STUDY OF BULL SPERMATOZOA

Quantitative Electron Microscopy

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ABSTRACT

The electron microscope has been used to determine the characteristic dimensions and the distribution of the dry mass in bull spermatozoa. The most important result is that all characteristic data are occurring in logarithmic distributions. Furthermore, no correlation between such parameters as head weight and tail weight or head length and tail length was found. The occurrence in logarithmic distributions and the non-correlation of parts in the assembly of a spermatozoon are considered to reflect significant biologic principles. Methodologically, a new procedure is added to quantitative electron microscopy permitting the recording of the mass cross-section (total mass per unit length) of an object. This approach makes possible determinations of the distribution and the total mass of very long and narrow structures.

INTRODUCTION

Presently several major theories are under discussion attempting to explain the movement of spermatozoa. The quantitative physical data on the respective species of spermatozoa supporting the theories were obtained with the aid of a light microscope. In the majority of species, however, two dimensions of the propellant structure of spermatozoa are smaller or just slightly larger than one wave length of visible light. Astonishingly little quantitative information is available in the electron microscopy literature. The obvious need for more precise and, preferably, quantitative data on sperm structure and physiology was recently emphasized in a review article by Bishop (1). One of the methods of choice to provide these is electron microscopy, which is capable not merely of rendering morphologic facts but of furnishing quantitative physical information as well. This paper reports such data on the dry mass, geometry of sperm structures, and their correlation. Fawcett's nomenclature (2–4) of sperm structure is used in this article.

MATERIALS

Bull semen samples for quantitative study were obtained from two purebred Holstein bulls[1] (Nos. 43-50, 43-54). Samples were collected in centrifuge tubes with the aid of an artificial vagina, chilled on crushed ice, and so transferred to the laboratory. Portions of the semina were then diluted 1:5 with saline (0.9 per cent NaCl) and immediately centrifuged at low speed (~500 *g*) for 5 minutes. After the supernatant was decanted the sediments were resuspended in saline by gently inverting the centrifuge tube several times. Centrifugation was then repeated. Finally the sediment was resuspended in an equal volume of saline. Smears were prepared from this suspension by the procedure previously described for erythrocytes (5). This involved rapid heat fixation of the smear at 100°C and a 5 minute rinse in 95 per cent ethyl alcohol or distilled water.

As in the quantitative study of erythrocytes (5), Parlodion was used to support the specimens, since

[1] We are obliged to Dr. Charles A. Kiddy and Mr. Norman Hooven, Jr., Dairy Cattle Research Branch, Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Maryland, for generous supplies of bull semina.

it has the property of losing about 50 per cent of its mass during the initial observation time in the electron beam.[2] Taking advantage of this property of Parlodion, we were able to prepare relatively thick and tough membranes, which were easy to handle during the preparation of smears. When exposed to the electron beam the major proportion of the nitrous groups are sublimated from the film, thus leaving the membranes uniformly thin and very transparent, a decisive advantage, especially in the quantitative assay of relatively thick biologic objects.

In selecting spermatozoa for measurement, special attention was given to avoiding abnormalities. Spermatozoa with kinked or coiled middle pieces and tails; with swelling, doubling, or abaxial attachment of middle pieces; or with double heads were excluded from measurement. All *sizes* of sperm heads, however, including those that may, by some investigators, be counted as slightly microcephalic or macrocephalic, were measured.

METHODS

A Siemens Elmiskop I was used throughout for the quantitative work and an RCA EMU 3D for morphologic studies. The high voltage was set at 100 kv; objective and condenser apertures of 100 μ diameter were used.

Units of 12 plates were developed together (5 minutes, Kodak D-72, 1:1, 20°C); each 12th plate represented a weight standard.

Size and Area

Determination of size and area requires the comparison of the dimensions of the object with the dimensions of a suitable standard. The units of the standard must have the same order of magnitude as the dimensions of the object, and object and standard must be enlarged sufficiently to permit reasonably accurate comparison. (These truisms are sometimes overlooked.) Therefore, object and standard were photographed under identical electron microscopic conditions. The micrographs were subsequently enlarged with a final magnification of about 5,000 and recorded either graphically or photographically. Actual measurement was then carried out with the aid of a ruler, allowing for not more than ± 1 per cent read-off error. As a size standard, an aluminum oxide replica[3] of a diffraction grating, cross-ruled at 2,160 lines per millimeter in 2 perpendicular directions (0.463 μ per division), was used (6). These gratings have a practical tolerance of ± 1 per cent, so that an error of ± 2 per cent for the magnification is certainly on the safe side. Fig. 1 presents the essence of this procedure. For area determination, a planimeter was used. In order to reduce the intrinsically higher error of the planimetric procedure, the sperm heads were enlarged together with a micrograph of the standard at a final magnification of 12,000 for area determination. An accuracy of ± 2 per cent was maintained. Recordings of the scanning procedure, to be described later, provided convenient ways to assess the geometry of the object.

Mass Determination

All electron microscopic mass determination is based on the fact that the photographic transmission of an electron micrograph, taken under appropriate conditions, is proportional to the mass per area of the object producing this transmission. Photometric procedures are available to evaluate electron micrographs in terms of either mass per area or mass.

Electron microscopic and photographic conditions, as well as the principal procedure for mass determination with the electron microscope, have been described earlier (7).

Calibration

Polystyrene latex spheres are used for calibration of the weight determination procedure because their density and shape are known and their size can readily and accurately be determined with the cross-grating; furthermore, their size is of the order of magnitude of the object under study.

Scanning the image of a polystyrene latex sphere through its center with an aperture that is small, compared to the size of the image, not only produces the relation between photometer reading (R) and the mass per unit area (W/A), but renders crucial proof of the linearity of this relation and thus the applicability of the method. Provided the shape of the sphere is unaltered, the recorded distance x from the center of the sphere is a direct measure of the mass per area $2y$ radiated by the electron beam. The center was found as the spot of maximum transmission. A straight scan through this spot renders both the mass distribution and the diameter $2r$ of the sphere.

The results of 20 such sphere scannings under standardized conditions for quantitative evaluation are presented in Fig. 2. This presentation also indicates the mathematical relations among distance, radius, and secant. Proof that contamination and possible alterations of the spherical shape during the standard exposure to the electron beam are immaterial is contained in the straight line of the plot. A mutual compensation of flattening and contamination cannot, of course, be excluded.

