# MEASUREMENT OF THE SIZE DISTRIBUTION OF ZYMOGEN GRANULES FROM RAT PANCREAS

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ABSTRACT Zymogen granules are obtained in pure form and processed for electron microscopy. Thin sections are photographed and diameters measured with a Zeiss particle size analyzer. Since sectioning cuts any given particle in random way, these diameters are not the true diameters of the particles. The true size distribution is obtained by comparing the observed diameter distribution with a generated diameter distribution. The generated distribution is constructed from an assumed parent distribution (of true diameters) by the Monte-Carlo technique. "Goodness of fit" is judged by the value of "chi-squared" resulting from the comparison. Appropriate adjustments of the parameters of the true distribution are made on the basis of minimizing chi-square. A result of this process is that the zymogen granules follow a normal distribution: mean = 0.984 $\pm$0.005 $\mu$m, SD = 0.190 $\pm$0.005 $\mu$m. A second preparation of granules was made and diameters were measured directly with a scanning electron microscope. The distribution was again found to be normal, thus supporting the first result.

## INTRODUCTION

Determining the three-dimensional shape of objects when the available data are only two-dimensional is a problem encountered frequently in the biological sciences as well as in other fields such as physics and geology, and solutions to it depend upon the particular details of the system under investigation (Underwood, 1970). In the present case the system consists of a collection of spheres, the zymogen granules from rat pancreas, whose diameters are roughly 1 $\mu$m. This size range is just at the limit of resolution of the light microscope so that one is forced to use the electron microscope in order to gain reliable size information. Measurements must be made on photographs of sections observed in the microscope and will of necessity yield information only in the plane of the section (i.e., two-dimensional information). The particles are not all of the same diameter; rather they form a distribution of diameters and it is this distribution that has been determined.

Investigation of this problem has made use of an isolation procedure which concentrates the particles in an essentially pure form without other cell contaminants.

The preparation of granules is fixed, embedded, and cut into thin sections in the way customary for the electron microscope. Each section consists of a plane section taken through a distribution of spheres and therefore contains a distribution of circles of varying diameters. Each sphere sliced actually results in two concentric circles due to the finite thickness of the section. However only the larger of these circles is the one measured because the smaller one is masked by the electron-dense material within the granule. As will be discussed below, this causes no essential difficulty. The diameters differ for two reasons: (*a*) the plane cuts through spheres of differing diameters, and (*b*) the plane cuts any given sphere at a random distance away from its center. Our problem can now be concisely stated; given the distribution of diameters of circles as measured from the electron microscope section, determine the distribution of diameters of spheres from which the section was cut. The problem has interest for two reasons: (*a*) the distribution of diameters of the zymogen granules must reflect the process of their origin and thus knowledge of the size distribution would contribute to the understanding of the mechanism of production and subsequent export. (*b*) The method used to solve this problem has some advantages relative to other published methods and therefore may prove useful to other investigators working on related problems (DeHoff, 1965; Underwood, 1970; DeHoff and Rhines, 1968; Hennig and Elias, 1970).

The following paragraphs describe the methods used to prepare the zymogen granules, the measurement of sizes using the electron microscope sections, the procedure for obtaining the parent distribution, the results of the fitting technique, and finally a discussion of these results.

## METHODS

### *Preparation of Zymogen Granules*

This method follows those of Hokin (1955) and Greene et al. (1963) with modifications made by Longphre.[1] Female white Wistar rats, fasted for 17 h, were lightly anesthetized with ether and the pancreas was removed, the fat cleaned off with scissors, and rinsed and homogenized in cold buffer (0.45 M sucrose, $10^{-4}$ M MgCl, $10^{-4}$ MES (2-(*N*-morpholino)-ethanesulfonic acid) [Good et al., 1966], pH 4.6). Two pancreata were diced and homogenized in 35 ml of buffer in a Dounce homogenizer (Blaessig Glass Specialties, Rochester, N. Y.); 10 strokes with the loose pestle followed by 30 strokes with the tight pestle; the pH of the homogenate was measured to be 6.7. The homogenate was spun in an International centrifuge (IEC head 823, International Equipment Co., Needham, Mass.) according to the following schedule: spin 1; 10 min at 1,500 rpm; spins 2 and 3 (previous supernatant spun), 10 min at 1,500 rpm; spin 4 (previous supernatant spun), 15 min at 4,000 rpm. The final pellet was gently resuspended in sucrose buffer.

