The basic stereological formulas for estimating volume ($V_v$) and surface ($S_v$) densities are strictly valid only for true infinitely thin sections; the use of "ultrathin" sections of finite thickness $T$ introduces systematic errors, mostly in the sense of overestimation of the parameters. These errors depend on the size and shape of the structural elements and on $T$. Correction factors for this effect of $T$ are derived by considering model structures that simulate the shape and arrangement of subcellular organelles: (a) spherical vesicles, (b) disks as models for rough endoplasmic reticulum (RER) cisternae, (c) cylindrical tubules as models for smooth endoplasmic reticulum (SER) tubules, microvilli, etc. For vesicles, a model of discrete convex spherical particles is assumed; the correction factors consider loss of caps due to grazing sections and size distribution of the vesicles. The disk and tubule models are used in connection with the new integral geometric formulas of R. E. Miles which consider random aggregates of "interpenetrating" particles so that the resultant structure is non-convex and thus approximates in nature the networks characteristic of endoplasmic reticulum (ER). Some practical examples relative to liver cells show that the errors due to section thickness may be of the order of 20–40% or more. Computation formulas as well as graphs are given for the determination of the correction factors for $V_v$ and $S_v$. 

KEY WORDS: stereology, section thickness, correction factors, organelle volume density, membrane surface density.
organelles under consideration as well as on their dimension relative to section thickness, and both these conditions were known to change when intact cells are homogenized as done for subcellular fractionation. Thus, endoplasmic reticulum (ER) of liver cells changes from broad cisternae and tubules in the intact cells to spherical microsomal vesicles of variable diameter in the subcellular fractions. Similar changes may affect the plasma membrane and mitochondria. We have therefore attempted to seek ways by which such errors could be compensated by appropriate corrections, adaptable to the specific conditions of the preparations.

The errors due to finite section thickness have been repeatedly estimated since their first recognition in 1927 by Holmes (9), whence their common designation as "Holmes effect." Most studies have dealt with spherical particles of homogeneous size (3, 5–7, 9); infinitely large plates and cylinders were also considered by Hennig (8). General formulas were derived by Cahn and Nutting (2) and DeHoff (4), but their direct applicability is limited because they require the estimation of several stereological measurements which all depend on section thickness; furthermore, they are strictly applicable only in cases where the components can be represented by convex particles which are very loosely spaced, which is often not the case with cellular components. This latter restriction has been eliminated by Miles (10) who has derived general formulas relating true parameters of structure in an aggregate of nonconvex elements to apparent stereological measurements obtained in sections of finite thickness. The formulas of Cahn and Nutting (2) and DeHoff (4) are special cases of those of Miles (10).

In this paper, we shall derive correction factors for estimates of volume \( V_v \) and surface density \( S_v \) of membrane-bounded cellular organelles obtained on sections of finite thickness by exploiting the theoretical formulas of Miles (10), and partly those of Cahn and Nutting (2) and DeHoff (4). Approximate correction factors for some specific classes of organelles will then be evaluated.

**THEORETICAL DERIVATION OF CORRECTION FACTORS**

**Expected Effects of Section Thickness on Stereological Estimates**

The basic formulas of stereology assume that the measurements are done on true sections of thickness zero. Under this condition, the areal density of profiles, \( A_A \), and their boundary length density, \( B_A \), are simply related to volume and surface density, respectively, by (14):

\[
A_A = \frac{V_v}{T}
\]

\[
B_A = \frac{\pi}{4} S_v.
\]

In reality, an ultrathin section is a slice of finite thickness \( T \) whose entire content is projected onto the micrograph. Granting perfect contrast (totally opaque objects in a translucent matrix), the observed areal and boundary length densities, \( A_A' \) and \( B_A' \), respectively, are larger than those predicted by the basic formula:

\[
A_A' > \frac{V_v}{T}
\]

\[
B_A' > \frac{\pi}{4} S_v.
\]

We shall call this effect "overprojection" of profiles.