The straight line optimally fitted to the measure-

[2] Unpublished results.

[3] Some of these replicas were prepared at the Kodak Research Laboratories. The hospitality and assistance of Mr. R. P. Loveland, Mr. C. F. Oster, Jr., and Mr. D. C. Skillman are gratefully acknowledged.

ment points of Fig. 2 yields a rate of conversion from 10^{-6} gm/cm² to 5.9 arbitrary reading units (slope of the curve). This value is in good agreement with measurements of the electron microscopic contrast reported and discussed earlier (7, 8). The coefficient of variation was determined to ±3.5 per cent and is also indicated in Fig. 2. The range of linearity covers 60 × 10^{-6} gm/cm², and is thus sufficient for weight determinations of bull spermatozoa whose maximum weight per area is in the order of 50 × 10^{-6} gm/cm². This can be seen in Fig. 3, where the typical "elevations" of a scan recorded longitudinally through the head are presented. The aperture was small, compared to the dimensions of the head, to optimize resolution.

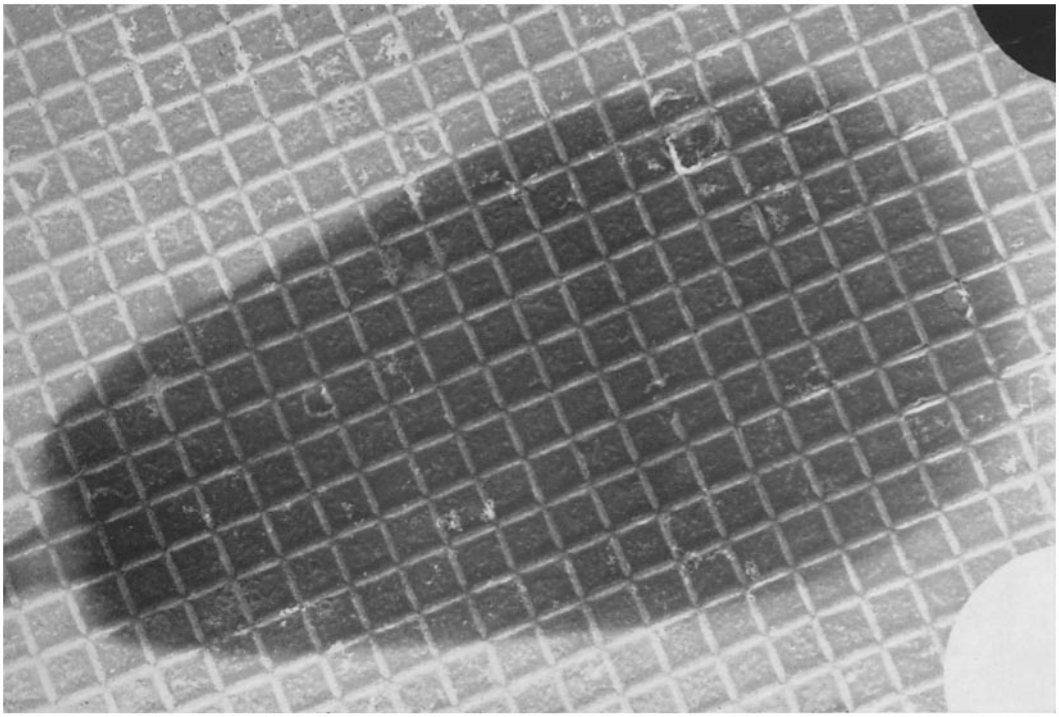

FIGURE 1 Comparison of cross-ruled grating (2,160 lines per mm in each direction) with the projected outlines of a sperm head. Length and area of a sperm head can easily be determined, with a ruler and a planimeter, respectively. × 16,000.

Total Mass of Head

In order to determine the total mass of the sperm head, its mass profiles are not evaluated singly but summed up in one measuring step by means of an integrating photometer. This procedure has been described in detail earlier (7).

In Fig. 4 an example is given of how a measuring aperture is fitted around the sperm head to select the object area to be measured. The measurement separates the head from the middle piece in the region of the neck structures, thus including a small portion of the latter in the weight determination of the head. Included in the measurement is the materlia of the acrosome or galea capitis. For the sake of having a clear background, sperm cells surrounded by debris were excluded from measurement. The total dry mass of the head is then calculated by multiplying the reading (R), already converted into mass per unit area, by the area which the measuring aperture of the photometer defines in the object plane of the electron microscope. Hence three errors are involved in the final absolute determination of mass. First, the measurement as such; *i.e.*, the read-off value (R) has an error of ±2 per cent. This figure was derived from repeated measurement of an identical particle. Secondly, the rate of conversion is accurate only up to ±3.5 per cent. Thirdly, the correct determination of the aperture area mentioned is given through the second power of the magnification value, so that another ±4 per cent inaccuracy is

