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THIRTY YEARS OF EXPERIENCE IN ULTRASTRUCTURAL QUANTITATIVE MORPHOLOGY AND STEREOLOGY OF THE LIVER

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ABSTRACT

Against the background of the author's own research results, an account is given of developments, over the past 30 years, in the use of quantitative methods in optical light and electron microscopy of the liver. The liver is used as an example to show that on top of the generally known methods of stereology additional, quantitative approaches, some of them in combination with each other, are necessary to characterise biological structures. Such approaches have been developed by the author, for example, to determine relationships between cell organelles, on the one hand, and specific bonding on the cell membrane, on the other, with microvilli being an example. Combination of quantitative results with qualitative assessment of cellular components is of decisive importance to cellular research and medical diagnosis, if conclusive findings are to be achieved.

INTRODUCTION

While the major principles underlying the methods of morphometry and stereology have been known for more than a century, widespread application of these methods in cellular research as well as in optical light and electron microscopy was initiated only through the pace-making studies of Elias and Weibel.

Yet, quantitative morphology was in existence even before the introduction of stereological methods and is valid for certain problems even in our days, since there is a number of quantitative questions to which no answers can be found by means of the arsenal of stereological methods.

DIVERSITY and RELATIVITY of QUANTITATIVE METHODS in ULTRASTRUCTURAL RESEARCH

Some developments and trends in quantitative morphology will be outlined, with reference being made to experience obtained by the author from his own ultrastructural research on the liver.

1. Quantitative investigations of the liver, up to the early fifties were concerned with cell counts and, in particular, with the amount of binuclear cells (2, 5, 8). This problem is not even today tackled by means of stereological methods. The problem of binuclearity and multinuclearity of hepatocytes is related to the fact that all stereological liver findings are related to mononuclear cells and that, therefore, too high cell counts still are assumed, so that absolute numbers of cell organelles and cell volumes are derived which differ from reality. The amount of binuclear cells in the liver of mouse, for example, was found to increase from its normal level of 22.2 per cent to the order of 46.8 per cent, following five days of absolute fasting. The author and his co-workers have calculated the values for both mononuclear and binuclear cells in an attempt to find solutions to the problem (2, 8). Stereological findings for differentiation of both volume and organelle population of mononuclear and binuclear hepatocytes were first presented by them in 1983 (36).

Processes for liver homogenisation together with phase-contrast microscopy enabled determination of the number of mitochondria in one gram of liver tissue as well as conversion to the mononuclear hepatocytes (1). The author thus succeeded in determining, under normal and pathological conditions and, particularly, in situations of feed deficiency and starvation, values which were equivalent to those obtained today by means of stereology (1, 5-8).

The author's own quantitative investigations of mononuclear hepatocytes gave 1,630 $\pm$ 70 mitochondria for the adult mouse and 1,530 $\pm$ 90 for adult rat. By consideration of binuclear cells, figures would be 2,970 for mouse liver and 2,450 for rat liver (8).

The stereologically determined value of mononuclear cells (42) in adult rats, five month of age, was 1,595 $\pm$ 39.8 and was thus in agreement with values obtained from earlier studies.

Hence, even the quantitative morphological methods used about 30 years ago were good enough for measurement of several parameters, including alteration and change in cell counts (2), certain cellular components of the liver, such as cell nucleus volume (5, 8), number and size of nucleoli (3-5, 8), number and volume of mitochondria (1, 5-8), as well as correlations between cellular components and cells, in other words, measurements undertaken today by means of systems of automated microscopic image analysis, less tedious and more elegant but with results that are not basically different from what had been achievable in the past.

2. Over the past two decades, the whole range of stereological methods proved to be of decisive importance to wider and more systematic knowledge of liver structures, as a whole, and of hepatocytes, in particular. It was primarily in correlation with functional and biochemical findings that new insights were gained into the function of the liver under normal and pathological conditions.

Knowledge has been built up by ultrastructural stereology about relationships between different components of the liver as well as about relative and absolute values in the composition of hepatocytes under normal and pathological conditions. Substantive variations among hepatocytes even under normal conditions have been conspicuous findings, in that context.