If the contrast conditions are imperfect, i.e., if the objects are only slightly denser than the embedding matrix, some profiles which do not penetrate across the entire section thickness may be lost. This effect, called "truncation," applies mainly to grazing sections in which only a shallow cap profile is included in the section. This truncation effect will tend to reduce the overestimation resulting from overprojection; in some cases, it may even surpass it, resulting in an underestimation of the parameters.

If the apparent areal or boundary length densities, \( A_A' \) and \( B_A' \), are used to estimate \( V_v \) and \( S_v \), respectively, the estimates will be affected by a certain bias or systematic error which depends on the shape of the objects and on their size relative to section thickness.

**Approach to Derivation of Correction Factors**

To estimate the magnitude of this error, we shall examine model structures, made of geometrically well-defined objects, for which \( A_A' \) and \( B_A' \) can be calculated as a function of section thickness \( T \), and \( V_v \) and \( S_v \), respectively. The model objects will be chosen in such a way that they simulate the real objects as faithfully as possible. In view of correcting measurements on cell constituents, the
The following shapes have been found to be useful (Fig. 1): (a) spherical vesicles of diameter \(d\) as models for cytoplasmic vesicles, lysosomes, and microsomal vesicles in fractions; (b) thin disk-shaped membrane spaces of thickness \(d\) and diameter \(D = \delta \cdot d\) as models for cisternae of RER and Golgi stacks; in part also for smooth portions of the plasma membrane; (c) long cylindrical tubules of diameter \(d\) and length \(L = \lambda \cdot d\) as models for SER and microvilli. Model structures are constructed by assembling such elements by some process of randomization. Two basic possibilities exist which we shall call models A and B, respectively.

**MODEL A: STRUCTURES MADE OF DISCRETE CONVEX OBJECTS:** The first method for constructing model structures by means of these elements is to assemble them by a random process which allows all orientations and all positions, with the exception that the objects may not overlap. With \(N_v\) = numerical density of objects, \(\bar{v}\) = mean object volume, \(\bar{s}\) = mean object surface, and \(\bar{H}\) = mean tangent diameter of the objects, the apparent areal density of profiles on a slice of thickness \(T\) is (2):

\[
A_{\alpha}' = N_v \left( \bar{v} + \frac{T}{4} \cdot \bar{s} \right)
\]

\[
= V_v + T \cdot S_v, \tag{3a}
\]

and the apparent boundary length density (4):

\[
B_{\alpha}' = N_v \cdot \pi \left( \frac{\bar{s}}{4} + T \cdot \bar{H} \right) \tag{4a}
\]

\[
= \frac{\pi}{4} S_v + N_v \cdot T \cdot \pi \bar{H}. \tag{4b}
\]

The true model parameters are, evidently,

\[
V_v = N_v \cdot \bar{v}, \tag{5}
\]

\[
S_v = N_v \cdot \bar{s}. \tag{6}
\]

Models of this type suffer from the limitation that the random process of assembling the objects must avoid overlaps; they are valid only for cases where \(V_v\) is very small so that the chance of overlap is negligible.

**MODEL B: STRUCTURES MADE OF NON-CONVEX OBJECTS:** This limitation is avoided by making use of models as proposed by Miles (10). One starts again with convex elements of specified shape, defined by: \(\gamma\) = mean volume of the element, \(\sigma\) = mean surface of the element, \(\zeta\) = mean tangent diameter of the element, and \(\nu_v\) = numerical density of the elements.

In assembling these elements, their centroids are placed at points of a poisson random process in three-dimensional space, and they are allowed to take any orientation in space. In contrast to model A, the elements are allowed to overlap; the resulting objects are now not necessarily convex as they result from the union of partly overlapping (interpenetrating) elements. Note that the dimensions of the elements of structure have no real value, as part of their volume or surface is "lost" due to overlap.

