

# STUDY OF MITOCHONDRIA IN RAT LIVER

## Quantitative Electron Microscopy

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### ABSTRACT

The electron microscope has been used to determine the weight distribution of isolated subcellular particles from normal rat liver. The following results are reported: (1) There exist at least two well defined weight populations of subcellular particles; their respective median weights are  $1.3 \times 10^{-14}$  and  $11 \times 10^{-14}$  gm. The lighter fraction is considered to consist of lysosomes, the heavier of mitochondria. (2) The mitochondrial fraction shows a log-normal distribution of the particle weight. (3) By the introduction of morphologic criteria, the mitochondrial fraction is divided into two groups, one consisting of a spherical, the other of an oblong type of particle. The data found support the following concepts: (a) Mitochondria increase their weight from a certain size up by linear growth. (b) Mitochondria divide. The division is not necessarily symmetric; in all cases, however, one part of the division product is a spherical particle. It is felt that these results constitute a valuable demonstration of the general capabilities of quantitative electron microscopy and may stimulate many other useful applications of this technique in cytology, bacteriology, and virology.

### INTRODUCTION

The size of mitochondria in fresh liver tissue lies in the range of the wave length of visible light. The characteristics of *individual* mitochondria cannot, therefore, be determined with light-optical techniques. Mean quantities as to mitochondrial volume and volume changes can be obtained from suspensions of whole *populations* of mitochondria only (light scattering); assumptions as to the shape of the mitochondria have yet to be made.

In quantitative centrifugation techniques a similar situation is encountered. Even elaborate methods cannot assess the distribution of size and/or weight of a mitochondrial population. The theoretical and experimental difficulties have been presented extensively by de Duve, Berthet, and Beaufay (1).

Centrifugation, however, is presently the only available preparatory method, and is employed together with enzymatic classification and/or electron microscopic morphology to "define" the fraction of the mitochondria.

The electron microscope can overcome many of the problems imposed by the size of the mitochondria. It has been applied to the study of isolated mitochondria as well as those in sections. Only geometric data have been derived from the micrographs, however. Because of their irregular shape, cumbersome planimetry of reconstructions from serial sections would be necessary to furnish more detailed information, not to mention the number of particles that would have to be measured to permit statistical analysis.

A new procedure using the electron microscope has been developed and described elsewhere (2) to determine quantitatively the dry weight of small biologic objects in the range from  $10^{-12}$  to  $10^{-18}$  gm. In short, the approach is the following: A biologic object is brought onto a membrane-coated grid and photographed in the electron microscope (Fig. 1). The plate is developed and subsequently evaluated in a special photometer (2). The photometric evaluation is based on the fact that, under certain conditions, the photo-

mensions of the granule are to be determined. For the determination of weight alone, neither swelling nor shrinkage of the object need be considered, provided that they do not involve loss of substance.

The aim of this paper is to analyze quantitatively the fraction of subcellular particles of rat liver homogenates and at the same time to demonstrate the useful applicability of the electron microscope to physical measurements of small biologic objects.

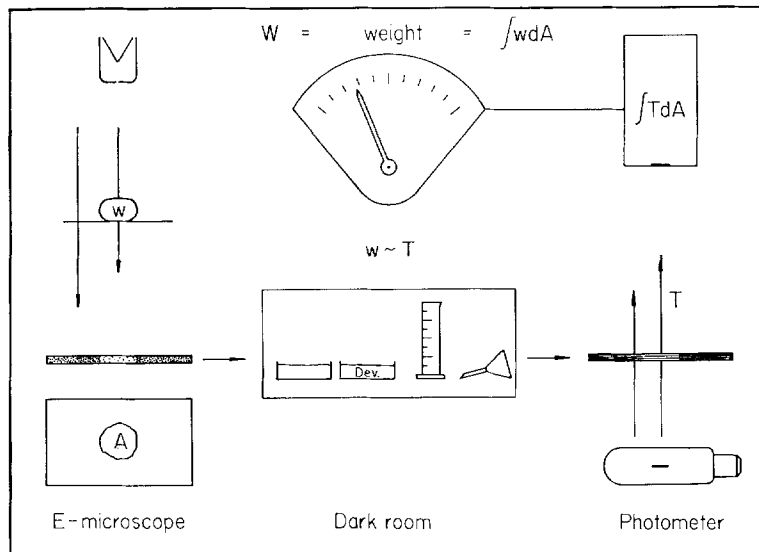


FIGURE 1

Scheme of the procedure for quantitative electron microscopy.  $w$ , weight per area;  $W$ , weight;  $T$ , photographic transmission;  $A$ , area.

graphic transmission through the micrograph is proportional to the mass per area of the object. Hence, the mean transmission, averaged by transilluminating the entire image area, is an equivalent of the dry mass of the object.

It was not the primary aim of this study to achieve optimal morphologic preservation as is usually the case with electron microscopy of sectioned material, but rather to provide basic data on mitochondria for the numerous biochemists interested in mitochondrial function. Hence, the preparation of the material follows the biochemical line. It is evident that no fixative containing heavy metal could be used, as it would change the weight of the object. The finer morphologic detail is of little concern when the weight and the rough di-

## MATERIALS AND METHODS

### Material

Wistar rats weighing from 250 to 320 gm were used in this study. They were fed commercially prepared food pellets and water *ad libitum*. Groups of six were kept in 1- by 2-foot wire cages at a controlled temperature of 24°C. Animals were killed by stunning and bleeding without previous fasting. The livers were quickly excised and dropped into cold sucrose, where they remained for at least 5 minutes to insure thorough cooling of the organ. For the isolation of normal mitochondria 0.44 M sucrose was used (Merck low-residue sucrose). It was decided to use this sucrose concentration because practically all of the enormous amount of biochemical work has been carried out with either 0.25 M (the so-called

“isotonic”) or 0.44 M sucrose. The latter concentration has frequently been used since Witter, Watson, and Cottone (3) reported a good preservation of mitochondria without change in their enzymatic activity. They found that further increase of sucrose concentration to 0.88 M entailed “morphological alterations ascribable to dehydration” and “alteration in enzymatic activity.”