### *Preparation of Zymogen Granules for Electron Microscopy*

*Scanning Electron Microscope.* The fixative used was 4% glutaraldehyde in 0.1 M phosphate buffer (pH 6.6). This stock solution was mixed with an equal volume of the su-

[1] Longphre, W., and F. Lamy. Manuscript in preparation.

crose buffer suspension of zymogen granules, resulting in a final mixture of 2% glutaraldehyde, 0.05 M phosphate buffer, 0.225 M sucrose. This final suspension was slowly stirred overnight in the cold room. Care was taken to keep the osmolarity of the solutions constant at near 550 mosmol. To prepare a sample for the scanning electron microscope, 1 ml of the glutaraldehyde suspension was spun down and the pellet resuspended in distilled water. This process was repeated three more times and the final pellet resuspended in distilled water. A drop of this suspension was spread over a clean cover slip and allowed to dry. Then a thin layer of gold (400 Å) was vacuum evaporated onto the cover slip containing the dried granules. Another sample was prepared by freeze drying a cover slip of the water-suspended granules before coating with gold. Observations were made on an Ultrascan model SM-2 scanning electron microscope.

*Transmission Electron Microscope.* Zymogen granules prepared as a pellet (see above) were resuspended in a Veronal-buffered osmium tetroxide solution (1% osmium tetroxide in 0.88 M sucrose) for 12 h, repelleted, put through a standard dehydration schedule, and embedded in Vestopal W (E. F. Fullam, Inc., Schenectady, N. Y.). Sections approximately 1,000 Å in thickness were taken with glass knives and stained with uranyl acetate (3 min) followed by counterstaining with lead citrate (60 s). Observations were made on a Philips electron microscope 100B.

### *Measurements of Diameters*

Diameters were measured on photographs of electron microscope sections with a Zeiss particle size analyzer model TGZ-3 (Carl Zeiss, Inc., New York). The over-all magnification of the photograph was 14,700 times (1,600 from the microscope and 9.1 from the enlarging process). The microscope magnification was carefully checked with a diffraction grating replica grid and is accurate to within 2%. The particle size data were accumulated in 21 bins of approximately 1.2 mm each beginning at about 2 mm. This bin size corresponds to a real dimension of 0.082 $\mu$m. In a separate test of the particle sizing instrument, sytematic errors were found to be less than 3% for diameters above 3 mm and less than 1% for diameters above 4 mm. Since fewer than 2.5% of the zymogen granule diameters had values 4 mm or below, it was not necessary to use special thin transparencies for these measurements. Almost 4,000 diameters were measured. The number of events in any bin was assumed to belong to a Poisson distribution and so the error associated with the number $N_k$ for the $k$th bin was taken to be $\delta N_k = (N_k)^{1/2}$. The data were usually plotted in terms of the frequency: $f_k = (N_k/\Delta_k) \pm (\delta N_k/\Delta_k)$ where $\Delta_k$ is the width of the $k$th bin. The machine obtains the diameter by matching a test area against the area of the circle being measured. The feature compensates for any lack of roundness that might be present due to sectioning pressures of fixation distortions. These effects proved to be relatively minor in practice.

Diameters of particles examined with the scanning electron microscope were measured on photographs (see Fig. 1 *b*) using a template containing circles of graduated diameters. The measurements were then classified and the standard statistical calculations made on the resulting histogram.

### *Finding the "Parent Distribution"*

The subject of size distribution determination received an extensive treatment by Wicksell (1925) and has been reviewed most recently by Elias et al. (1971). Most of the methods discussed build the parent distribution directly from the data. These methods result in a numeri-

cal description of this parent distribution but do not generally yield its functional form. In our approach we start with an assumed parent distribution function (e.g., normal, Poisson, etc.) which contains a number of, as yet undetermined, parameters. For a given set of values of these parameters this function forms the basis for the production of a set of generated data using the Monte Carlo technique (Meyer, 1956, and see Appendix II). The generated data are created by mathematical simulation of the original experimental procedure, including the slicing and measurement processes. Some of the details are presented in Appendix II. One point that deserves mention now however is that only one circle is generated each time a sphere is "sliced." This corresponds to the larger of the two concentric circles produced for every real slice (as mentioned in the Introduction) and therefore to the one actually measured. The raw data and the generated data are then compared according to the maximum likelihood method, with the chi-squared function serving as a goodness-of-fit criterion, (Orear, 1958; Cramer, 1955). This process is continued using adjusted values of the parameters, until a minimal value of chi-squared is found. This final set of parameters is taken to be the one that best fits the experimental data. The errors in the determination of this "best set" are also obtained from the $\chi^2$ function by considering the error matrix formed from the second derivatives of $\chi^2$ when it is expanded in a Taylor series about its minimum.