For such a model, the true volume and surface densities of the objects are (10):

\[
V_v = 1 - \exp\{-\nu_v \cdot \gamma\} \tag{7}
\]

\[
S_v = \nu_v \cdot \sigma \cdot \exp\{-\nu_v \cdot \gamma\}. \tag{8}
\]

Similarly, the apparent areal and boundary length densities on slices of thickness \(T\) are:

\[
A_{\alpha}' = 1 - \exp\{-\nu_v \left[ \gamma + T \cdot \frac{\sigma}{4} \right]\} \tag{9}
\]

\[
B_{\alpha}' = \pi \cdot \nu_v \left[ \frac{\sigma}{4} + T \cdot \zeta \right] \cdot \exp\{-\nu_v \left[ \gamma + T \cdot \frac{\sigma}{4} \right]\}. \tag{10}
\]

It is noticed that the terms in square bracket are similar to those found in Eqs. 3 and 4 for model A. Indeed, as the number of elements becomes very small, overlap becomes very rare and Eqs. 9 and 10 reduce to Eqs. 3 and 4, respectively.

**CORRECTION FACTORS:** With any of these two models, it is possible to define, as a function

\[
\text{exp}(m) = e^m.
\]

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of shape, size, and frequency of the elements, the true volume and surface density as well as the apparent parameters as estimated on sections of thickness \( T \). Under the condition that the model is a faithful representation of the real structure, we can assume that the ratio of true to apparent parameter in the model is approximately equal to the ratio of true to apparent parameter in the real structure. Thus, the ratios,

\[
K_v(T) = \frac{V_v}{V_v'} = \frac{V_v}{V_v' A_{v'}}
\]

and

\[
K_s(T) = \frac{S_v}{S_v'} = \frac{4 \cdot B_{v'}}{\pi}
\]

constitute correction factors by which the true volume and surface densities can be estimated from the apparent parameters determined on sections of thickness \( T \):

\[
V_v = K_v(T) \cdot A_{v'} = K_v(T) \cdot P_{v'}
\]

\[
S_v = K_s(T) \cdot 4 \cdot B_{v'} = K_s(T) \cdot 2I_{v'}
\]

where \( P_{v'} \) is the density of test point hits and \( I_{v'} \) the density of intersections on test lines, used for estimating the parameters by point counting procedures (14).

The correction factors \( K_v \) and \( K_s \) can be derived by introducing Eqs. 3-10 into Eqs. 11 and 12.

**Correction Factors for the Sphere Model**

**Correction for Overprojection:** For those cases in which spherical vesicles best describe the elements of structure (microsomal vesicles, lysosomes, Golgi vesicles, etc.), the elements can be assumed to be discrete convex objects; hence model A applies. For the simplest case of vesicles of equal size \( d \), the correction factors are derived by substituting,

\[
v = \frac{\pi}{6} d^3
\]

\[
s = \pi d^2
\]

into Eqs. 3 and 4. They are found to be:

\[
K_v(T) = \frac{2d}{2d + 3T} = \frac{2}{2 + 3g}
\]

\[
K_s(T) = \frac{\pi d}{\pi d + 4T} = \frac{\pi}{\pi + 4g}
\]

where \( g = T/d \) is relative section thickness. These are the factors derived by Holmes (9) and Hennig (6, 7).

**Correction for Overprojection and Truncation:** In the case of spherical vesicles, the loss of small cap sections (truncation) becomes an important consideration. Assume that the vesicle has to penetrate at least by a “cap height” \( h_o \) into the slice before a profile can be detected (Fig. 2); the radius of the “smallest visible cap,” \( r_o \), is then found from:

\[
h_o = R - \sqrt{R^2 - r_o^2} = R(1 - \sqrt{1 - \rho^2})
\]

where \( \rho = r_o/R = 2r_o/2R \) is the relative radius (or diameter) of the smallest visible cap section.