After cooling, the liver was blotted, weighed, finely minced with scissors, and homogenized (1,000 RPM/5 minutes with a Teflon pestle-glass homogenizer). About 10 gm of liver homogenate was diluted with sucrose to 80 ml. All preparations were carried out in a cold room at 0°C. The temperature of the homogenates at no time exceeded +3°C. Centrifugations were carried out in a Spinco Model L centrifuge, with rotor no. 30. The following simple centrifugation scheme was adopted (after a considerable number of trial runs had demonstrated the complete separation of nuclei and whole cells as well as the ribonucleoprotein fraction from the homogenate): Spin at 3,000 RPM/10 minutes (790g at 7.8 cm  $R_{av}$ <sup>1</sup>); resuspend, 2,000 RPM/10 minutes (350g at 7.8 cm  $R_{av}$ ); resuspend again, 2,000 RPM/10 minutes (350g at 7.8 cm  $R_{av}$ ); discard pellet (nuclear); pool supernatants and spin at 20,000 RPM/10 minutes (34,800g at 7.8 cm  $R_{av}$ ) three times, each time discarding the supernatant and resuspending the pellet in fresh sucrose. After the last run the pellets are combined and resuspended in 5 ml sucrose. This suspension is then mixed with three times its volume of 10 per cent buffered formalin (pH 7.2) and fixed for 20 minutes. Light fixation with formalin has been used since it was found that the surface tension during the drying of unfixed preparations on a Formvar-coated grid crushed the particles.

Small droplets of the fixed suspension are then pipetted onto Formvar-coated copper grids. After standing 2 minutes, the droplets are blotted with the torn edge of filter paper, and after further drying in open air the grids are washed by gently dropping triple-distilled water on them. Again the water is removed by blotting with filter paper. The grids are now ready for electron microscopy.

### The Preparations

Homogenization was incomplete in that 18 to 20 per cent of the cells constantly remained unbroken. This was evident from differential counts with a phase contrast microscope of at least 400 free and cellular nuclei in the nuclear pellet. The percentage of unbroken cells may have been somewhat lower since free nuclei rapidly engorged sucrose and reached a refractive index close to that of 0.44 M sucrose, a

<sup>1</sup> Average distance of liquid column from center of centrifuge axis.

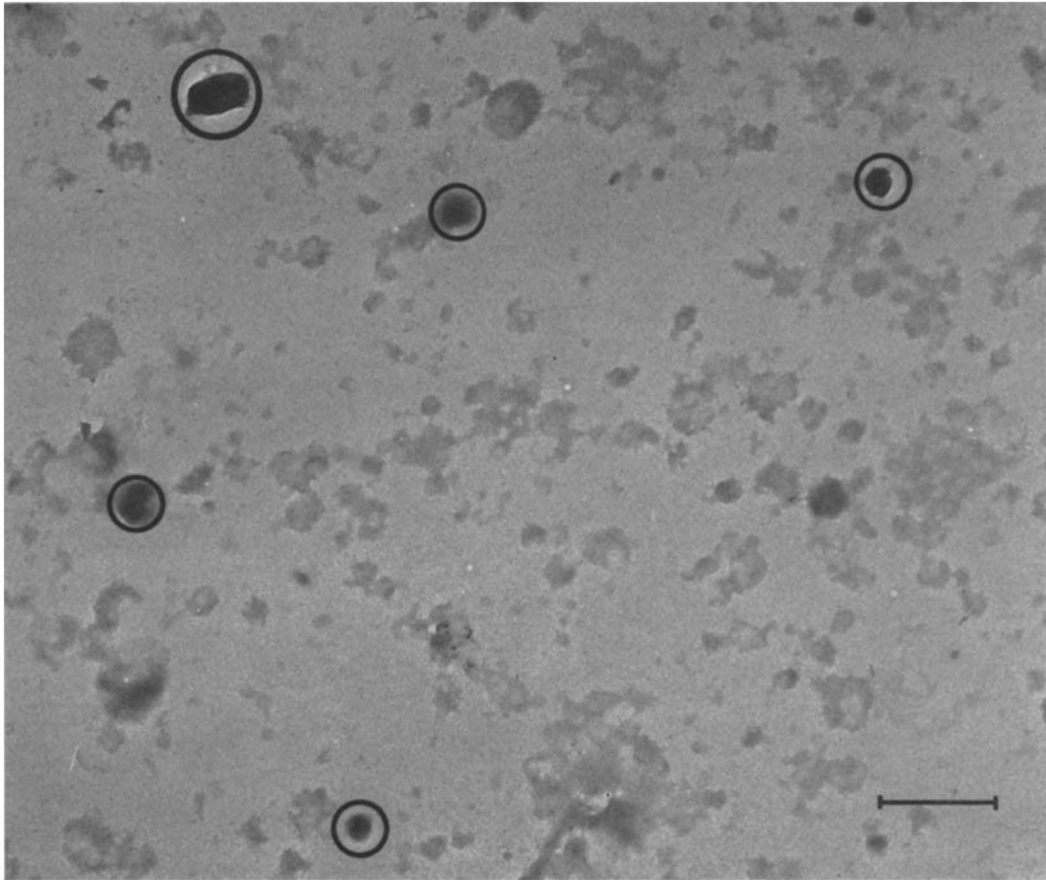
circumstance which made them difficult to discern. On the contrary, whole cells were easy to count since the refractive indices of mitochondria and fat globules in their cytoplasm provided a characteristic appearance. Morphologic evidence was not found that the unbroken cells constitute a particular group of cells. They probably were prevented from rupturing by the buffering effect of previously isolated nuclei, cell membranes, and connective tissue elements as well.