An incorrect parent distribution function will still yield a minimal value of $\chi^2$ when introduced by the above process. However, in that case the resulting value for the minimal $\chi^2$ will exceed its expected value. This expected value is equal to the number of data points minus the number of parameters in the fitting function. Furthermore, the chances of getting any observed values of $\chi^2$ at minimum is easily determined and so it is thus possible to decide if the chosen parent distribution function is a good one.

## RESULTS

Fig. 1 *a* is an electron micrograph of a sample of the purified zymogen granule pellet. Fig. 1 *b* is a scanning electron micrograph of another preparation of these granules. Notice the relatively unstructured surface of the particles. No significant features were observed even at the highest magnification used (50,000 times).

Fig. 2 depicts the results of measuring transmission electron micrographs with the Zeiss comparison device. These data have not been adjusted in any way and are taken directly from the measurement table. Fig. 3 shows the result of fitting the data in Fig. 2 using the maximum likelihood procedure and a normal distribution as the parent distribution function. The raw data used for fitting were smoothed at one point; the datum point with the arrow was adjusted as shown. It was felt that this one point might possibly represent the presence of another species of particle even though no other evidence was found to indicate this. Fitting the raw data including the point in question gave almost identical results for the parameters but the goodness-of-fit measure was poorer. Also shown in Fig. 3 is the parent distribution function found to give the best fit to the smoothed raw data. This distribution has a mean of $0.984 \pm 0.005$ $\mu$m and a SD of $0.190 \pm 0.005$ $\mu$m. The chi-square obtained for these best fit values of the parameters is 22.0. This is to be compared with a value of 18 to be expected when 21 data points are used to determine two parameters. A total of 30,000 "measurements" were made in obtaining the generated data curve shown in