To derive correction factors, we must calculate the apparent areal and boundary length densities from the apparent numerical density of profiles, \( N_v' \), and their apparent mean area \( \bar{a}' \) and boundary length \( \bar{b}' \):

\[
A_v' = N_v'(a')
\]

\[
B_v' = N_v'(b')
\]

This cannot be derived by direct application of Eqs. 3 and 4 because they do not account for truncation effects. Instead, \( N_v', a', \) and \( b' \) must be arrived at by an integration procedure allowing for all positions of the sphere relative to the section. Fig. 2 shows that a profile is formed if the sphere center is within a “superslice” of thickness \( T + 2R \). If a profile becomes recognizable only after the sphere has penetrated into the section by \( h_o \), recognizable profiles are formed if the center is in a “reduced superslice” of thickness \( T + 2R - 2h_o \). According to Floderus (5), the number of profiles observed is then:

\[
N_v' = N_v'(T + 2R - 2h_o)
\]

\[
= N_v' \cdot R(g + \sqrt{1 - \rho^2})
\]

As shown in Fig. 2a, the radius of a profile, \( r \), depends on the vesicle radius \( R \) and on the position of the vesicle center relative to the section. Two cases are to be distinguished (Fig. 3):

(a) The vesicle center is outside the slice;
measuring the position by $x$, where $x = 0$ is at a distance $R$ above the slice, the profile radius is:

$$r_1(x) = \sqrt{R^2 - (R - x)^2}; 0 < x < R, \quad (21)$$

and the profile area and boundary length:

$$a_1(x) = \pi[R^2 - (R - x)^2] \quad (22)$$

$$b_1(x) = 2\pi \cdot \sqrt{R^2 - (R - x)^2}. \quad (23)$$

(b) The vesicle center is within the slice; in this case the equatorial plane of the vesicle is seen, so that,

$$r_2(x) = R = \text{const}; R < x < (R + T/2), \quad (24)$$

and

$$a_2(x) = \pi R^2 \quad (25)$$

$$b_2(x) = 2\pi R. \quad (26)$$

The mean apparent profile area and boundary length are obtained by integrating Eqs. 22–26 in the range (Fig. 3),

$$h_a < x < (R + T/2),$$

or

$$R(1 - \sqrt{1 - \rho^2}) < x < R(1 + g).$$

Note that this range extends to the midplane of the slice; the other half of the superslice is symmetrical and thus repeats the same relations. This yields:

$$a' = \pi R^2$$

$$b' = \pi R$$

Using for the true volume and surface density,

$$V_v = N_v \cdot \frac{4}{3} \pi R^3 \quad (29)$$

$$S_v = N_v \cdot 4\pi R^2, \quad (30)$$

and substituting Eqs. 18–20, and 27–30 appropriately into Eqs. 11 and 12, we derive the correction factors for truncated vesicles as:

$$K_v = \frac{2}{(2 + \rho^2) \sqrt{1 - \rho^2 + 3g}} \quad (31)$$

$$K_s = \frac{\pi}{2(\rho \sqrt{1 - \rho^2 + \sin^{-1}(\sqrt{1 - \rho^2 + 2g})}). \quad (32)}$$

These factors are plotted in Fig. 4. Although they have not been derived by Eqs. 3 and 4 describing model A, they are compatible with this model because they tend to the factors given by Eqs. 15 and 16, respectively, if there is no truncation, i.e., as $\rho \to 0$. 

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EFFECTS OF SIZE DISTRIBUTION OF VESICLES: If the vesicle size is variable, these correction factors must be modified by introducing the appropriate moments of the size distribution. Designating by $E(R^n)$ the $n$th moment of the size distribution,\(^{9}\) correction factors equivalent to those given in Eqs. 15 and 16 are found to be:

$$K_V = \frac{4 \cdot E(R^3)}{4 \cdot E(R^3) + 3 \cdot T \cdot E(R^2)}$$

$$K_S = \frac{2 \pi \cdot E(R^2)}{2 \pi E(R^2) + 4 \cdot T \cdot E(R^1)}$$

Expressing the moments in relation to the $n$th power of the mean radius, a dimensionless size distribution factor,