Under phase contrast the freshly isolated cytoplasmic granules appeared as round or elongated bodies freely moving and tumbling in the medium. No clumping was observed. The size ranged from 5  $\mu$  (length of rods) down to the limits of resolution of the phase contrast microscope.

The centrifugation scheme was chosen to separate first the nuclei and whole cells from the homogenate and leave the other cytoplasmic constituents in suspension. A check with the phase microscope confirmed that only a few free mitochondria were found in the nuclear-whole cell pellet. These may well have been released from cells broken during the resuspension of this pellet.

Next, the so-called “mitochondrial fraction,” which is the classic material of biochemical studies, was spun down, leaving the so-called microsome fraction in the supernatant. To check the supernatant for the presence of remaining small mitochondria and other cytoplasmic particles in that same size range, it was spun at a higher centrifugation rate, namely, 20,000 RPM/10 minutes (34,800g at 7.8 cm  $R_{av}$ ). The pinkish pellet, being the heavier part of the classical “microsome fraction,” showed little indication of remaining particles in the phase microscope. In the electron microscope, however, numerous spherical or ovoid “electron-opaque bodies” were found (Fig. 2), which could not be considered to be microsomes and which probably had been prevented from sedimenting with the mitochondrial fraction by retention in the maze of microsomal structures. The centrifugation scheme was, therefore, devised to collect the mitochondrial fraction together with this particulate material found at 20,000 RPM, *i.e.*, the mitochondrial fraction and the heavier part of the microsome fraction were spun down together at 20,000 RPM/10 minutes.

The completeness of isolation of all cell granules was assured not only with the light and electron microscope but also with specific staining. The nuclear pellet as well as the granule pellet were treated with the Janus green B stain (4). Any staining observed in the nuclear pellet could be attributed to mitochondria in unbroken cells. The granule pellet to be investigated with quantitative electron microscopy displayed three relatively distinct layers of staining. At the very bottom a few erythrocytes and



**FIGURE 2**

Electron-opaque bodies (some marked with circles) found in the supernatant after the mitochondrial fraction was spun down at higher centrifugation rate. These particles have been included in the study to avoid arbitrary selective preparation.  $\times 11,000$ .

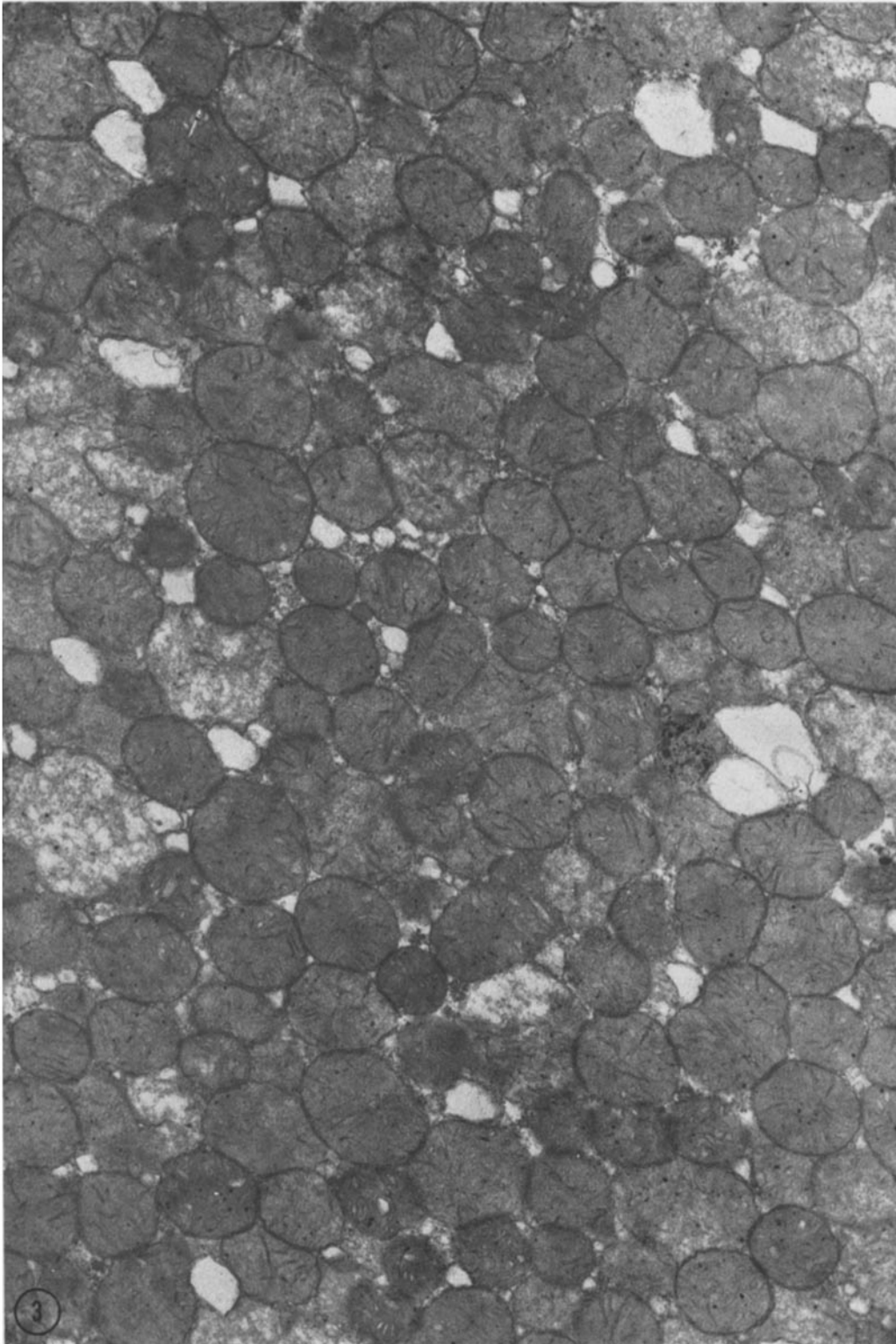
nuclei were associated with a whitish, somewhat transparent accumulation of glycogen. The layer lying on top of the latter appeared dark blue and was identified as the mitochondrial fraction. The uppermost layer showed a gradient of blue color fading to pink at the very top of the pellet. Upon standing, the blue color in the mitochondrial layer and the adjacent parts of the top layer faded and became a brownish grey. The uptake, distribution,