Hepatocytes have been characterised in the following way in great detail by the author and his co-workers, using the established methods of stereology and taking into consideration relevant results reported by other authors:

1. Volume of all hepatocytes (25, 29, 36, 41, 42)
2. Quantitative characterisation of cellular components
2.1. Cell nucleus (25, 30, 42)
2.2. Mitochondria (21, 24, 31, 33-39, 42, 44-46)
2.3. Peroxisomes (28, 36, 37, 42)
2.4. Endoplasmic reticulum, ribosomes, polysomes (13, 15, 19, 20, 27, 31, 33, 34, 36, 39, 42, 44-46)
2.5. Golgi apparatus (27, 36, 37, 42)
2.6. Lysosomes (27, 31, 33, 36, 37, 42, 46)
2.7. Glycogen (26, 36, 42)
2.8. Lipids (26, 36, 42)
2.9. Groundplasm (36, 42)
2.10. Plasmamembrane (25, 29, 32, 36, 37, 42, 43, 47)

3. However, quantitative characterisation of hepatocytes gave rise to new scientific problems which had been unknown before. Improvement of existing and development of new methods proved to be necessary, in that context. The author's interest and research efforts were then primarily focussed at the following complexes:

(a) An approach to quantitative characterisation of specific hepatocytes under extraordinary conditions typical of defined pathological changes, for example, formation of minihepatocytes and macrohepatocytes, had to be devised with reference to hepatocyte average populations (36, 42).

(b) A way of differentiation had to be prepared among all regions of hepatocytes, according to different functional performances. In that context, the vascular pole (supranuclear sinusoidal zone) of the hepatocyte was found to differ, for various parameters of its quantitative composition and modes of reaction, from the lateral zone and the zone adjacent to biliary canaliculi (24, 42, 47). This had substantive bearings upon the concept of the "perisinusoidal functional unit"(48).

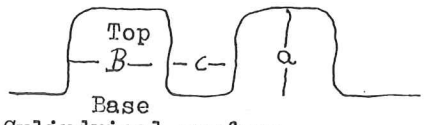
(c) Only part of the relationship between cell organelles, as an expression of functional interactions, can be quantitatively measured by means of conventional stereology. The author and his co-workers, starting and proceeding from

stereological results, have methodically studied quantitative characterisation of relationships between mitochondria and granular endoplasmic reticulum. Changes likely to occur in the course of development and due to pathological processes have been described (11, 14, 16, 42, 44).

(d) The dynamics of certain cell organelles under functional conditions and in response to pathological effects can be determined only by combination of stereology with other quantitative methods. This applies, for example, to relations between granular endoplasmic reticulum, agranular endoplasm reticulum, ribosomes, and polysomes. Knowledge of the relations which exist between free and membrane-linked ribosomes and polysomes is likely to widen understanding of protein biosynthesis processes in the hepatocyte and was a prerequisite for exploration of the same processes and relations in other cell types, such as exocrine pancreas cells and ganglial cells of the central nervous system (10, 13, 15, 18-20, 27, 42).

(e) The plasma membrane is to a great extent variably structured in different regions of hepatocytes. This is one of the reasons why no optimum solutions has so far been found for stereological measurement of its part and its differentiated modes of functional formation. The author and his co-workers have responded to the challenge by developing a combined method based on the assumption of the smooth hepatocyte surface as a rotational ellipsoid. Also, due consideration is given to differentiated formation of surface structures, with particular reference being made to number, length, and volume of microvilli in the sinusoidal zone and the zone adjacent to biliary canaliculi (25, 29, 32, 37, 42).