$$m_n = \frac{E(R^n)}{[E(R)]^n}$$

is obtained. Likewise, section thickness can be related to $E(R)$ such that,

$$T = g \cdot 2 E(R).$$

Eqs. 33 and 34 then become:

$$K_V = \frac{4m_3}{4m_3 + 3g m_2}$$

$$K_S = \frac{\pi m_2}{\pi m_2 + 4g}.$$ 

\(^{9}\) The notation $E(\cdot)$ means expectation or theoretical mean value of the term in brackets. The second moment is the expectation value (or mean) of the square of the parameter, for example: $E(R^2) = \frac{\sum R^2}{N}$. 

It is evident that Eqs. 35 and 36 become equal to Eqs. 15 and 16 when $m_3 = m_2 = 1$, i.e., when sphere size is uniform.

To account for size distribution effects in the case of truncation, we can likewise express the minimal cap height, $h_o$, and the minimal cap radius, $r_o$, in relation to $E(R)$:

$$h_o = \chi \cdot E(R)$$

$$r_o = \rho \cdot E(R).$$

One then derives:

$$K_V = \frac{2 \cdot m_3}{2 \cdot m_3 + 3g \cdot m_2 - 3 \chi + \chi^3}$$

$$K_S \approx \frac{\pi m_2}{2\{(\rho + \sin^{-1} \sqrt{1 - \rho^2} m_2 + 2g - \rho(1 - \sqrt{1 - \rho^2})\}}$$

Whilst $K_V$ is exact, the expression for $K_S$ is approximate because some approximations had to be introduced during integration.

Correction Factor for Disk and Cylinder Model

ER is a complex of interconnected cisternae and tubules; it hence appears best approximated by a model made of nonconvex objects where discoid or cylindrical elements, respectively, are allowed to interpenetrate (model B). The system of formulas of Miles (10), i.e., Eqs. 7-10, permits calculation of the true parameters and the apparent densities observed on thick sections, provided the shape-size descriptors of the elements (vol-
Figure 4 Correction factors for volume and surface density of spheres, according to Eqs. 31 and 32.
ume, surface, mean tangent diameter) and their numerical densities can be estimated with some reliability.

**DISK MODEL:** The discoid elements are described by their thickness $d$, corresponding e.g. to the width of ER cisternae, and their assumed diameter $D = 8d$ (Fig. 1). The shape-size descriptors of the elements are:

$$
\gamma = \frac{\pi}{4} d^3 \cdot \delta^2
$$

$$
\sigma = \pi d^2 \cdot \delta \left( \frac{\delta}{2} + 1 \right)
$$

$$
\zeta = \frac{\pi}{4} d \cdot \left( \delta + \frac{2}{\pi} \right)
$$

Substituting this into Eqs. 7-10 and letting

$$
\xi = \nu \cdot \frac{\pi}{4} d^3,
$$

we derive the following correction factors:

$$
K_v(disk) = \frac{1 - \exp\left\{ -\xi \delta^2 \right\}}{1 - \exp\left\{ -\xi \left[ \delta^2 + g \cdot \delta \left( \frac{\delta}{2} + 1 \right) \right] \right\}}
$$

$$
K_s(disk) = \frac{\delta \left( \frac{\delta}{2} + 1 \right) \exp\left\{ -\xi \delta^2 \right\}}{\left[ \delta \left( \frac{\delta}{2} + 1 \right) + g \left( \delta + \frac{2}{\pi} \right) \right] \cdot \exp\left\{ -\xi \left[ \delta^2 + g \cdot \delta \left( \frac{\delta}{2} + 1 \right) \right] \right\}}.
$$

Note that $\xi$ is the “volume density” of a fictitious little cylinder of diameter and height $d$ circumscribed about the center point of the discoid element. Its value can be roughly calculated by dividing by $\delta^2$ the volume density of cisternae in the region of cytoplasm, in which RER is densely packed: $\xi = V_v/\delta^2$, its order of magnitude is $10^{-3}$ to $10^{-4}$. It should eventually be possible to estimate $\nu$ with more rigor, but the methods to do so are not fully developed at present. The values of $K_v(disk)$ and $K_s(disk)$ are plotted in Fig. 5.

