# Brief Notes

## A Note on the Statistical Determination of Shape of Chromatin Elements in Human Spermatid and *Tradescantia* Microsporocyte.* By Bernard R. Nebel, Sylvanus A. Tyler, and Carol J. Murphy. *(From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois.)‡*

The geometric interpretation of ultrafine structural detail in cellular components is, very often, a three-dimensionl abstraction based on measurements and observations of a two-dimensional projection from microphotographs. The information that can be gained using such methods would be more valuable if mathematical procedures were employed which would lead to consistent and reproducible results. Such procedures are not yet in common use; and the biological literature of electron microscopy contains many instances in which the interpretation of the same images differs widely among investigators. This is especially true when the components under examination are structures that are subject to periodic changes, such as chromatin structures during interphase and in mitosis, microsomal and fibrous materials, and RNA-protein deposits.

The problem of making valid inferences concerning the shape of a three-dimensional body from its two-dimensional configuration observed in a thin slice has numerous parallels in other fields. Two papers by Lenz (1, 2) are closely related to the present problem. The first one deals with the size distribution of spheroid elements in a solid matrix. The second deals with size distributions of sections through spheres. Elias *et al.* (3) dealt with the geometry of sectioned circular cylinders. A significant approach to the more general problem has been made by Smith and Guttman (4), and earlier by Chalkley, Cornfield, and Park (5), who devised methods using geometric probabilities and certain topological properties, that relate the physical measurements of a plane section with the surface-to-volume ratio of its three-dimensional counterpart. Both methods stem from an extension of "Buffon's Needle Problem" (6) but differ in the physical quantities that are required for estimating the surface-to-volume ratio of closed solids.

The material studied here consisted of a series of electron micrograph cross-sections of human late spermatids obtained from an oligospermic male. (The authors are indebted to Dr. Richard Landau of the Department of Medicine and Dr. Cornelius W. Vermeulen of the Department of Surgery at the University of Chicago for this and other biopsies. The Department of Surgical Pathology assisted with the reading of corresponding light microscope slides and furnished normal material for comparative light microscope measurements.) Fixation was carried out in the operating room with buffered osmic acid according to Palade. From a collection of 30 micrographs, 10 were selected for measurement. These presented sections at all angles; it was thus insured that no specific orientation was present. The specific problem dealt with in this communication arose in an attempt to ascertain from the cross-sectional images the shape of chromatin elements contained in these spermatids. Since both the method of Smith and Guttman (4) and Chalkley *et al.* (5) seem to be equally reliable and simple computationally for investigations of this kind, we shall, by choice, review the pertinent results of Smith and Guttman and apply them, to the problem of determining the surface-to-volume ratio of chromatin particles in these human spermatids. Through the use of the surface-to-volume ratios, inferences are made as to the shapes of these elements by comparison with several regular geometric solids.

Suppose that we wish to approximate the size and shape of the structures appearing as dark "spots" in micrograph 1. Let us assume that each "spot" represents a single, randomly oriented, three-dimensional body and belongs to a collection of elements whose members have similar shape and size. A grid ruled with parallel lines (this is not a requirement, but is usually most convenient), spaced a distance $d$ apart, is superimposed on the section, and the number of intersections of grid lines with boundaries of the

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"spots" is recorded. The dimensions of the grid should be such that the grid lines traverse the entire area under consideration and the grid width is greater than the maximum width of particles in the collection. Randomly alter the orientation of the grid over the particle field and at each grid position record the total intersections. (The use of several fields is recommended.) The smaller the number of intersections obtained per grid position, the larger should be the number of grid positions taken. Compute the mean number of intersections $N$ from these totals. Another quantity that is needed is the area occupied by the total number of "spots" within the portion of the section under investigation. An estimate of the surface-to-volume ratio of any closed solid can be found from average measurements made on plane sections by using a slight variation of Equation 8 from reference 4.