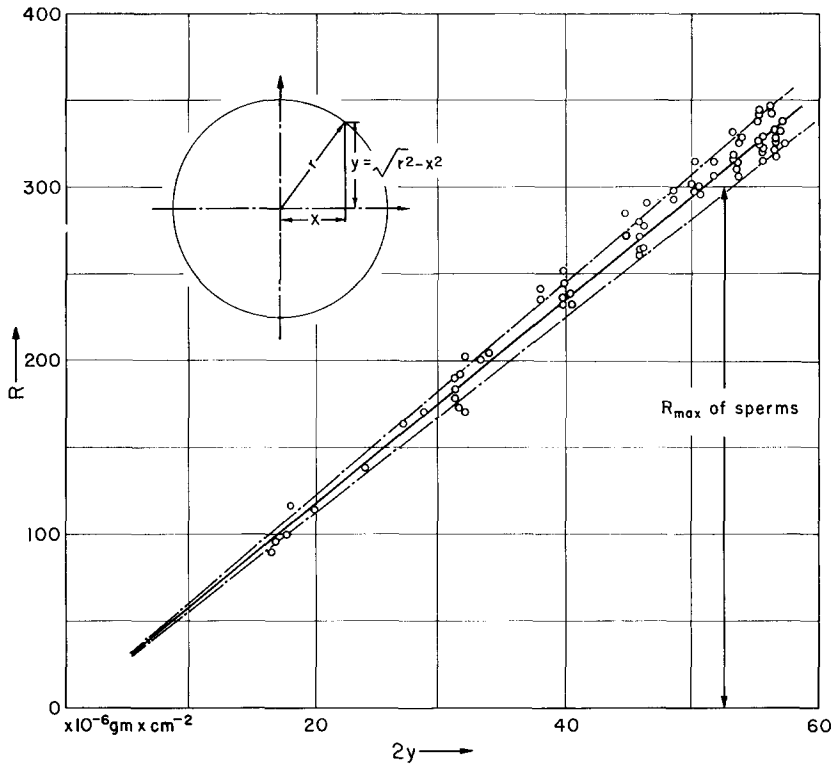

FIGURE 2 Standardization curve. Relation between values (R), read off the photometer, and mass per area ($2y$), transluminated in the electron microscope. Scanning the image of a polystyrene latex sphere through its center with an aperture that is small, compared to the size of the image, not only produces the relation between photometer reading (R) and the mass per unit area (W/A), but renders crucial proof of the linearity of this relation and thus the applicability of the method. Provided the shape of the sphere is unaltered, the recorded distance x from the center of the sphere is a direct measure of the mass per area $2y$ radiated by the electron beam. The center was found as the spot of maximum transmission. A straight scan through this spot renders both the mass distribution and the diameter $2r$ of the sphere. The result of 20 such sphere scannings under standardized conditions for quantitative evaluation are presented in Fig. 2.

encountered. Presuming statistical independence of all those errors, their geometric addition results in a total error of ±6 per cent for *absolute* mass determination.

It must, however, be pointed out that for *relative* measurements, comparisons, discussions of distributions, and so on, only the much smaller error of the R values need be considered.

Total Dry Mass of Sperm and Dry Mass per Unit Length

The extreme difference in morphologic configuration of sperm head and sperm tail precluded the use of integrating measurements for the determination of the tail mass and thus the total mass of the spermatozoon. In order to determine this total mass and the masses of the major morphologic components a special procedure was developed. In principle, the image of a straight spermatozoon on the electron micrograph is moved through a slot-shaped aperture of the photometer while the cross-sectional transmission is concurrently recorded. The recording thus represents the mass cross-section of the sperm; *i.e.* the dry mass per unit length. Simple planimetry of such recordings permits the derivation of the mass of any desired part of the object. Thanks to the variability in scanning speed, speed of the paper drive of the recorder, and amplifier gain, the area of the scans could be adjusted to keep planimeter errors below ±2 per cent. For the purpose of this study, the weights of head, middle and principal pieces, and end piece were determined.

In order to maintain proper sensitivity of recording, head and tail structures were each scanned with an

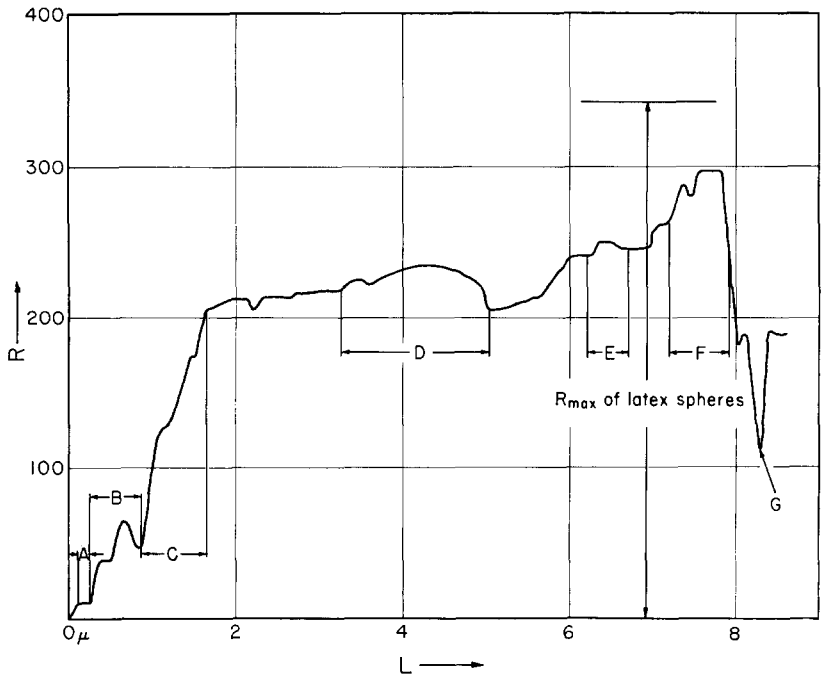

FIGURE 3 Typical recording from a longitudinal photometric scan through the center of a sperm head (scanning aperture 0.2 mm, sperm head 8 mm).

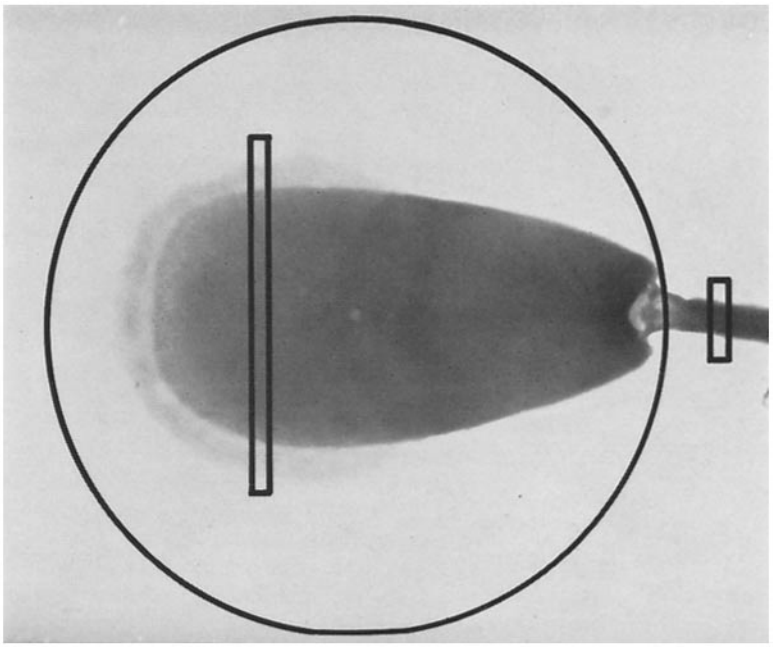

FIGURE 4 Transmission micrograph of a sperm head. The ink circle indicates the way in which a measuring aperture is fitted around the sperm head to select the object area to be measured. The rectangular areas drawn across the head and the tail show the fitting of the slot aperture for the recording of the total mass per unit length. × 8,000.

aperture just wide enough to encompass the full width of their structures (Fig. 4).