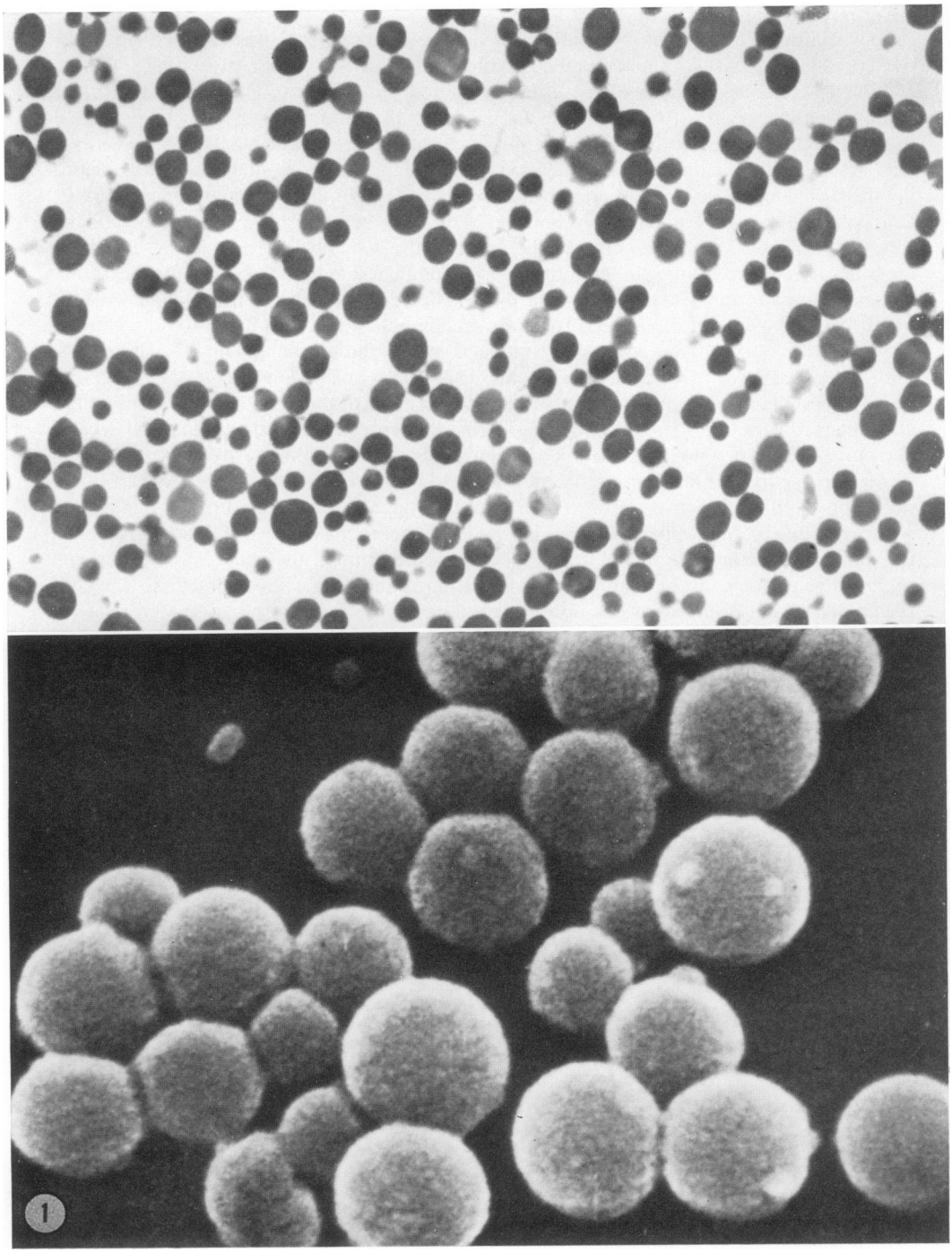

FIGURE 1 (*a*) Transmission electron micrograph of zymogen granules from rat pancreas. Magnification: × 4,140. (*b*) Scanning electron micrograph of a second preparation of zymogen granules. Magnification: × 23,300.

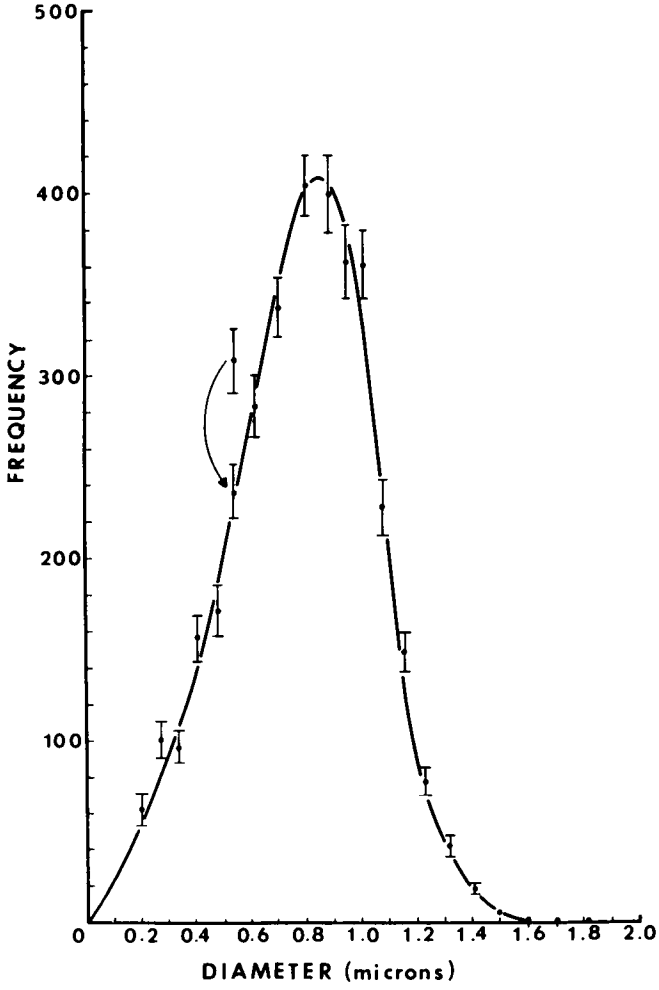


FIGURE 2 Raw data of zymogen granule diameters measured on transmission electron micrographs. The frequency is expressed in terms of number per unit length. For the purpose of fitting, the data were smoothed at one point (as shown by the arrow). The curve is hand drawn through the smoothed data points. The total number of events, mean, and variance are: unsmoothed data: 3,953, 0.78 $\mu$m, 0.26 $\mu$m; smoothed data; 3,876, 0.79 $\mu$m, 0.26 $\mu$m.