and decolorization of Janus green B were thus further evidence that practically all of the mitochondria were included in the material to be analyzed by quantitative electron microscopy.

Granule pellets were also resuspended in cold 0.44 M sucrose, fixed for 20 minutes in neutral formalin, spun again to a firm pellet, fixed in Dalton's chrome osmium fixative, poststained according to Parsons (11), and embedded in Epon. Fig. 3 shows a thin

**FIGURE 3**

Section from pellet obtained at 20,000 RPM/10 minutes (34,800g at 7.8 cm  $R_{av}$ ). Post-staining according to Parsons (11). The majority of the mitochondria show excellent preservation, a few are slightly swollen. Smaller, chiefly spherical, bodies and "empty" vesicles are seen among the mitochondria.  $\times 19,000$ .



section of such material, illustrating that the majority of the cell granules collected after the 10-minute centrifugation at 20,000 RPM clearly consists of elements with the well known morphologic characteristics of mitochondria. The degree of preservation is markedly better than that reported by others using similar concentrations of sucrose (3). Fig. 4 is an electron micrograph from a typical formalin-fixed suspension of rat liver particles.

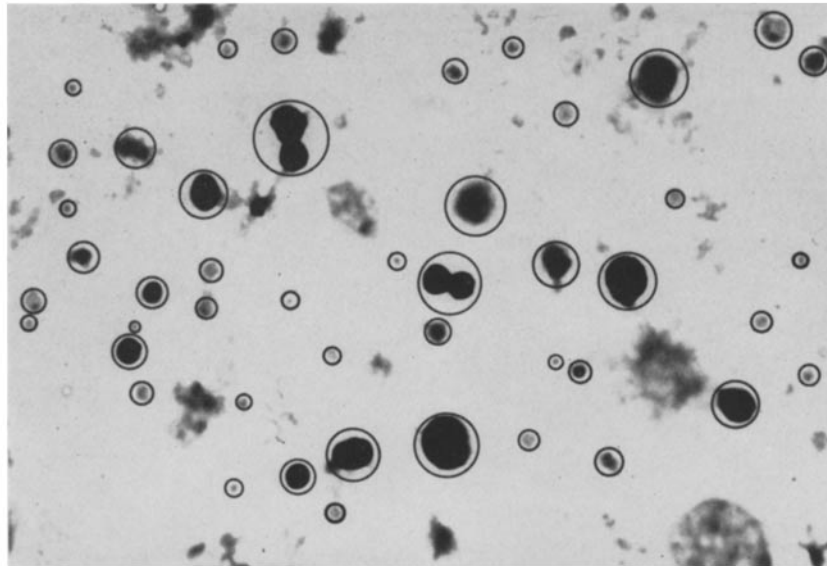


FIGURE 4

Typical preparation of formalin fixed rat liver particle suspension. A few particles are encircled to indicate the principal way of selecting them for measurement.  $\times 8350$ .

#### ELECTRON MICROSCOPY

A Siemens electron microscope was used throughout this study. The high voltage was set at 100 kv; objective and condenser apertures of  $100 \mu$  were used.

Batches of 12 plates were developed together (5 minutes, Kodak D-72, 1:1,  $20^{\circ}\text{C}$ ); each twelfth plate represented a weight standard (latex-sphere preparation). Prints were made from each plate; objects suitable for measurement were marked on the print so that a correlation between individual object and measurement was established. The criteria for the selection of particles for measurement were the following:

- (a) All particles on the micrograph being larger than  $1 \mu$  (smallest diameter) at an electron microscopic magnification of 20,000 times are measured.
- (b) The particle has to lie singly.
- (c) Obvious debris or objects with uncertain outlines are avoided (Figs. 2, 4).

#### RESULTS

In Fig. 5 the weight distribution of liver particles determined in three normal rats are represented. Each distribution consists of more than 900 particles. It was found that a plot of the integral frequency (*i.e.*, the frequency with which mitochondria occur whose weight is equal to or smaller

than a given one) on probability paper with a logarithmic abscissa yields a very clear and informative representation.

The values from the three animals (indicated in the graph as circles, squares, and triangles) coincide very well, especially in the straight-line part of the heavier particles. The more apparent deviations in the distribution of the lighter particles concern, however, only a small percentage of particles. The deviations become more prominent in this form of presentation only since the division of the ordinate for lower percentages is expanded.