**CYLINDER MODEL:** Tubules of SER, for example, are simulated by a “network” of cylindrical interconnected tubules of diameter $d$ and length $L = \lambda \cdot d$ (Fig. 1) for which we find:

$$
\gamma = \frac{\pi}{4} d^3 \cdot \lambda
$$

$$
\sigma = \pi d^2 \cdot \left( \lambda + \frac{1}{2} \right)
$$

$$
\zeta = d \cdot \left( \frac{\pi}{4} \cdot \frac{1}{2} \lambda \right)
$$

When calculating the correction factor for membrane surface density, it appears more appropriate to consider only the membrane contribution from the “cylindrical shell,”

$$
\sigma_o = \pi d^2 \cdot \lambda,
$$
i.e., to consider them to be “open-ended tubules.”

Introducing these parameters of the elements into Eqs. 7-10 and handling the numerical density of elements as for the disks we derive:

$$
K_v(cylinder) = \frac{1 - \exp\left\{ -\xi \lambda \right\}}{1 - \exp\left\{ -\xi \left[ \lambda + g \left( \lambda + \frac{1}{2} \right) \right] \right\}}
$$

$$
K_s(cylinder) open = \frac{\lambda \cdot \exp\left\{ -\xi \lambda \right\}}{\left[ \lambda + g \left( 1 + \frac{2}{\pi} \lambda \right) \right] \cdot \exp\left\{ -\xi \left[ \lambda + g \left( \lambda + \frac{1}{2} \right) \right] \right\}}.
$$

These correction coefficients are plotted in Fig. 6. As for disks, there is, at present, no rigorous method for estimating the true value of $\xi$; it can merely be estimated roughly by dividing by $\lambda$ the volume density of tubules in the cytoplasmic region of SER, for example: $\xi = V_v/\lambda$. For practical purposes, the possible error introduced by this procedure is acceptable because $\xi$ affects both exponentials in numerator and denominator to nearly the same extent.

**EFFECT OF TRUNCATION:** The correction coefficients for disks and cylinders do not correct for truncation. This appears unnecessary, for truncation would occur only by grazing sections hitting

---

Note that $\sigma_o$ is substituted for $\sigma$ only in those parts of Eqs. 8 and 10 which are outside the exponential, whereas $\sigma$ is retained in the exponentials in Eqs. 9 and 10.
Figure 5 Correction factors for volume and surface density of disks, according to Eqs. 39 and 40.
Figure 6  Correction factors for volume and surface density of cylinders, according to Eqs. 41 and 42.
the edge of disks or the end of cylinders. Provided \( D \gg d \) and \( L \gg d \), such grazing sections are very rare events; their effect can be disregarded.

In a previous communication (16), we had attempted to consider the effect of truncation on the section thickness correction for disks and cylinders. These factors were, however, only approximations; they were not based on the model of Miles (10).

PRACTICAL ESTIMATION OF SOME CORRECTION FACTORS FOR HEPATOCYTIC ORGANELLES

To calculate specific correction factors for the most important classes of organelles, we have measured their critical dimensions and the thickness of the sections.

Material and Methods

The measurements were done on sections of intact tissue of the material used for the companion study. The livers were derived from fasted male Sprague-Dawley rats. Small dices were briefly fixed by immersion in glutaraldehyde, post-fixed in \( \text{OsO}_4 \), and stained en bloc with uranyl acetate, as described in detail in the companion paper (1).