In practice the micrograph is mounted on a motor-driven modification of the regular slide carrier of the integrating photometer (Figs. 3 and 6 *b* of reference 8). This arrangement allows easy alignment of the image of the sperm perpendicular to the aperture slit (Fig. 5). A 1,000-mesh silver grid, in turn calibrated by aid of the 2,160 line/mm cross-ruling, is photographed in series with the sperm preparation and scanned repeatedly in the manner described in order to introduce a magnification standard in the transmission records.

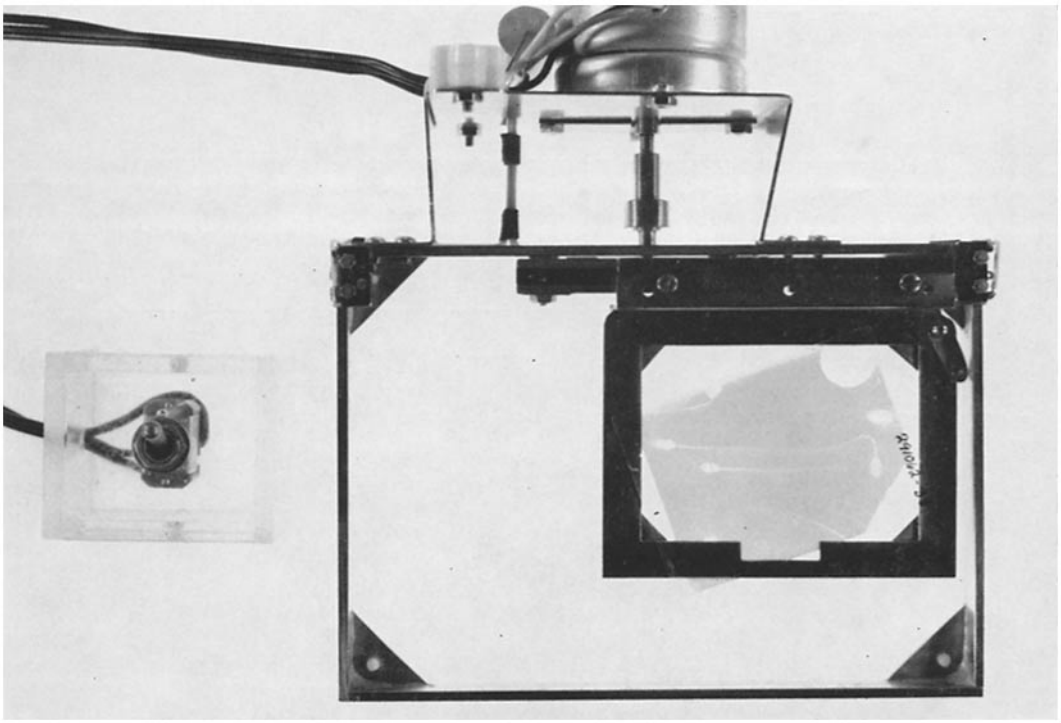

FIGURE 5 Photograph of the scanning device used on the object stage of an integrating photometer for quantitative electron microscopy (8). The heavy frame, carrying the electron micrograph, is driven from right to left, thereby moving the image through scanning slot.

RESULTS

The results are derived from geometric, photometric, and morphologic evaluations of bull sperm images on electron micrographs. A study of correlations between the measurements on different sperm parts follows.

The spermatozoa of one bull (Holstein No. 43-54) were studied for most of the quantitative data. Those of another (Holstein No. 43-50) were used to render control data on certain critical determinations.

Probability paper offers the most suitable way of presenting the data (5, 9). A Gaussian distribution appears as a straight line whose slope is simply related to the standard deviation of the distribution. This representation provides not only certain values characteristic for the distribution, but also proof of normality. For two reasons the logarithm of the value whose frequency is to be presented was taken rather than the value itself. First, the geometric variables length, area, and volume (mass) are all powers of a length. Therefore, the logarithms of these variables can validly be compared. Secondly, biologic quantities are distributed in a log-normal (symmetric) fashion; that is, a linear representation of the respective values results in a skewed distribution with a tail towards higher values. Applying the logarithmic representation might then be considered as symmetrizing the original distribution.

For very narrow distributions a possible asym-

metry or skewness may be obscured, so that the logarithmic and the linear representation render indistinguishable results. (The reader is referred to the appendix in reference 9.)

Geometric Features of the Bull Sperm

Table I summarizes the results of geometric determinations. Results are given as medians;

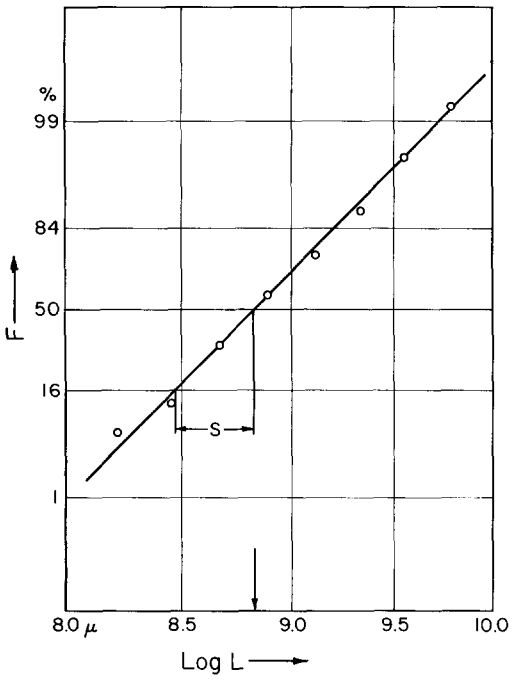

FIGURE 6 Distribution curve of the length of sperm heads. The cumulative frequency (*F*) is represented on probability paper. Instead of the length, its logarithm (*log L*) is used as the abscissa, for reasons explained in text. Arrow indicates median value; s, the standard deviation of distribution.

the s values are the standard deviations pertaining to the log-normal distribution of the particular value.