Surface enlargement is calculated by the following method (32):



S surface
N number
MV microvilli

Cylindrical surface

$S_{Sin-eel}$ percentage of surface of rotational ellipsoid($/um^2$)

MV area	$\frac{(B + 2x\ 1/2\ c)^2}{4}$
$MV/_{/um^2}$	$/um^2/MV$ area
N_{MV}	$MV/_{/um^2} x_{/um^2} S_{Sin-ell}$
S_{MV-tot}	$N_{MV} x S_{MV}$
$S_{Sin-corr}$	$S_{Sin-ell} + S_{MV-tot}$
Multiplication factor	$\frac{S_{Sin-corr}}{S_{Sin-ell}}$

The overall surface of the hepatocyte must be known together with the sizes of the sinusoidal zone and the zone adjacent to biliary canaliculi. The following values would then be obtained for an adult hepatocyte of rat liver:

Surface, as rotational ellipsoid,	2,567 /um^2
Part of sinusoidal surface	20.8 %: 534 /um^2
Part of surface adjacent to biliary canaliculi	3.5 %: 90 /um^2
Number of sinusoidal microvilli	2,170
Number of biliary canaliculi microvilli	340
Enlargement of sinusoidal surface	x 1.7 = 929 /um^2
Enlargement of surface adjacent to biliary canaliculi	x 1.4 = 125 /um^2
Corrected surface of hepatocytes	3,192 /um^2

This method has made it possible, for the first time, to obtain information on surface enlargement of the cell membrane of the vascular pole in hepatocytes under different conditions. The number of microvilli at the vascular pole of a hepatocyte, consequently, goes up from 1,074 at birth to 4,679 at the age of 27 months.

TRENDS IN DEVELOPMENT OF QUANTITATIVE METHODS FOR ULTRASTRUCTURAL RESEARCH

1. The use of quantitative methods, particularly stereology, in morphological studies on the basis of optical light and electron microscopy has added a brandnew quality to many results and their objectivation.

2. The building of stereology cannot even today be considered as complete. Mathematical approaches can be further improved, and methods can be devised for possible automation. Methods can be also developed with specific applicability to defined problems.

3. While our knowledge about the cell has been expanded by stereology to hardly determinable dimensions, it cannot replace in biology and medicine the qualitative methods of optical light and electron microscopy. In medico-biological research and in medical diagnosis the unity of quantitative and qualitative results is the very basis for new insights, since stereological findings may be attributable to a wide range of different causes and pathological mechanisms.

4. Hence, at least at the ultrastructural level, fully automatic measurement of parameters and evaluation of results is not possible today and will not be practicable in the future. The experienced examiner, selecting and deciding the structural details for assessment and interpreting results against the background of unity of quantitative and qualitative findings, will continue to be indispensable in biology and medicine. Recognition of this unity and its importance may be considered to be the most substantive progress and achievement which have resulted from all efforts to develop stereology for more understanding of processes of life.

