_{VMB}	0.013	—	cm^3/cm^3
Ground substance	volume	V_{VGS}	0.549	0.012	cm^3/cm^3

Table 4

Compartment	Parameter	Symbol	Characteristics of mean mitochondrion		Dimension
			Mean	s.e.	
Mitochondrion	volume	V_{VM}/N_{VM}	0.837	0.052	μm^3
Outer membrane	surface	S_{VMO}/N_{VM}	12.791	2.108	μm^2
Inner membrane	surface	S_{VMC}/N_{VM}	30.304	4.731	μm^2

the microbodies 1.2%, respectively (Fig. 5). The corresponding values for the reference system 'hepatocyte' and 'hepatocytic cytoplasm' are given in Figure 5 and Tables 2 and 3.

Per unit volume of cytoplasm the surface density of the SER (3.0 m^2/cm^3) exceeds that of the RER (1.2 m^2/cm^3). Standard errors are within the expected limits (10% of the mean, Table 3). The surface density of the outer mitochondrial membrane is estimated at 1.7 m^2/cm^3, that of the inner membrane at 4.0 m^2/cm^3 (per unit volume of cytoplasm, Table 3).

One cubic centimeter of liver tissue contains $102 \cdot 10^6$ hepatocyte nuclei ($\beta = 1.38$), $170 \cdot 10^9$ mitochondria ($\beta = 1.45$) and $74 \cdot 10^9$ microbodies ($\beta = 1.45$, Table 1).

The average volume of the 'mononuclear' hepatocyte was calculated at 11,300 μm^3, that of the nucleus at 790 μm^3. Further values are given in Table 5. The average 'mononuclear' hepatocyte contains about 2,200 mitochondria and 1,050 microbodies (Table 5).

Table 5

Component	Parameter	Symbol	Characteristics of mean ("mononuclear") hepatocyte		Dimension
			Mean	s.e.	
Hepatocyte	volume	V_{VH}	11,305	803	μm^3
Nucleus	volume	V_{VN}	798	27	μm^3
Mitochondria	volume	V_{VM}	1,905	168	μm^3
	number	N_{VM}	2,225	402	
outer membrane	surface	S_{VMO}	17,814	1,595	μm^2
inner membrane	surface	S_{VMC}	42,219	3,085	μm^2
Microbodies	volume	V_{VMB}	132	10	μm^3
	number	N_{VMB}	1,050	14	
Rough endoplasmic reticulum	volume	V_{VRER}	1,380	180	μm^3
	surface	S_{VRER}	12,756	673	μm^2
Smooth endoplasmic reticulum	volume	V_{VSER}	802	46	μm^3
	surface	S_{VSER}	31,184	2,374	μm^2
Fat	volume	V_{VF}	47	16	μm^3
Lysosomes	volume	V_{VLY}	206	20	μm^3
Ground substance	volume	V_{VGS}	6,035	509	μm^3

Table 6. Laboratory data

	Volunteer			
	1	2	3	4
Age (years)	24	24	24	27
Sex	male	male	male	female
Bilirubin (mg%)	0.7	0.6	0.6	0.6
SGPT (iU)	16.4	7.4	8.1	25.2
SGot (iU)	10.3	7.4	9.6	18.4
Alk. Phosphatase (iU)	112	103	93	74
Cholesterol (mg%)	233	227	188	271
HB_s-Ag (blood)	neg.	neg.	neg.	neg.
Immunofluorescence liver tissue (HB_s-, HB_c-Ag, C'3, IgG, IgM)	neg.	neg.	neg.	neg.

The mean mitochondrial volume is estimated at 0.83 μm^3 (Table 4). That of the microbodies was 0.13 μm^3.

The absolute surface of the inner mitochondrial membrane per 'mononuclear' hepatocyte was found to be about 2.5 times larger than that of the outer membrane (Table 5).

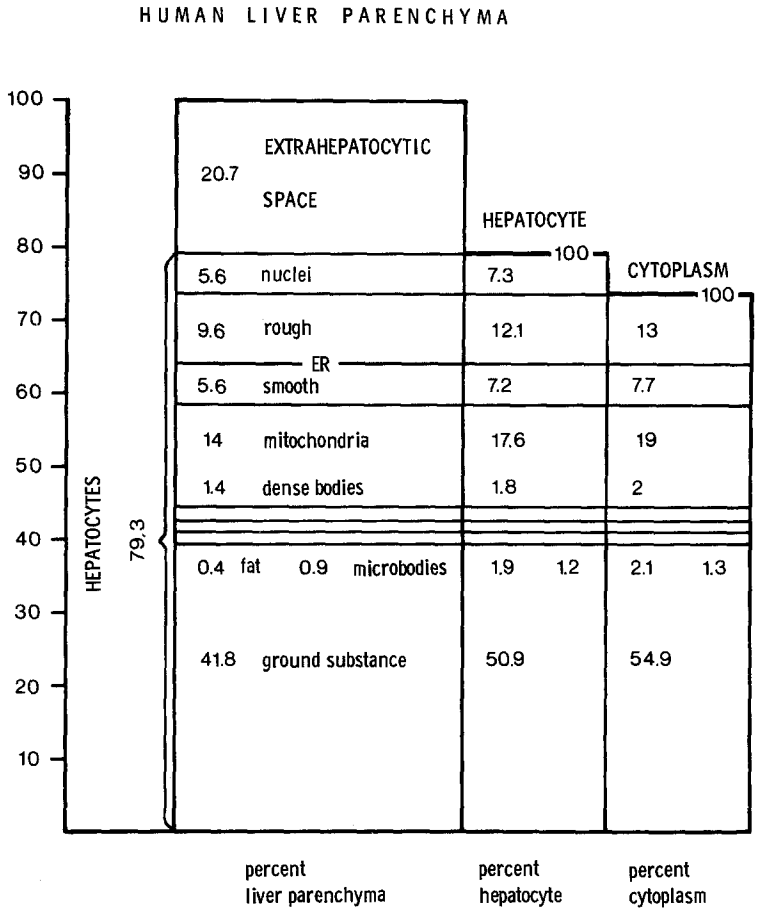

Fig. 5. Volumetric composition of liver tissue, hepatocyte, and hepatocytic cytoplasm in normal volunteers (mean of 4 individuals)