$$\frac{S}{V} = \frac{2\bar{N}d}{A} \qquad (1)$$

in which $S/V$ is the surface-to-volume ratio, $A$ is the total area occupied by the projections, $\bar{N}$ is the total number of intersections of grid lines with the boundary (number of single or tangential intersections of grid plus twice the number of double intersections), and $d$ is the grid width. If it is further assumed that the structures are approximately the same shape with sizes varying within small limits, and eduction as to both the shape and dimensions of the particles is possible. A comparison of the derived surface-to-volume ratio with that of several regular geometric solids is given in Table I, and a significance test (Table II) of the paired differences between $S/V$ ratios

TABLE I

*Analysis of Chromatin Particle Shape ($S/V$ Ratios) in 10 Samples of Human Sperm*

| Sample No. | Derived value of $S/V$ | Value of $S/V$ for sphere | Value of $S/V$ for prolate spheroid | Value of $S/V$ for cylinder |
|---|---|---|---|---|
| 1 | 2.8 | 2.0 | 3.4 | 1.3 |
| 2 | 2.0 | 2.0 | 2.3 | 1.3 |
| 3 | 2.2 | 2.5 | 2.8 | 1.7 |
| 4 | 2.3 | 2.7 | 2.9 | 1.8 |
| 5 | 2.9 | 3.0 | 3.1 | 2.0 |
| 6 | 2.0 | 2.2 | 2.2 | 1.4 |
| 7 | 2.9 | 2.7 | 3.1 | 1.8 |
| 8 | 3.8 | 4.1 | 4.6 | 2.7 |
| 9 | 3.6 | 3.8 | 3.8 | 2.5 |
| 10 | 3.5 | 3.0 | 3.4 | 2.0 |

for these indicates that the more probable shape of the particles is globular. The $S/V$ ratios for the regular solids used were determined from the following expressions:

$$\text{Sphere:} \frac{S}{V} = \frac{4\pi r^2}{4/3\pi r^3} = \frac{3}{r} \qquad (2)$$

$$\text{Prolate spheroid:} \frac{S}{V} = \frac{3}{2}\left(\frac{1}{a} + \frac{\sin^{-1}e}{be}\right) \qquad (3)$$

$a$ = one-half major axis
$b$ = one-half minor axis
$e$ = eccentricity

$$\text{Cylinder:} \frac{S}{V} = \frac{2\pi rl}{\pi r^2 l} = \frac{2}{r} \qquad (4)$$

$r$ = average radius
$l$ = length

TABLE II

*Paired Significance Test of the Deviation from the Derived Value of $S/V$ for Selected Geometric Solids*

| Geometrical solid | No. of differences $n$ | Mean difference $x_d$ | Standard error $\hat{\sigma}_{\bar{x}_d}$ | Statistical probability $t$ | Statistical probability $p$ |
|---|---|---|---|---|---|
| Sphere | 10 | 0 | 0.123 | 0 | $p > 0.90$ |
| Prolate Spheroid | 10 | −0.360 | 0.087 | 4.14 | 0.001 $<p$ $< 0.005$ |
| Cylinder | 10 | 0.119 | 0.950 | 7.98 | $p < 0.0005$ |

TABLE III

*Average Length of Major ($M$) and Minor ($m$) Axes of Dark "Spots"*

| Sample No.* | No. in sample | $\bar{M}$ (2a) | $\bar{M}$ | $\bar{m}$ (2b) | $\bar{m}$ |
|---|---|---|---|---|---|
| 1 | 20 | 2.35 | 0.17 | 1.60 | 0.11 |
| 2 | 20 | 3.50 | 0.14 | 2.45 | 0.17 |
| 3 | 20 | 2.85 | 0.17 | 1.90 | 0.18 |
| 4 | 20 | 2.50 | 0.18 | 1.95 | 0.15 |
| 5 | 20 | 2.24 | 0.18 | 1.81 | 0.11 |
| 6 | 20 | 2.90 | 0.18 | 2.65 | 0.13 |
| 7 | 20 | 2.65 | 0.20 | 1.75 | 0.14 |
| 8 | 20 | 1.80 | 0.12 | 1.15 | 0.06 |
| 9 | 20 | 1.95 | 0.17 | 1.20 | 0.10 |
| 10 | 20 | 2.45 | 0.17 | 1.55 | 0.13 |

* Average number of particles evaluated per cell: 307. Sample number represents classes of cells in various stages of late spermatid maturity, thus intercell variability is larger than the intracell variability.