PUBLICATIONS OF THE AUTHOR TO THE PROBLEM

(1) David H: Untersuchungen über die Mitochondrienzahl in den Lebern von Hungermäusen. Virchows Arch.Pathol. Anat. 1957; 330: 316-324
(2) Phan N v, David H: Zur Frage der Genese und Zahl groß- und zweikerniger Leberzellen bei Mäusen in und nach absolutem Hunger. Z. Zellforsch. 1958; 48: 653-660
(3) David H: Ribonukleinsäurestoffwechsel und Nukleolusgröße in den Lebern von Hungertieren. Z. ges. Inn. Med. 1959; 14: 63-77
(4) David H: Korrelationen zwischen Größe und Zahl der Nukleolen und zytoplasmatischer RNS in den Lebern verschiedener Tiere während und nach absolutem Hunger. Verh.Dtsch. Ges. Pathol. 1958; 42.Tgg. 417-421
(5) David H: Die Morphologie der Hungerleber. Habil.Schrift Med. Fak. Berlin 1960
(6) David H: Zur Mitochondriengröße in der Leber von Hungermäusen. Acta biol. med. germ. 1960; 4: 159-165
(7) David H: Zur submikroskopischen Morphologie der Mitochondrien der Hungerleber. Verh.Dtsch.Ges.Pathol. 1960; 44.Tgg. 229-232
(8) David H: Die Leber bei Nahrungsmangel und Mangelernährung. Akademie Verlag Berlin 1961
(9) David H: Submikroskopische Ortho- und Pathomorphologie der Leber (Textbuch und Atlas). Akademie Verlag Berlin 1964
(10) David H, Metzler E: Anordnung und Aufbau der Polysomen in der intakten Rattenleberzelle. Acta biol. med.germ. 1967; 18: 529-540
(11) David H; Quantitative elektronenmikroskopische Befunde über die Relation Mitochondrien : Endoplasmatisches Retikulum in Leberzellen nach Aktinomyzinmedikation. IV. Eur. Reg.Conf.on Electron Microscopy Rom 1968; I: 603-604
(12) David H, Uerlings I: Strukturveränderungen von Lebermitochondrien nach kurzfristiger Erwärmung bis 50°C in vitro und in vivo. Acta biol. med.germ. 1968; 20: 65-76
(13) David H, Uerlings I: Granuläres endoplasmatisches Retikulum und Ribosomen von Leberzellen nach Erwärmung bis 50°C in vitro und in vivo. Acta biol. med.germ. 1968; 20: 77-89
(14) David H: Aktinomyzinbedingte qualitative und quantitative Veränderungen des perimitochondrialen granulären endoplasmatischen Retikulums der Mäuseleber. Acta biol. med. germ. 1969; 22: 393-401
(15) David H: Morphometrische Untersuchungen membrangebundener und freier Ribosomen und Polysomen der Leber der Maus nach Aktinomyzinapplikation. Acta biol.med. germ. 1969; 22: 787-795
(16) David H, Nocon J: Struktur und quantitatives Verhalten des perimitochondrialen granulären endoplasmatischen Retikulums der Mäuseleber. Z.Zellforsch. 1969; 94: 56-61

(17) David H: Zellschädigung und Dysfunktion. in: Protoplasmatologia, Handbuch der Protoplasmaforschung. Bd. X,1 Springer Verlag Wien, New York 1970
(18) David H, Uerlings I: Ribosomen-Membranbeziehungen und ribosomale Substrukturen in intakten Rattenleberzellen. Acta biol.med.germ. 1970; 25: 343-350
(19) El Gamal M, David H: Quantitative Bestimmungen freier und membrangebundener Ribosomen in Leberzellen von Ratten bei absolutem Hunger. Exper. Pathol. 1970; 4: 291-296
(20) David H, Dreher R: Zum Verhalten membrangebundener Ribosomen der Leber unter verschiedenen Fixationsbedingungen. Z. med. Labortechn. 1972; 13: 309-311
(21) David H: Quantitative Morphology of Mitochondria. Biol. Zbl. 1975; 94: 129-153
(22) David H: Quantitative Ultrastructural Data of Animal and Human Cells. VEB Georg Thieme Verlag, Leipzig 1977
(23) David H: Cellular Pathobiology in: "Electron Microscopy in Human Medicine" Vol. 2, 1-148; ed., by J.V. Johannessen Mc Graw-Hill New York 1978
(24) David H: Quantitative and Qualitative Changes of the Mitochondria in Hepatocytes During Postnatal Development of Male Rats. Exper. Pathol. 1979; 17: 359-373
(25) David H: Veränderungen des Volumens und der Oberfläche von Leberzellen männlicher Ratten während der postnatalen Entwicklung. Acta biol.med.germ. 1979; 38: 935-952
(26) David H, Uerlings I: Quantitative ultrastrukturalle Befunde über das Verhalten von Lipiden und Glycogen in der Rattenleber während der postnatalen Entwicklung. Zbl. allgem.Pathol.u.pathol. Anat. 1979; 123: 85-103
(27) David, H: Quantitative changes of the endoplasmic reticulum, ribosomes and GERL in the hepatocytes during postnatal development of male rats. Pathol. Res. Pract. 1980; 166: 381-399
(28) David H: Morphometric analysis of peroxisomes in the liver cells of male rats during postnatal development. Exper. Pathol. 1980; 18: 321-328
(29) David H: Methodische Probleme und Ergebnisse der Volumen- und Oberflächenbestimmung von Leberzellen von Ratten während der postnatalen Entwicklung. Gegenbaurs morphol. Jahrb. 1980; 126: 285-292
(30) Specht U, David H: Ultrastrukturell-morphometrische Untersuchungen der Rattenleber in der Postnatalperiode. 1. Veränderungen des Extrahepatozytärvolumens, der Hepatozytennuclei, des Zytoplasmas und der Kern-Plasma-Relation. Gegenbaurs morphol. Jahrbuch 1980; 126: 293-299
(31) David H: Zum Verhalten von Zellorganellen im Entwicklungs- und Alternsprozeß von Herz und Leber. Gegenbaurs morphol. Jahrb. 1981; 127: 564-573