Length determinations of bull sperm heads from the two bulls produced markedly similar values, namely, 8.83 μ and 8.78 μ, respectively, differing only by 0.56 per cent.[4] One of these populations of head lengths is plotted on probability paper (Fig. 6), yielding a straight line, *viz.*, a logarithmic Gaussian distribution.

[4] The length does not include the acrosome. It is defined between the tip of the head and its farthest axial extension.

Because of the possibility of artefacts in drying, the projected area of the head (Table I) was selected as a more significant measurement than the width, having values that again conform closely to a logarithmic Gaussian distribution (Fig. 7).

Geometric determinations of tails were done on the recordings of the scanning procedure,

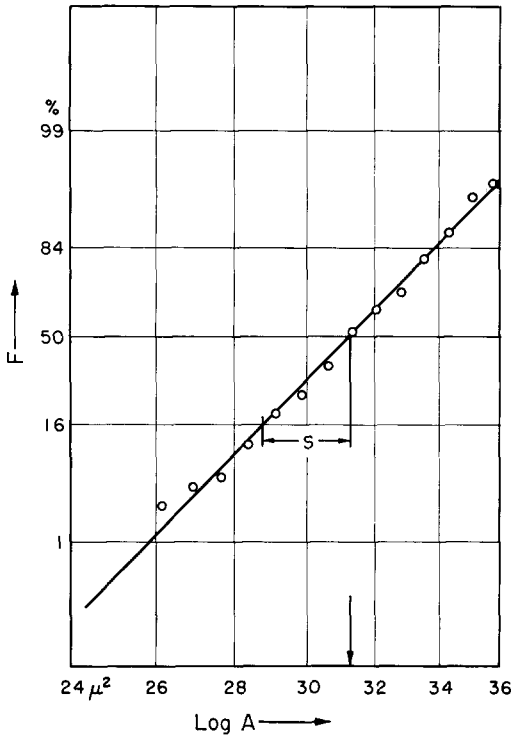

FIGURE 7 Distribution curve of logarithms of the projected area (*log A*) of sperm heads. Arrow indicates median value; s, the standard deviation of distribution. For coordinates, see Fig. 6.

which were needed for the mass determination anyway. This renders highly accurate data, since the tails yield recordings 50 cm long. It was necessary to have tail samples stretched out; these were difficult to find, since they are ordinarily bent or curled, and so 65 had to be considered sufficient. A plot of these determinations on probability paper (Fig. 8) demonstrates that also the tail lengths of bull spermatozoa are log-normally distributed.

Dry Mass of Sperm Heads

In Table II the determinations of dry mass of sperm heads from two bulls are compiled. More

than 150 measurements from each animal yield very close values of the median masses, which, being within the error limits of the standardization, can be considered as identical. It should be pointed out again that the standard deviation is influenced only by the smaller error of the measurement as such, whereas the absolute values carry the additional error of the standardization.

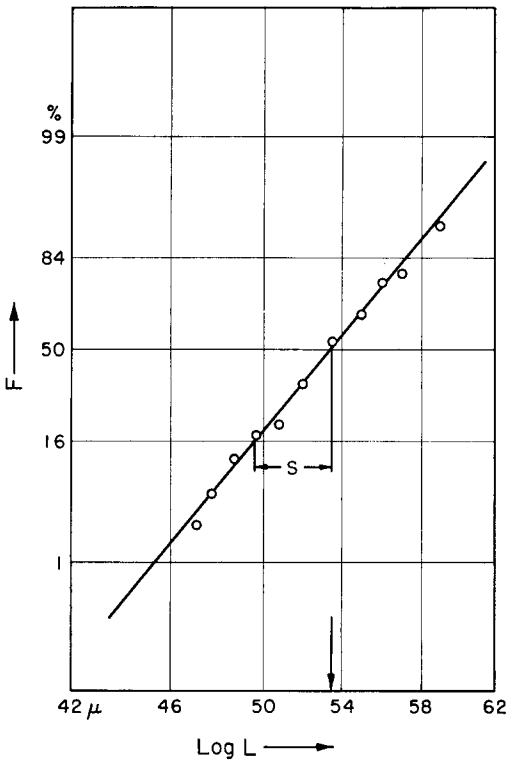

FIGURE 8 Distribution curve of logarithms (*log L*) of the length of sperm tails. Arrow indicates median value; s, the standard deviation. For coordinates, see Fig. 6.

Therefore, the relatively large standard deviation of ±0.07 signifies true variations of dry mass within the sperm population (Fig. 9, bull No. 43-54). Furthermore, this standard deviation exposes the natural asymmetry of the distribution of mass in a population of sperm heads. In the log-normal case, 68 per cent of all values measured are centered symmetrically about the median value within the limits of ±0.07. Translating the logarithms into mass values does not affect the median value (which simply becomes the antilogarithm), but these limits then lie 16.5 per cent under and 19.7 per cent above the median.

Table II also contains the magnification and area in the object plane of the electron microscope defined by the measuring aperture of the photometer. This value, together with the calibration constant, renders the absolute dry mass values.

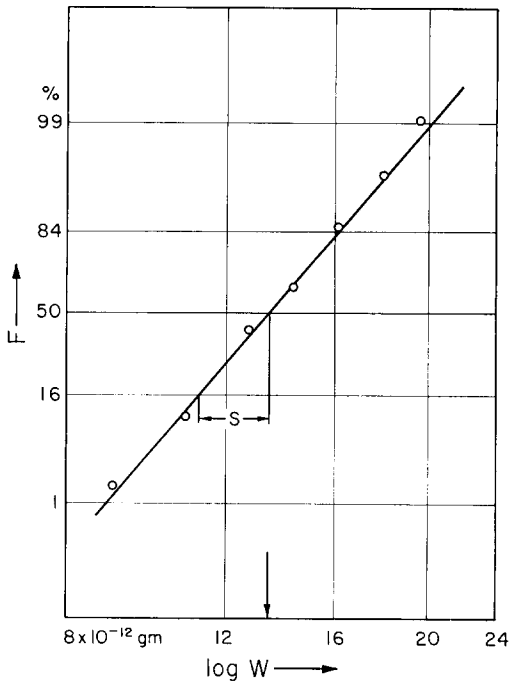

FIGURE 9 Distribution curve of logarithms of the dry mass (*log W*) of sperm heads. Arrow indicates median value; s, the standard deviation. For coordinates, see Fig. 6.