Random micrographs were recorded from random sections and evaluated at a final magnification of 96,000. A set of test lines superimposed on the micrographs served as a guide for random sampling of sites for measuring the critical width \( d \) of RER cisternae, SER tubules, and mitochondrial cristae (Fig. 7): wherever a test line intersected a profile, \( d \) was measured if the bounding membranes were sharp, indicating that the profile was cut about perpendicularly. At least 50 measurements were recorded for each component. The disk diameter \( D \) and the tubule length \( L \) were estimated from the approximate length of “straight” profiles, i.e., the distance between nodes in the network of tubules in SER, or between “kinks” in the profiles of RER cisternae.

Section thickness was measured by the fold method proposed by Small (11). It consists of micrographing long folds in the sections that have parallel edges and a fine midline, which indicates the juxtaposition of the two pleats of the fold. In effect, these folds represent two transverse views of the sections; an example of such a fold is shown in Fig. 8. Folds were recorded at a magnification of about 70,000 and measured. The average thickness of the silver-gray sections used here and in the companion paper was found to be 36.7 nm, with a standard deviation of \( \pm 4.6 \) nm (range 30-45 nm). Using this fold method, we found sections exhibiting gray, silver, and gold interference colors to measure 32, 46, and 81 nm, respectively.

Results

Table I presents the results of these measurements. It should be noted that the width \( d \) includes the luminal space plus the thickness of the two bounding membranes; for RER cisternae, the layer of ribosomes was also included wherever it was present at the measuring sites, because ribosomes are an important aid in identifying RER profiles, particularly when sectioned obliquely.

The estimates of \( D \) and \( L \) are to a certain extent arbitrary; therefore only rounded figures are given. A more precise estimate is not of great importance in using correction factors given in Eqs. 39-42, because if RER, for example, is subdivided into smaller imaginary disks, this increases their number \( \nu_r \), and the effects on the correction factors nearly cancel; assuming disks of diameter \( D = 1,000 \) nm instead of 2,000, the calculated correction for volume density would be smaller by 1% only, that for surface density by 3%.

The calculation of correction factors by Eqs. 39-42 requires the calculation of the relative shape factors \( \delta = D/d \) and \( \lambda = L/d \), of relative section thickness \( g = T/d \), and of the value of \( \xi \). For the latter, a crude estimate of volume density of the component is required which is divided by \( \delta^\nu \) or \( \lambda \), respectively. With these values at hand, the correction factors are easily computed on any desk or pocket calculator that allows for exponential functions. Alternatively, a more rapid but cruder estimate can be obtained from the set of curves given in Figs. 4-6; for many purposes, this will be more than adequate.

The values of the correction factors calculated in Table I for some hepatocyte organelles show that we expect the true volume density of SER tubules to be some 37% smaller than the raw estimate, obtained by a point count on a section of 40 nm thickness, would indicate. This overestimate is even greater for mitochondrial cristae, but smaller for RER cisternae. To demonstrate the effect on these factors of shape changes in one and the same structure, Table I includes mitochondrial cristae in fractions which are swollen; the resulting errors are smaller. The expected errors are smaller.
**Figure 7** Measurement of critical dimension $d$ (marked by double arrows) of RER cisternae (rer) SER tubules (ser), and mitochondrial cristae (mic) for rat liver cell. Profiles with sharp membrane trace are measured at intersections with sampling lines. $\times 76,000$.

**Figure 8** Electron micrograph of fold used for estimation of section thickness (half-distance between black arrows). The white arrows make the cleft where the two pleats are apposed. $\times 191,500$. 
**TABLE I**