(32) David H: Methodische Probleme der Berechnung der Mikrovilli von Leberzellen. Acta histochemica Suppl. XXVI 1982; 357-360
(33) David H: Morphometrical findings in the liver cell during its postnatal developments and connections with quantitative findings in the heart. in: Morphometry in Morphological Diagnosis. Y. Collan, T. Romppanen (ed.) Kuopio University Press, Kuopio 1982; 115-122
(34) Daimon T, David H, v.Zglinicki T, Marx I: Correlated ultrastructural and morphometric studies on the liver during prenatal development of rats. Exper.Pathol. 1982; 21: 237-250
(35) Specht U, David H: Morphometrische Untersuchungen des Chondrioms in der Postnatalperiode der Rattenleber. Acta histochemica, Suppl. XXVI 1982; 401-404
(36) David H: Populations of megahepatocytes and minihepatocytes and their components in livers of old rats. Exper. Pathol. 1983; 24: 77-82
(37) David H: Quantitative characterisation of ageing hepatocytes. Acta Stereologica 1983; 2: 408-412
(38) David H, Uerlings I: Quantitative ultrastructure of the rat liver by immersion and perfusion fixations. Exper. Pathol. 1983; 23: 131-141
(39) Daimon T, David H: Stereological analysis of the hepatocyte of the rat during prenatal development. J. Electronmicrosc. 1983; 32: 223-224
(40) David H, Uerlings I, Wolff H, Gellert K, Otto G: Alterations of the ultrastructure of the sinusoids after conservation and transplantation of the liver. Proceedings Eur.Congr. Electron Microscopy 1984; 2269-2270
(41) Daimon T, David H, v.Zglinicki T, Marx I: Morphometric study on the absolute volume of differentiating hepatocytes in rats. Exper.Pathol. 1984; 26: 141-149
(42) David H: The hepatocyte - Development, differentiation, and ageing. Exper.Pathol. 1985; Suppl. 11: 1-148 VEB Gustav Fischer Verlag Jena
(43) Gellert K, David H, Otto G, Uerlings I, Wolff H: **Alteration of the ultrastructure of the pig liver after a continous perfusion of six hours in vitro. Exper. Pathol. 1985; 28: 245-250**
(44) Singh KP, David H, v.Zglinicki T: Association between mitochondria and rough endoplasmic reticulum (rer) in rat hepatocytes. Pathol. Res. Pract. 1985; 180: 49-53
(45) Singh KP, Krause W, David H, v.Zglinicki T: Effects of chloroquine on hepatic organelles in rat. Exper. Pathol. 1985; 28: 119-124
(46) David H, Reinke P, Bimmler M: Postnatal development of hepatocytes following oxygen deficiency in utero. Exper. Pathol. 1986 (in press)
(47) David H, Reinke P: Der vaskuläre Pol des Hepatozyten - Struktur, Funktion und Pathologie. Zbl. allgem. Pathol. u. pathol. Anat. 1986; (in press)

(48) Reinke P, David H: Struktur und Funktion der Sinusoidwand der Leber "Die Perisinusoidale Funktionseinheit" - eine Übersicht. Z. mikrosk.anat.Forsch. 1986 (in press)

(49) David H, Reinke P: Die perisinusoidale Funktionseinheit der Leber (ein neues Konzept der Pathogenese von Leber-erkrankungen - Stand der Erkenntnisse und Forschungsaufgaben). Studie 1986