The existence of at least two populations (fractions) of particles is indicated by two easily discernible slopes in the plot. This fact becomes very clear if the entire populations depicted in Fig. 5 are grouped in two fractions, one of which consists

of particles lighter, and the other of particles heavier, than  $3 \times 10^{-14}$  gm. This weight was not chosen arbitrarily. Only dividing the population at  $3 \times 10^{-14}$  resulted in one perfectly straight line for the heavier particles of three animals (Fig. 6), indicating that they have a log-normal distribution of weight and constitute a particular fraction. This fraction is considered to consist of mitochon-

are compiled. The median weight<sup>2</sup> of the heavy fraction, for all three animals, lies at around  $11 \times 10^{-14}$  gm. These values agree very well with those determined by de Duve *et al.* (1). The median weight of the light fraction is about  $1.3 \times 10^{-14}$  gm, with greater deviations from animal to animal for reasons already mentioned. There is thus a difference of one order of magnitude between the

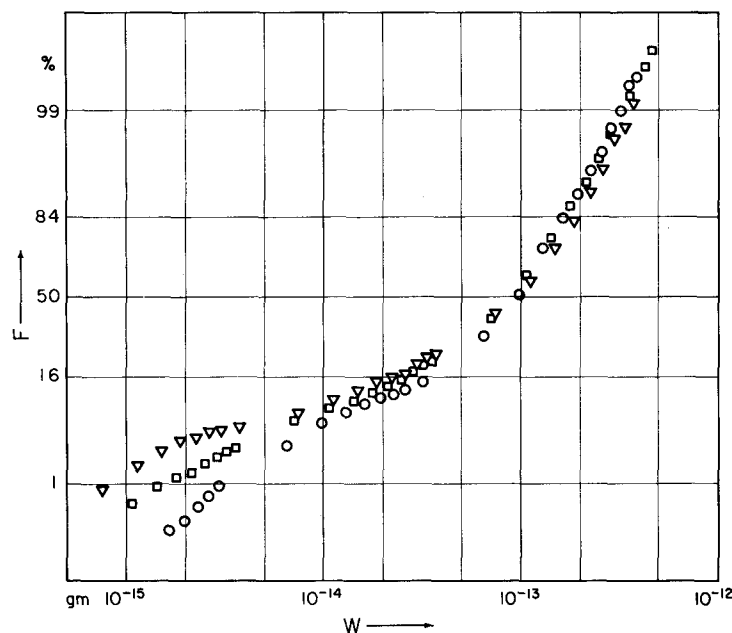


FIGURE 5

Weight distribution of the isolated particles. Circles, squares, and triangles signify populations from different animals.  $F$ , integral frequency;  $W$ , weight.

dria, since the bulk of the particles that have been analyzed exhibit indeed the morphologic characteristics of mitochondria (Fig. 3).

The lighter fraction is not so clearly defined, and other experiments (starvation, partial hepatectomy) show that it is subject to considerable variation. Therefore, the differences from animal to animal in Fig. 6 may be due to small variations in the physiology of their livers. This fraction is correlated to the lysosome fraction of de Duve (1) and Novikoff (5). The designation of the lighter fraction as lysosomes, however, is tentative and awaits further (enzymatic) studies to test this suggestion. A still smaller population of particles can be isolated in this way, but the significance of this is yet unknown.

In Table I the characteristics of both fractions

two fractions. The identity of the heavy fractions from three animals is confirmed by nearly equal standard deviation values  $\sigma$  of 0.21. It should be pointed out that this standard deviation is related to the logarithm of the weight; it means that 68 per cent of the particles of the heavy fraction have a weight that lies within the limits of  $6.8$  to  $17.8 \times 10^{-14}$  gm. For the light fraction root-mean-square values have not been calculated because a certain heterogeneity cannot be excluded. The heavy fraction—the mitochondria—represent roughly 80 per cent by number of the entire population. The following will deal with this fraction only.

The heavy fraction is characterized by its very uniform log-normal distribution, which varies

<sup>2</sup> See Appendix.

little from animal to animal. In biologic material a log-normal distribution is generally found, for the simple reason that the occurrence of the value "zero" for fundamental characteristics such as weight or size must be zero. This basic requisite is fulfilled *per se* by the log-normal distribution, whereas the normal distribution yields finite occurrences even for the variable being zero.

The electron micrographs of the particles of

the heavy fraction contain more information than just their weight. If a new fundamental parameter is introduced, namely, the geometric appearance, two fractions with almost equal occurrence are found. One consists of round particles while the other is characterized by oblong particles.

In Fig. 7, examples of these two forms have been mounted together and demonstrate that they can clearly be distinguished. The distributions re-

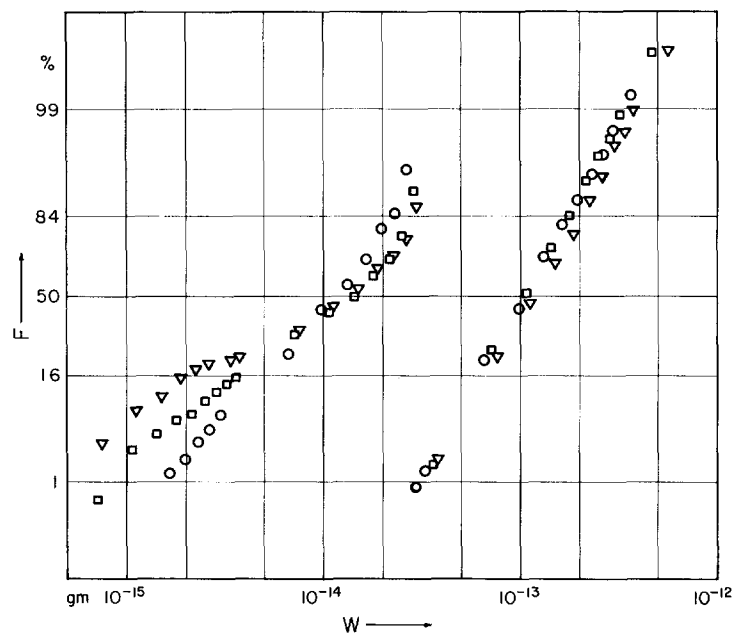


FIGURE 6

Separation of populations from Fig. 5 into a heavy and a light fraction. The heavy fraction shows a distinct log-normal distribution (straight line) and is referred to as the mitochondrial fraction.