Fig. 3. 68% of the granules have diameters between 0.794 and 1.174 $\mu$m. This spread of 0.38 $\mu$m is about 40% of the average diameter.

Fig. 4 depicts the results of measurements made with the scanning electron microscope. Altogether, 253 granules were measured and used to compute the mean and variance of the distribution. The results of this calculation gave: $\langle x \rangle = 7.28$ mm, $s = 1.23$ mm. These direct results must be corrected for a systematic error of 0.85 mm inherent in the template used for measurement and for the magnification of the

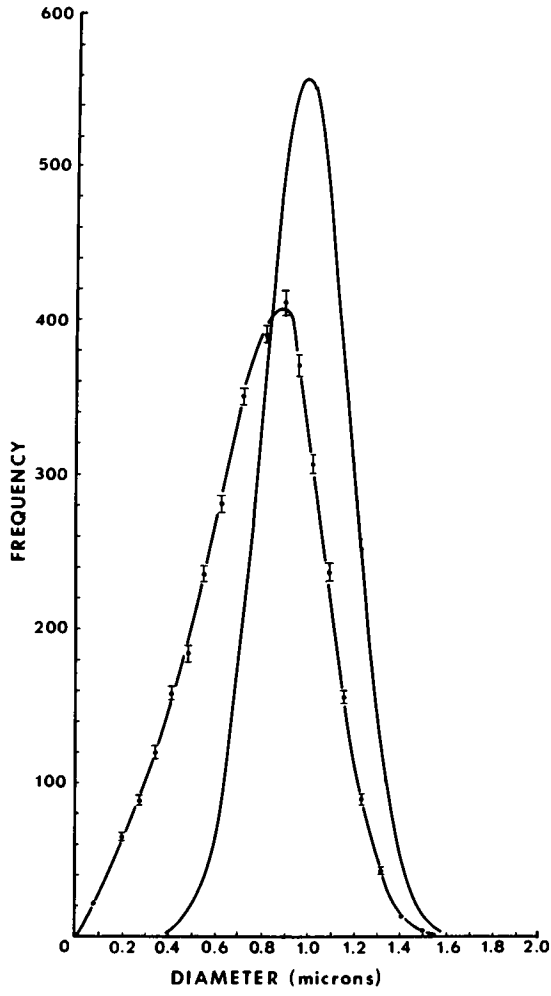


FIGURE 3 Results of fitting the experimental data. The points with error bars represent the generated distribution function which best fits the smoothed raw data; the curve is hand drawn through these points. The mean and variance are 0.77 and 0.27 $\mu$m, and 30,000 events were used in the generation process. The other curve shown is the parent distribution (normal) used to produce the generated data. The mean and standard deviation are 0.98 and 0.19 $\mu$m. The areas under the two curves are equal.

microscope ($M$ = 9,500 $\pm$2%). The results after these corrections are made are: $\langle x \rangle$ = 0.86 $\mu$m, $s$ = 0.13 $\mu$m. Also shown in Fig. 4, as an insert, is a plot of the data on probability paper. As can be seen, the data fit well to a straight line as would be expected for a normal distribution. As a more meaningful test of normality, the chi-square was computed using the values of the mean and variance obtained above, with the result: $x^2$ = 3.4. For this case, where there are 13 degrees of freedom, such