**Estimation of Correction Factors for Hepatocytic Organelles**

| Model structure    | RER | SER | Tissue | Fractions |
|--------------------|-----|-----|--------|-----------|
| Cristae mitoch.    |     |     |        | Disk      |
| $d$, nm (± SD)     | 46 ± 13.4 | 66 ± 27.6 | 28 ± 8.5 | 65 ± 29.7 |
| $D$ or $L$, nm     | 2,000 | 400 | 200    | 200       |
| $h$ or $\lambda$   | 43   | 6   | 6.7    | 3.1       |
| Section thickness  |     |     |        |           |
| $T$, nm            | 36.7 | 36.7 | 36.7   | 30.2      |
| $g = T/d$          | 0.804 | 0.561 | 1.321  | 0.462     |
| Density estimate   |     |     |        |           |
| $N_v$              | 0.05 | 0.03 | 0.01   | 0.01      |
| $\xi$              | 2.7·10⁻⁶ | 5.10·10⁻³ | 2.2·10⁻⁴ | 1.04·10⁻³ |
| Correction factors |     |     |        |           |
| $K_v(T)$           | 0.711 | 0.628 | 0.541  | 0.726     |
| $K_x(T)$           | 0.986 | 0.702 | 0.757  | 0.824     |

for surface than for volume density.

Further examples for the application of these correction procedures are found in the companion paper (1).

**DISCUSSION**

Correction of errors due to finite section thickness has hitherto received inadequate attention in the application of stereological methods in cell biology. The principal reason was that available methods were only partially suited to be applied to the structure of cell constituents. After the original recognition of this type of error by Holmes (9), correction methods have been developed mainly for spherical objects (3, 6, 7, 9). Hennig (8) has furthermore considered infinitely large plates and infinitely long cylinders. More general treatments of the problem were introduced by Cahn and Nutting (2), DeHoff (4), Underwood (12), and most recently by Miles (10), but the formulas proposed did not easily lend themselves to a direct practical application in electron microscope cytology (13, 14).

The errors introduced by section thickness depend essentially on the shape of the structural elements and on their size relative to section thickness. In view of comparing measurements of membrane surface made on intact hepatocytes and on subcellular fractions derived therefrom (1), a consideration of these errors became most important because the elements of one and the same membrane class exhibited different shapes and sizes in intact cells and fractions. We have therefore sought to derive a correction method which would apply to the real structures and still was practical in its application. The rationale was that measurements were performed on micrographs "as if" they represented true sections; the error thus introduced was estimated theoretically on the basis of geometrical models which were made to approximate the real structure as closely as possible.

The model A assumes that the structural elements are discrete and convex (2, 4); it applies mainly to spherical vesicles. Model B allows for nonconvexity of the structure because the fictitious elements (cylinders or disks) may interconnect in a random fashion; it also admits that profiles may overlap on the projected image, i.e., on the micrograph (10). This model is particularly well suited to describe structures such as ER.

It should be noted by comparing Eqs. 7-10 with Eqs. 3-6, that model B tends to model A as the numerical density of elements becomes very small.

Compared to preexisting methods, the set of correction factors here derived presents distinct advantages because it provides great flexibility with respect to the choice of appropriate structural models. The main drawback is that reliable estimates of one crucial parameter, the numerical density of the fictitious elements in model B, $\nu_v$, cannot be easily obtained at present; but somewhat crude estimates of order of magnitude are possible and appear to yield satisfactory results.

The formulas presented may appear unduly complex, although the correction factors can evidently be computed on any modern calculator, or even read from the graphs of Figs. 4-6 with
reasonable accuracy. Slightly simpler formulas could be derived by introducing appropriate shape factors into the formulas of Cahn and Nutting (2) and DeHoff (4) (Eqs. 3–6); but this would have the distinct disadvantage that all dimensions of the structural elements must be estimated with greater precision than in the proposed approach. It is evident from the practical example given, as well as from the corrections required in the companion study for comparing data on intact tissue to measurements on fractions (1), that the errors introduced by section thickness are appreciable; as a rule of thumb, it appears mandatory to henceforth correct for them if the section thickness is \( > \frac{1}{10} - \frac{1}{5} \) of the critical smallest dimension of the structural elements, designated by \( d \) in this study. The overestimates expected for volume density are generally larger than those for surface density estimates. It should be noted, however, that the correction procedures proposed are valid only if the point and intersection counts were done at high magnification and on well-contrasted specimens where all profiles in the section—even grazing ones—are recognized, because, except for the spherical vesicle case, this correction does not allow for truncation by contrast deficiency.

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