TABLE I  
Quantitative Data on Weight Distribution of Rat Liver Cell Particles

Animal	1	2	3
Weight of animal (gm)	258.1	272.0	252
Weight of liver (gm)	10.1	12.1	10.0
Particles measured	1051	906	924
<i>Heavy Fraction</i> $> 3 \times 10^{-14}$ gm			
Per cent	87	77	80
Median weight ( $\times 10^{-14}$ gm)	10.1	11.7	10.7
$\sigma$ (log W)	0.214	0.217	0.206
<i>Light Fraction</i> $< 3 \times 10^{-14}$ gm			
Per cent	13	23	20
Median weight ( $\times 10^{-14}$ gm)	1.1	1.3	1.4



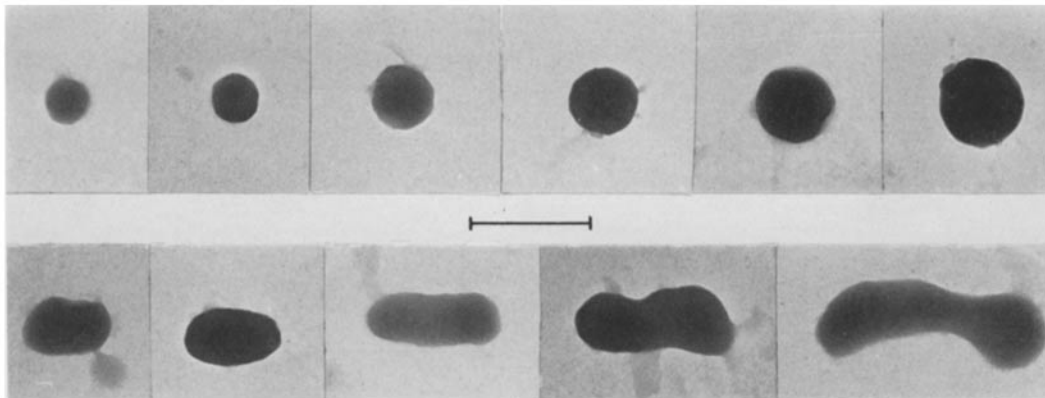


FIGURE 7  
Examples of the two morphologic types of mitochondria.

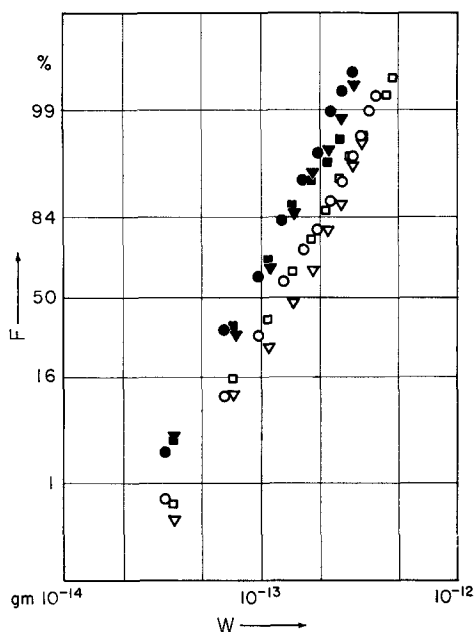


FIGURE 8  
Weight distribution of the round and the oblong mitochondria.  $F$ , integral frequency;  $W$ , weight. Open symbols refer to oblong mitochondria. Solid symbols refer to round mitochondria.

sulting from this "morphologic labeling" are represented in Fig. 8. Both fractions show a log-normal distribution of their weight population with almost identical slopes (root-mean-square values). The median weight, however, of the oblong particles, indicated by open symbols in the graph, is for all three rats larger by a factor 1.6

than that of the round particles. The pertinent data are given in Table II.

The length,  $D_1$ , and the width,  $D_2$ , of particles heavier than  $3 \times 10^{-14}$  gm have been measured on the micrograph and correlated to the weight of the individual particle. In the case of round particles both dimensions coincide. In Fig. 9 the logarithms of  $D_1$  and  $D_2$  are plotted versus the logarithms of the weight. The plot shows that mitochondria from a certain weight up are elongated. On the average the smaller axis of the oblong particles is the same as the average diameter of the round mitochondria. Applying the Gaussian method of least squares, a regression line for  $\log D_1$  and  $\log W$  with a slope of 0.89 is found, confirming an axial arrangement of the dry mass in the particle. The correlation coefficient for the variables  $\log D_1$  and  $\log W$  is 0.78, indicating a fairly strong correlation.

The fact that the distribution of the oblong and round particles shows almost identical root-mean-square values excludes the possibility of interpreting the oblong particles as a mere statistical accumulation of two or more round particles. This fact furthermore indicates a principle distribution scheme for the weight of mitochondria through the entire liver regardless of type and location of the respective cell.