Distribution of Mass in the Spermatozoon

The narrowness and length of the tail made the application of integrating photometry less feasible. The weight of the tail, therefore, was derived by planimetry of the recordings of scans with a slot-like aperture over the entire sperm (as in Fig. 4).

It must be borne in mind that a given reading is determined by the geometric extension of the particular region of the sperm and the concentration of dry matter in it; *i.e.*, it presents a cross-section of the dry mass per unit length.

Since the absolute mass of each head was already known from integrating photometry, the

ratio of the planimeter readings from head and tail could be used to calculate the absolute mass of the tail. Likewise, the relationship between the masses of other sperm parts could be established without difficulty.

In Table III the mean values of the mass ratios and the absolute masses in bull spermatozoon

It is located at the level of the anterior border of the base plate of the head (Fig. 10).

Correlation of Measurements

The multitude of individual measurements makes it possible to investigate whether any strong correlation exists between the various

TABLE I

Geometric Data on Bull Spermatozoon

Part	Parameter	Result	±	Number of measurements	±s*
Head	Length	8.83 μ	0.25	167	0.018
	Control bull	8.78 μ	0.25	172	0.019
	Area	31.3 μ^2	1.4	162	0.026
Tail	Length	53.4 μ	1.20	65	0.018
Middle piece	Length	11.0 μ	0.3	53	0.030
	Width	0.63 μ	0.02	53	0.021
Principal piece	Width near middle piece	0.58 μ	0.02	51	0.020
	Width near end	0.46 μ	0.02	48	0.023

* s Standard deviation of log-normal distribution of the particular parameter.

TABLE II

Dry Mass of the Head of Bull Spermatozoon

Bull	Median mass	±	No. of measurements	±s*
43-50	13.0 $\times$ 10^{-12} g	0.70	162	0.067
43-54	13.35 $\times$ 10^{-12} g	0.70	167	0.070

*s Standard deviation of the log-normal distribution of the head masses.

are compiled. These values possess a distribution whose standard deviation clearly exceeds that of the error of measurement. The total median mass of a sperm is found to be 18.20 ± 0.90 × 10^{-12} gm.

A recording typical for its maxima and minima is shown in Fig. 10. The "elevations" are representative, with regard to their height and location, of all 65 recordings. The integrated mass curve is shown in the same figure. The intersection of the integral curve with the 50 per cent line indicates the center of gravity for the entire sperm.

size and mass values. For this purpose it suffices to deal with the deviations of the respective value from the median values of the entire population.

This relationship between the masses of head and tail is plotted in Fig. 11. The scattered pattern, even without calculation of a correlation coefficient, is striking evidence that no correlation exists. A head lighter than the median mass may very well be pushed by a tail much heavier than the median tail mass, and vice versa. In Fig. 12 the relation of head mass to tail length is presented with similar results. No correlation exists.

DISCUSSION

Quantitative Data, Geometry, and Mass

In order to bring the results of the present study into proper perspective with regard to earlier work reported in the literature, the available data are compiled in Table IV. It becomes obvious at first glance that electron microscopy has contributed surprisingly few quantitative data. Furthermore, these have been noted coinci-

dentally rather than intentionally providing physiologically significant measurements.

The average length of 219 bull sperm heads is given as 10.2 μ by Van Duijn (10) and as 8.5 to 10 μ by Bretherton and Rothschild (11), while this study reports 8.8 μ as the median of the length distribution; *i.e.*, a value about 10 per cent lower. The same is the case for the projected area, which is slightly smaller than earlier figures of Van Duijn (10) and Leuchtenberger *et al.* (12). The difference might be attributed to the exclusion of the acrosome from our length measurements on electron micrographs. These two parts are often distinguished only with difficulty in preparations for interference or phase-contrast microscopy. The exclusion of the acrosome from our measurements results in a sharper definition of the length of the head, since the acrosome appears to be subject to varying degrees of changes during preparation. No measurements of the widths of heads were carried out.

Measurements of dry masses of sperm from bull, ram, and boar are published in ranges from roughly 6.5 to 9.0 $\times$ 10^{-12} gm. We have consistently found a figure that is between 25 and 30 per cent higher than this range and the published values for bull spermatozoa in particular. There is no ready and unequivocal explanation for this discrepancy. A possible source of error could be the difficulty of determining the specific refractive increment of the tightly packed nucleoprotein. Another potent source of error in determining total mass by interference microscopy is the inaccuracy of measurements on projected areas of small objects. Also, the microradiographic data of Müller *et al.* (14) give values that are significantly higher than their values on sperm head masses obtained with interference microscopy. Figures lower than those reported here were found by Burge and Silvester (15) in their first report determining the dry mass of ram sperm heads with both the electron and the interference

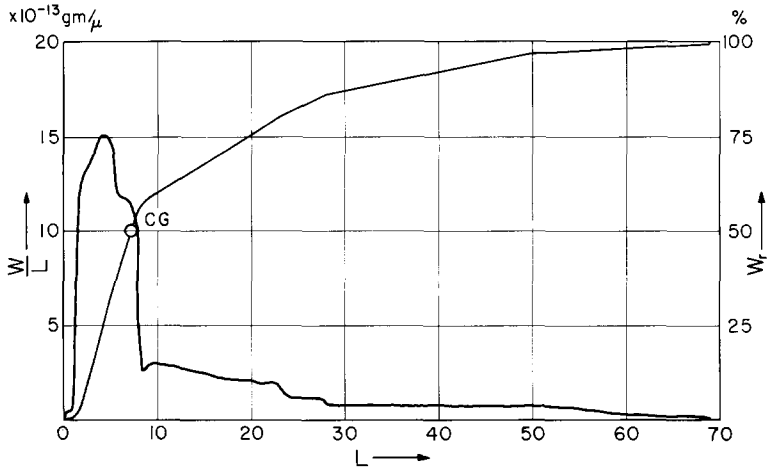

FIGURE 10 Example of a recording of the mass per length (W/L) from a longitudinal scan over the entire sperm with a slotted aperture. Simultaneously the total mass (W) as a function of the length (L) is shown. The intersection of the integral mass curve with the 50 per cent line of the W coordinate indicates the location of the center of gravity (CG). At about 23 μ a slight "elevation" marks the annulus, and at about 28 μ a "dip" marks the termination of the first pair of the outer fibers.