Discussion

a) Results

In the stereological analysis of needle biopsies the method of sampling is of great importance. Despite the fact that regional intralobular differences for the cell organelles and their enzymes have been shown (Novikoff, 1959; Cossel, 1964; Novikoff and Essner, 1960; Reith et al., 1968; Reith and Schueler, 1972), a zonal stereological differentiation was not considered. The aim of the present investigation is to establish base-line data suitable for correlation with biochemical findings. These latter data are as a rule derived from liver homogenates and are therefore representative for the entire liver lobule.

The base-line data of human hepatocytes obtained from biopsies of clinically and histologically normal volunteers show strikingly small variations Within both the individual biopsy and the group of four biopsies, the standard

errors (s.e.) are less than 10% of the mean. The only exception is the volume density of fat (per unit volume of hepatocyte; see Table 2). This is explained partly by the low volume occupied by fat (less than 2%) and partly by the wide topographical difference in distribution of fat in the liver lobule.

Since we could not show any significant difference between the results of the principal parameters in the individual biopsy, the ultrastructural volumetric composition of the hepatocyte within the liver lobule may be regarded as approximatively homogeneous. Furthermore, the low variations of the stereological data between the biopsies demonstrate the usefulness of needle biopsy material for ultrastructural stereology of the liver cell, at least in normal liver and probably also in diffusely distributed liver cell alterations. However, for the morphometric analysis of focal parenchymal alterations several needle biopsy specimens may be necessary. A morphometric comparison between needle and block biopsies in dog liver confirms the feasibility of quantitative analysis using needle biopsies provided that the sample size is large enough (Hess et al., 1973). Therefore, in morphometric analysis using needle biopsies as many samples as possible should be taken.

There is no fundamental difference between human beings as presented here and rats, mice (Rohr and Riede, 1973) and dogs (Hess et al., 1973): Both, the rough endoplasmic reticulum and especially the mitochondria occupy roughly equal relative volumes. In the different species subjected to morphometry (man, dog, different rodents) the size of the individual mitochondrion (0.7–0.8 μm^3) shows a high degree of constancy. This finding is surprising in view of the fact that the numerical density of mitochondria represents an approximate value due to the variation in shape.

Only the mean individual cell volume of human hepatocytes with approximately 11,000 μm^3 is clearly larger than in rodents. In the present study the individual cell volumes were determined by the method of Giger (1967), thus permitting a comparison with the values for dogs evaluated by Hess (1973).

We would like to suggest that the constancy of volume densities of cell compartments in the different reference systems such as liver tissue or cytoplasm, especially that of mitochondria in man, dogs and rodents, might reflect a similarity of intermediary cell metabolism greatly independent of species.

b) General Considerations

Sampling. Factors known to influence liver enzymes and thus likely to bear on morphometric results include age, environment (diet, temperature, etc.), diurnal fluctuations, and zonal differences within the liver lobule. Therefore, for experimental studies in animals a strict standardization of all variables is mandatory and, as a rule, easy to perform. For the study of the human liver, however, it is difficult to define normal conditions and even more difficult to adjust human beings to postulated standards, especially in states of disease.

It is assumed that, with the precautions met in this study, a representative mean morphometric base-line for the "normal" human liver cell has been established. This makes it possible to compare several conditions with the presented reference data.

Pathological deviations may be distributed diffusely or zonally within the hepatic lobule, e.g. diffusely in drug-induced enzyme induction; or focally as in HB_s-Ag synthesis by irregularly distributed single cells or groups of cells in viral hepatitis (Gudat et al., 1975). Accordingly, the sampling strategy for both types might be different. In diffuse alterations a random sampling as applied in this study might be satisfactory. It is obvious that diffuse alterations such as drug-induced enzyme induction or toxicity are optimal objects for morphometric comparison with the presented normal base-line data. In focal lesions, however, a selected sampling of affected areas might be necessary to bring out pathological changes.

For the discussion of the interpretation and limitations of morphometry the reader is referred to recent reviews (Weibel, 1973; Rohr et al., 1975). The following considerations will be restricted to the aspect that pure morphometric data are inconclusive. This holds true especially for the liver which is the target for many agents. So far, no specific reaction pattern of the liver cell to any agent has been described. A given morphometric deviation in a single or several cell organelles resulting in a non-specific reaction pattern can be induced by a variety of causes. Conversely, the morphometric values observed after the application of a known substance are not necessary linked to the action of this agent, but may be caused by other factors. Interference of drug actions and metabolic disturbances due to underlying disease may render interpretation difficult in individuals with disease. Consequently, as an optimal approach a morphometric finding should be substantiated by biochemical determinations in liver tissue and/or blood. On the other hand, a pure biochemical approach which is in effect a diffuse sampling may be insensitive in detecting focal lesions. In these cases morphometry may be the only way to substantiate such lesions or may guide to the selection of the appropriate biochemical key parameter(s).

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