All particles that are not round have been described as oblong. Among these oblong particles, however, remarkable morphologic variations have been found and they demand special attention. Examples of the different morphologic types are given in Fig. 10. One type may be described as

dumbbell-shaped (*A*) and accounts for 8 per cent of the oblong fraction. Five per cent consist of two closely connected round particles (*B*), which are not simply two individuals lying accidentally together. A third type (*C*), here called compound mitochondrion, consists of an association of a round and an oblong particle. The compound particles

of the particles on the membrane-coated specimen grid. Several of these grids were rewashed with the intention of removing any residual sucrose. A comparison of the weight distribution of these two preparations, the normally treated and the rewashed ones, did not show any difference. The conclusion drawn from this experiment was that

TABLE II  
*Quantitative Data on Weight Distribution of Rat Liver Cell Particles, Applying "Morphologic Labeling"*

Animal	1	2	3
	<i>Round</i>		
Particles measured	407	339	319
Per cent heavy fraction	45	49	45
Median weight ( $\times 10^{-14}$ gm)	8.0	8.6	8.0
$\sigma$ (log W)	0.201	0.214	0.200
	<i>Oblong</i>		
Particles measured	500	353	399
Per cent heavy fraction	55	51	55
Median weight ( $\times 10^{-14}$ gm)	12.1	14.4	12.5
$\sigma$ (log W)	0.221	0.221	0.206
Ratio of median weights oblong/round	1.54	1.68	1.57

occur with a frequency of 7 per cent. Various intermediate forms of these three types are found.

#### *Influence of the Sucrose Concentration*

The concentration of sucrose in the isolation medium was suspected of influencing the results of weight determination in two respects: (*a*) The amount of sucrose remaining in or on the isolated particles could alter their true dry weight in proportion to the sucrose concentration. (*b*) The different tonicities might influence the shape of the particles.

To clarify these points, preparations of mitochondria from homogenates in four different sucrose molarities—namely, 0.125 M, 0.25 M, 0.44 M, and 0.88 M—were prepared. In the centrifugation procedures the specific gravity and viscosity of the different molarities have been taken into consideration.

To study the first point, mitochondria were isolated in the rather viscous 0.88 M sucrose and processed for quantitative electron microscopy as described above. This involves one gentle washing

the preparation technique used leaves only a small amount, if any, of sucrose beyond the sensitivity of the electron microscopic weight determination and it is therefore immaterial.

In order to consider the second point, the ratio of round to oblong particles was determined at three different sucrose concentrations (0.125, 0.250, 0.44 M). In all three cases the same ratio of both types was found on the micrographs, which indicated that the shape of the dried mitochondria is not simply a consequence of the tonicity of the isolation medium.

In the high concentration of 0.88 M sucrose, more oblong particles were found. This might have been brought about by the centrifugation procedure, which had been altered with respect to the increased viscosity only but not with respect to a possible shrinkage of the particles in the hypertonic medium. An extensive study on the effect of the isolation media is under way. It is felt, however, that the lack of a final clarification of this general problem does not restrict the aim of this paper.

### Accuracy of the Results

The determination of weight distribution involves errors inherent in the photometric procedure itself. It was found (2) that these errors were in the order of magnitude of  $\pm 8$  per cent. The inaccuracy of the median weights reported in this paper is merely a statistical error. Since in

### DISCUSSION

The quantitative data as to weight and morphology of isolated liver granules obtained during this first application of quantitative electron microscopy to a biologic problem demonstrate the capabilities of this method. They provide also ample ground for reconsidering the problem of growth

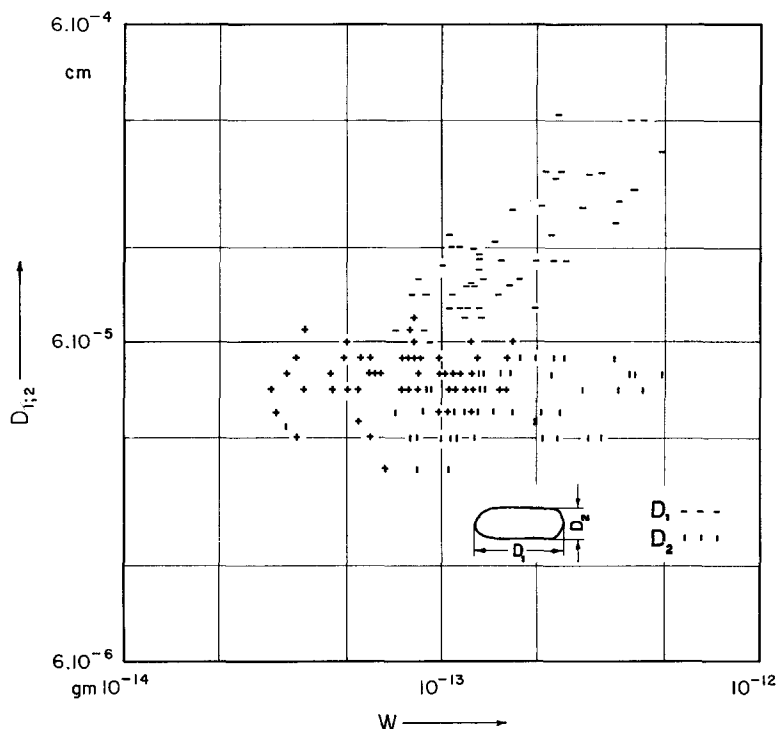


FIGURE 9

Correlation between weight of mitochondria and their geometric dimensions. (For the sake of clarity only 50 measurements are shown.)  $D_1$ , length;  $D_2$ , width.

all instances more than 500 particles were measured, errors in the median weights are of the order of  $(500)^{-1/2} = \pm 4.5$  per cent. The total error of the weight-determining procedure, being the geometric sum of the individual errors, remains under  $\pm 10$  per cent.

Errors in the determination of particle diameters are of the order of  $\pm 10$  per cent. It did not seem reasonable to improve the accuracy of the measurements since the oblong particles often have irregular shape, making it difficult to define the dimensions.

and multiplication of mitochondria. Practically all possible modes of increasing the mass and/or number of mitochondria have been advanced as theories. Evidence in favor of each has been reported. Recently Novikoff (6) has critically reviewed these questions, and it appears that there is not sufficient evidence available for any theory. There is, however, enough incentive to consider carefully all possible ways of mitochondrial replication, since on the one hand compelling evidence for the presence of RNA in mitochondria has been brought forward (7, 8), and on the other hand it has been shown that mitochondria may be one of

the possible carriers of non-chromosomal heredity (9).