TABLE III

Distribution of Mass in the Bull Spermatozoon

	Sperm	Head	Tail	Midpiece
Per cent of mass	100	63.7 ± 3.1	36.3 ± 1.0	17.3 ± 0.5
(10^{-12} g)	18.20 ± 0.85	13.35 ± 0.70	4.85 ± 0.3	2.31 ± 0.15

microscopes. A comparative series of ram spermatozoa measured in the course of our present study with quantitative electron microscopy indicated that the values for the dry masses of the sperm of this species may actually be some 30 per cent greater than values available in the literature.

Values for the length of the tail vary from 45 μ to about 65 μ (Table IV). The length of the sperm tail, 53.4 μ, reported by us seems almost to be a fair average of the values in the literature, while the weight of this sperm part, 4.85×10^{-12} gm, stays far below the 12×10^{-12} gm value of Bishop (1).

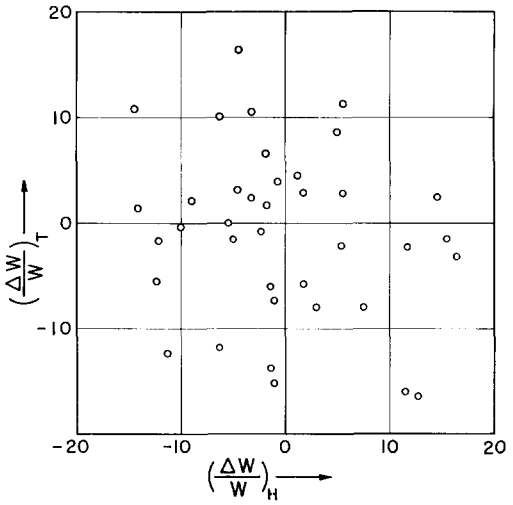

FIGURE 11 Trial of a possible correlation between relative deviations from the median mass values of tail $(\Delta M/M)_T$ and head $(\Delta M/M)_H$.

The Logarithmic Distribution

A particularly interesting finding of this study is the fact that spermatozoa of the bull, the heads as well as the tails, their masses as well as their geometric parameters, are truly distributed in distinct populations. This finding is in keeping with the earlier results of quantitative electron microscopy on mass distribution in biologic populations (5, 8, 9). Most noteworthy is the fact that these distributions are not normal but logarithmic-normal.

Using interference microscopy, Müller *et al.* (14) measured the mass of 167 heads of bull spermatozoa. Plotting their data on probability paper (Fig. 13 *a*) yields a logarithmic distribution, while their customary histogram shows a skew distribution (Fig. 13 *b*).

Measuring the lengths of 1,500 and the breadths of 264 heads of human spermatozoa with the light microscope, Van Duijn (16) also recognized a logarithmic distribution. He attributed this to a lower size limit, determined by the minimum volume required for accommodating the hereditary substance, but no theoretical upper limit, and concluded that the distribution ultimately arises from the growth state of the precursory cells, depending at any given moment on the size attained. This explanation does not, however, account for the distribution of Feulgen-positive material (DNA) reported by Leuchtenberger

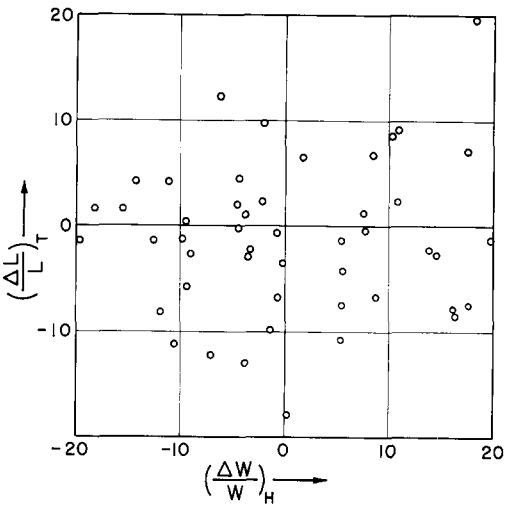

FIGURE 12 Trial of a possible correlation between the relative deviations from the median tail lengths $(\Delta L/L)_T$ to the relative deviations of the median mass of the head $(\Delta M/M)_H$.

et al. (17, 18) for bull and human spermatozoa (which can be shown to be logarithmic), since cell growth is not involved here.

Although our plot of the translatory speed of the sea urchin spermatozoon after data by Gray (19) yields a Gaussian distribution, and although the normal curve of Van Duijn's studies (20) appears to be Gaussian, a slight skewness was found in the large series of normal bull sperm by Rikmenspoel (21) and in the experimental material of Van Duijn (22, 23). Even though head mass and size are presently considered to be of little influence on motility, it remains to refinements of the Rikmenspoel technique or to other new approaches with higher sensitivity to