Differences between the measured lengths of major and minor axes are statistically significant based on the 5 per cent critical level of Student's $t$ statistic (Table III). This result indicates that the biological material investigated is somewhat asymmetrical and could be described more exactly as an ellipsoid with three slightly unequal axes. The formula for finding the surface area of an ellipsoid is given in reference (7).

In the example above, the dark "spots" are chromatin elements which, therefore, in the light of this study are nearly spheres with an average diameter of 500 A, and not cylinders or highly eccentric spheroids. Close scrutiny of the original micrographs indicates that the globular structures are not homogeneous, but have a fine internal structure which in this material cannot be resolved.

This result is of basic interest. Control biopsies from normal material were unfortunately not available. Measurements made under the light microscope on this biopsy compared with autopsy slides considered normal by the Department of Surgical Pathology of Billings Hospital, University of Chicago, show that the over-all dimensions of the present material are within the range of the normal spermatids. This agrees with Lisser and Escamilla (8, Fig. 5, p. 317) who show that with azoospermia caused by mechanical block the germinal elements may appear normal. The present attempt at a statistical analysis does not take into consideration the unequal distances between chromatin bodies (Fig. 1). Some of them appear in serial array as though they were beads on a string. Thus, the statistical analysis, although giving reliable information on the shape of the particles, does not decide about a structural arrangement of the particles relative to one another and the specific chromosome to which they pertain (9).

For a check on the method, a determination was made of the approximate shape of dark elements seen in an electron micrograph cross-sectional image of a *Tradescantia* microsporocyte nucleus (Fig. 2). These dark elements are continuous subunits of chromatids and are known to be cylindrical in shape (10). The empirical method gave consistent results. The derived value of $S/V$ is 1.55, which compares favorably with the value of 1.54 for a cylinder. The $S/V$ values for a sphere and a prolate spheroid are 2.06 and 1.72, respectively.

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## Explanation of Plate 209

FIG. 1. Electron photomicrograph of a human late spermatid from a clinical biopsy. Section thickness ~500 A; grid width 5 mm. × 37,000.