Although the data obtained during this study do not prove the division of mitochondria, they—of all possibilities—can most easily be brought into agreement with the concept that mitochondria divide. In the first place, the heavier median weight of oblong mitochondria suggests that in-

sphere persists. Finally a separation of rod and sphere takes place with some protein strands still holding them together. The association of a sphere with a rod is not an artifact; often their connection is broad and substantial. In most cases the sphere is joined with the end of the rod, but attachment to any other point of the rod has been observed. All possible divisions of mitochondria are thought

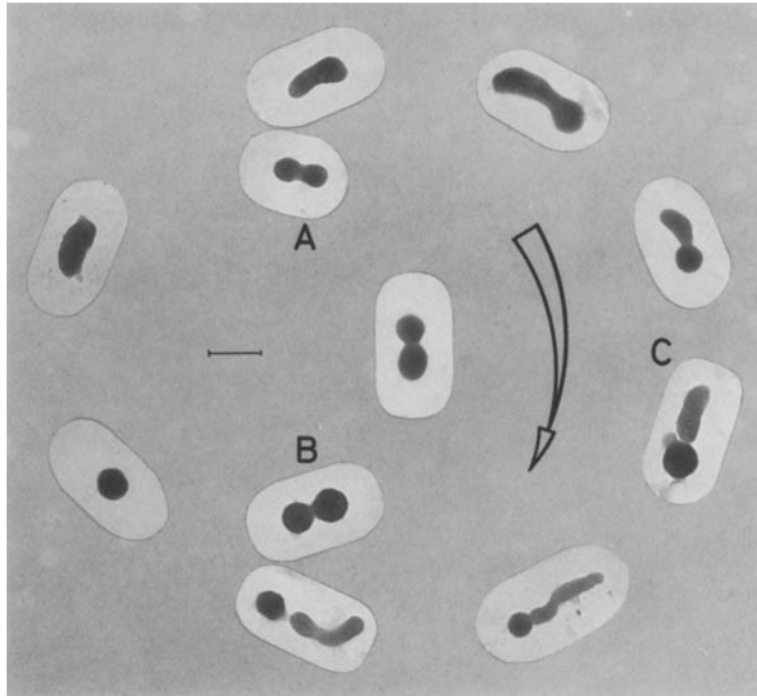


FIGURE 10

Variations in morphologic types of oblong particles: *A*, dumbbell-shaped particle; *B*, closely connected round particles; *C*, compound particles. Arrow indicates direction of two suggested cycles of hypothetical mitochondrial division. The reality probably lies between these two extreme circles.

crease of their mass from a certain size up produces linear growth. This leads to a duplication of mass, upon which division may occur. In the second place, there is morphologic support for this concept of mitochondrial growth and division.

In Fig. 10 two cycles of possible divisions are mounted together. The inner circle suggests the division of an oblong mitochondrion passing through a dumb-bell shape into two spheres in a symmetric fashion. The outer circle proposes a division in an extremely asymmetric way. A round particle assumes a club-shaped appearance. The rod extends increasingly while the original

to occur between the two extreme cycles of division, symmetric or asymmetric.

In the case of compound mitochondria it is not unequivocally clear whether the rod sprouts from the sphere or the sphere is pinched off from the rod. Whatever the mode of origin, however, the existence of prominently dense spheres may hint an essential role in the propagation of a structural template.

The finding that the weights of the individuals in the two particle populations are following a log-normal distribution is not particularly sur-

prising. The extensive karyometric studies of Bucher (10), already mentioned above, also report a log-normal distribution for liver nuclei.

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#### APPENDIX

There are several significant values by which to characterize a given frequency distribution of variable  $X$ . (a) The median  $X_m$  is defined so that it divides the distribution in equal parts, of which the one consists of smaller and the other of larger values than  $X_m$ . (b) The mode  $X_0$  represents the most frequently occurring value of  $X$ . (c) The mean value  $\bar{X}$  is simple the arithmetic average. (d) The standard deviation SD;

$$(SD)^2 = \bar{X^2} - \bar{X}^2$$

For the weight of mitochondria a log-normal distribution was found; *i.e.*, identifying  $X$  with the logarithm of the weight, the differential distribution of  $X$  is a Gaussian curve. This implies that all three characteristics—the median, the mode, and the mean—are identical.

The distribution of the weight, instead of the logarithm of the weight, however, is not normal. The small weights occur more frequently than the large ones; the distribution is skewed, with an asymmetric tail towards large values.

One may now be interested in the significant values of the linear distribution. Certainly there exists a simple relationship between the two sets of characteristics of the logarithmic respective linear distribution. Because of the importance of log-normal distribution in biology it seems worthwhile to compile these relationships.

	Logarithmic	Linear
Median $X_m$	$(\log W)_m = a$	$W_m = 10^a$
Mode $X_0$	$(\log W)_0 = a$	$W_0 = W_m e^{-b}$
Mean $\bar{X}$	$\overline{\log W} = a$	$\bar{W} = W_m e^{b/2}$
$(SD)^2$	$(\overline{\log W})^2 - a^2 = \sigma^2$	$\overline{W^2} - \bar{W}^2$
$\bar{X^2} - \bar{X}^2$	$b = (\sigma/0.43)^2$	$= \overline{W^2} (e^b - 1)$

The median value of the linear distribution is given as the antilogarithm of the median value of the logarithmic distribution. This fact explains the preference given to this value in the present paper. The SD value of the logarithmic distribution enters into all the remaining conversions of the characteristics.