TABLE IV

Measurements of Bull Sperm Structures from Literature

Year	Author	Method	Part	Parameter	Result	±
1960	Van Duijn (10)	Light microscopy	Head	Length	10.2 μ	0.5
1961	Bretherton (11)	" "	"	Length	8.5–10.0 μ	
1960	Van Duijn (10)	" "	"	Breadth	5.4 μ	0.35
1961	Bretherton (11)	" "	"	Breadth	4.5 μ	
1956	Leuchtenberger (12)	" "	"	Projected area	33.8 μ^2	0.24
1960	Van Duijn (10)	" "	"	Projected area	40.6 μ^2	4
1961	Blom *et al.* (24)	Electron microscopy	"	Thickness	0.3–0.5 μ	
1961	Bretherton (11)	Light microscopy	"	Thickness	0.7 μ	
1956	Leuchtenberger (12)	Interference microscopy	"	Dry mass	7.1×10^{-12} g	
1959	Müller (14)	Microradiography	"	Dry mass	7.87×10^{-12} g	0.46
"	" "	Interference microscopy	"	Dry mass	8.94×10^{-12} g	1.17
1949	Vendrely (29)	Chemical determination	"	DNA	3.2×10^{-12} g	
1956	Handbook of Biological Data (28)	" "	"	Total nucleic acid	48 per cent of dry weight	
1956	Leuchtenberger (12)	Microspectrophotometry	"	Arginine	2.07×10^{-12} g	
1949	Vendrely (29)	Chemical determination	"	Arginine	2.16×10^{-12} g	
1956	Handbook of Biological Data (28)	" "	"	Basic protein	28.7 per cent of dry weight	
1956	Handbook of Biological Data (28)	" "	"	Acidic protein	19.6 per cent of dry weight	
1941	Zittle (30)	" "	"	Nitrogen	52 per cent of total sperm	
1953	Barer (26)	Immersion refractometry	"	Solids	45 per cent	
1955	Nelson (27)	Chemical determination	"	Weight, lipid-free	51 per cent of total sperm	
1956	Leuchtenberger (12)	Microspectrophotometry	"	DNA	2.04×10^{-12} g	
1956	Handbook of Biological Data (28)	Chemical determination	"	DNA	3.3×10^{-12} g	

1961	Bretherton	Light microscopy	Tail	Length	45 μ
1962	Bishop (1)	" "	"	Length	60 μ
1961	Bretherton *et al.* (11)	" "	"	Diameter	0.3 μ
1953	Barer *et al.* (26)	Immersion refractometry	"	Solids	50 per cent
1962	Bishop (1)	Interference microscopy	"	Weight	1.2×10^{-11} g
1961	Bretherton (11)	Light microscopy	Midpiece	Length	15 μ
1960	Blom (25)	Electron microscopy	"	Length	15 μ
1961	Bretherton (11)	Light microscopy	"	Diameter	0.8 μ
1960	Blom (25)	Electron microscopy	"	Diameter	0.4–0.7 μ
1955	Nelson (27)	Chemical determination	"	Weight, lipid-free	16 per cent of total sperm
1941	Zittle (30)	" "	"	Nitrogen	12 per cent of total sperm
1961	Blom (25)	Electron microscopy	"	Angle of spiral	10–15°
"	" "	" "	Principal piece	Length	45 μ
"	" "	" "	" "	Diameter	0.2–0.4 μ
1955	Nelson (27)	Chemical determination	" "	Weight, lipid-free	33 per cent of total sperm
1941	Zittle (30)	" "	" "	Nitrogen	35 per cent of total sperm
1960	Blom (25)	Electron microscopy	Total sperm	Length	74 μ
1952	Lindahl (13)	Countercurrent centrifugation	" "	Specific gravity	1.280 (1.240–1.334)

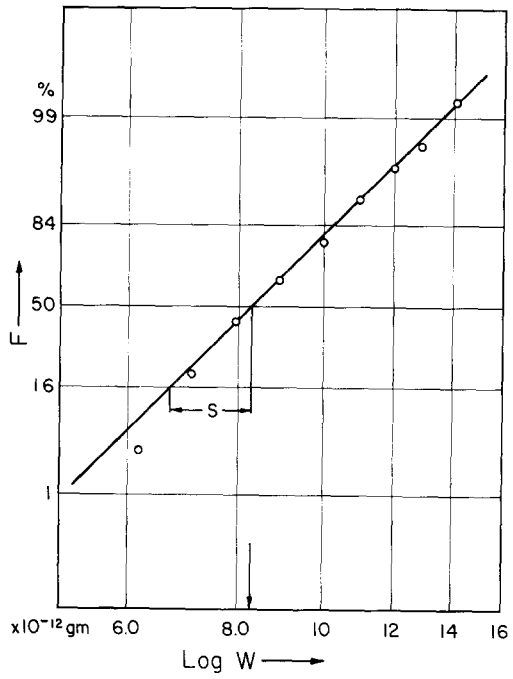

FIGURE 13 *a*

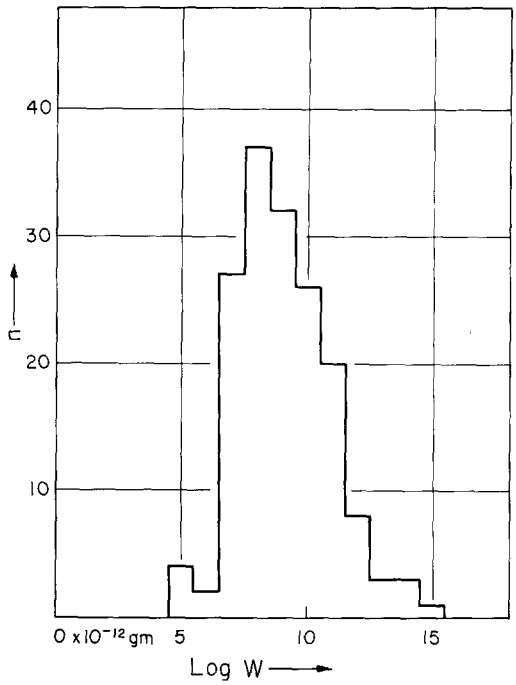

FIGURE 13 *b*

FIGURE 13 Histogram (*a*) of the mass of bull sperm heads, determined with the aid of interference microscopy by Müller *et al.* (14). The distribution is obviously skewed. Plot (*b*) of the data from (*a*) on probability paper, demonstrating the fact that (*a*) is a logarithmic distribution.

settle the question whether or not the distribution of translatory speed is also logarithmic.

While it is difficult to investigate in growing cells the principles of coordination in the intracellular manufacture of quantities of cellular elements because the influence of growth, differentiation, and level of function are so difficult to quantitate, the spermatozoon, being an end product, offers a favorable although specialized object for the study of these principles. It is reasonable to assume that no further *growth* occurs when the sperm has reached the seminal fluid. The only process to contend with is sperm maturation, the final phase in its differentiation, which results in some condensation or polymerization of existing sperm structures, implying a continuous rise in density. This process is regarded as dehydration (13) and will not affect the amount of dry substance of the sperm.

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