FIG. 2. Electron photomicrograph of part of the nucleus of a *Tradescantia* microspore in early prophase. Section thickness ~500 A; grid width 10 mm. × 12,300.
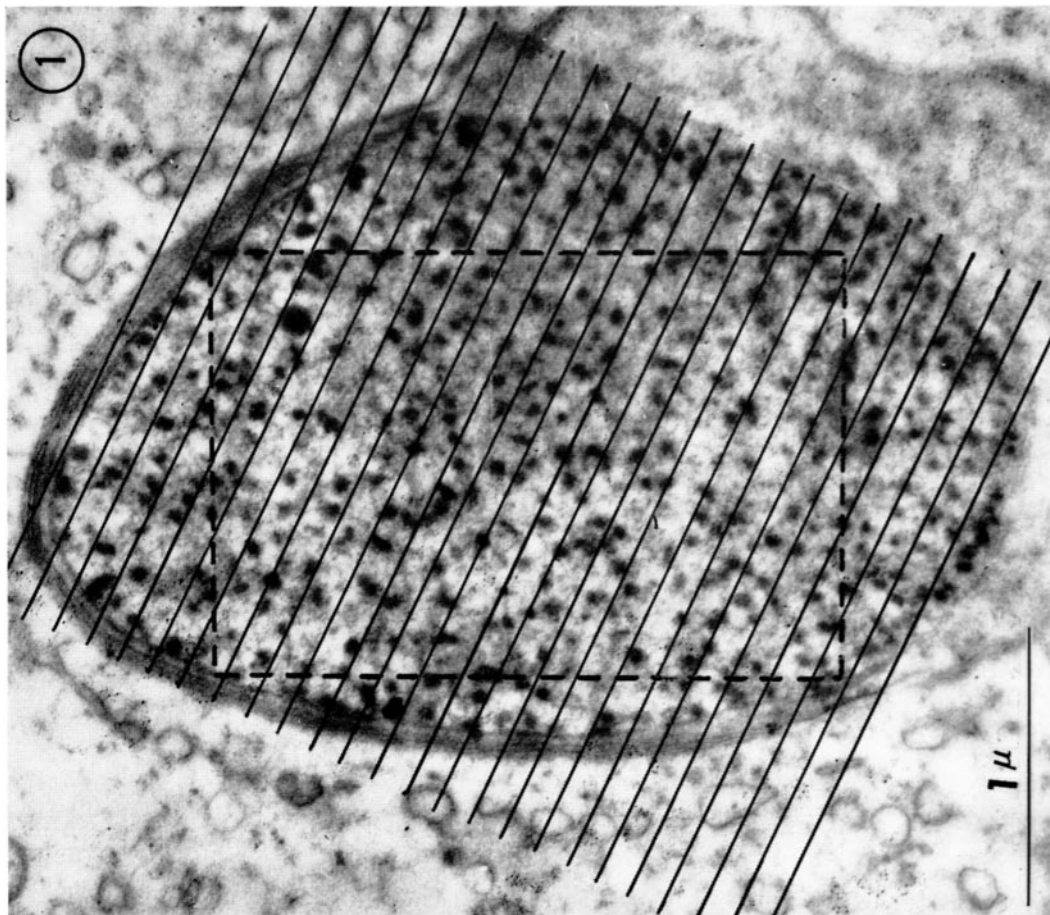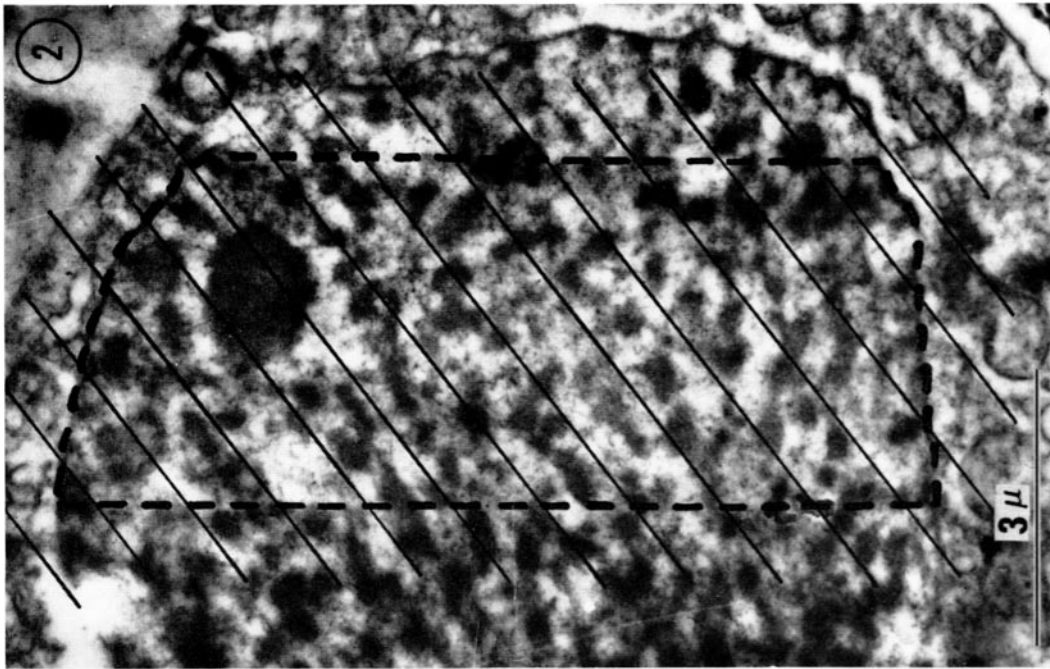
(Nebel *et al.*: Chromatin elements in human spermatid)