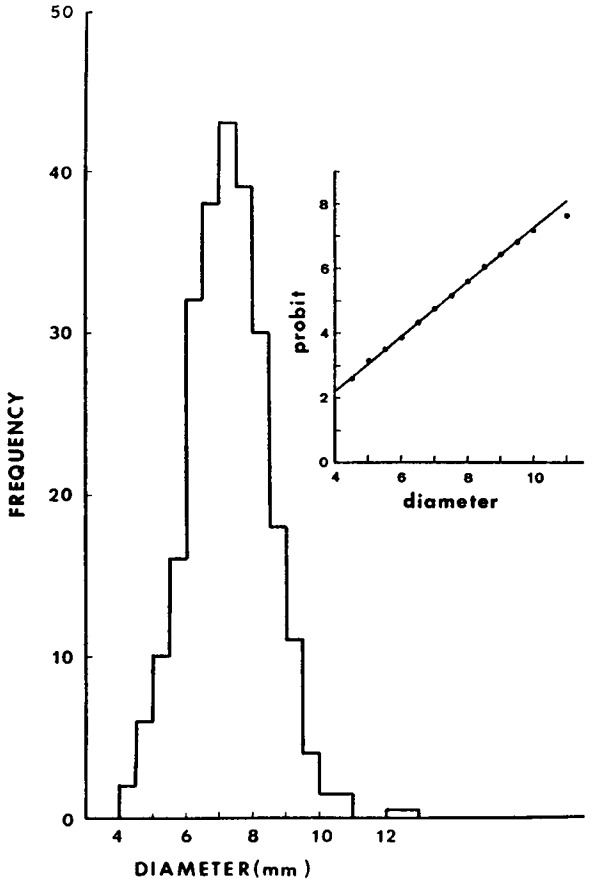


FIGURE 4 Raw data of zymogen granule diameters (from a separate preparation) measured on scanning electron micrographs. Number of events, mean, and variance are: 253, 0.86 $\mu$m, and 0.13 $\mu$m (see text). In the *inset* is shown the results of a probit transformation to test the observed distribution for normality. The resulting straight line supports the hypothesis that the observed distribution is normal.

a value indicates an excellent fit and supports the hypothesis that the observed sample is drawn from a normal population.

## DISCUSSION AND SUMMARY

The Monte Carlo technique combined with the maximum likelihood method proved to be very useful in determining the best fit to the observed data. The advantages of this include the feature that the entire observed distribution is being used in the comparison process. This contrasts with the more conventional methods where only two or three measures of the observed data are taken and then used in conjunction with an assumed model to compute its parameters. Also to be condidered is the

flexibility whereby different parent distribution functions can be inserted into the computer program and tried out using the chi-square test as an index of the goodness of fit. More conventional methods do not permit this convenient and quantitative comparison of one assumed distribution to another. We feel that, with ready availability of digital computers, such methods deserve widespread consideration.

An appraisal of the method described necessitates an examination of possible sources of error and discrepancies between the actual measuring process and the mock experiment performed by computer. In the actual measuring process, there is a bias against counting the very small circles (i.e., less than 2 mm in diameter). This arises from the lower cutoff in the particle sizing instrument, and also as a result of the finite section thickness. Although the Monte Carlo process could and did generate circles smaller than were encountered in the experimental data, they were not used in the comparison process; only generated data falling into bins corresponding to the allowed measuring bins were accepted. In this respect the computer experiment mimics precisely the actual one. A second possible source of error lies in the selection of spheres to be cut. According to a rigorous treatment of the problem (Wicksell, 1925) larger spheres have a greater chance of being cut by a factor of $r/r_0$, where $r$ is the sphere diameter, and $r_0$ is the average sphere diameter. This would tend to bias the distribution of circles towards larger diameters. Such a bias could have easily been introduced into the generating process but it was felt that practical considerations (Krumbein, 1938) had shown that the unbiased selection process gave better fits to the data and so that was the model chosen. The quality of the goodness-of-fit parameter (chi-square = 22 when 18 was the expected value) as well as direct comparisons between the generated and observed distributions support this choice and make unlikely the prospect that another scheme would result in a better fit.

A final remark can be made concerning the possibility that other functions might serve as parent distributions and generate observed distribution identical (to within statistical measures) with the one used here. It is conceivable that this possibility exists and in that case, in the absence of any previous theoretical framework for choosing one parent distribution over another, each can serve equally well as a model for the zymogen granule size distribution. This statement is true for any method used to analyze the observed data and only when the observations become more and more refined does there develop the possibility of distinguishing one proposed parent distribution from another.

Within the framework of remarks made above, it is clear that the observed distribution of zymogen granules in a plane section through the purified pellet is derived trom a parent distribution which is normal. Direct diameter measurements using the scanning electron microscope confirm and support this conclusion. There is no evidence for the presence of another population of particles, although this can not be absolutely ruled out since small amounts of contamination can be masked within

statistical fluctuations. The two groups of data give parameters which are not identical (within statistical limits). There are a number of possible factors contributing to these differences. One is that the methods used for fixation differ and therefore the amount of shrinkage could be different. Another is that there is a real difference in the two biological samples since they were separated in time and done on different size rats. Although this difference might be worthy of further investigation, the main result that the distribution is normal remains unaffected and still valid.

It is interesting to note that if the diameter distribution is transformed to a mass distribution (which follows the cube of the diameter), this is no longer normal but is strongly skewed towards small mass values and acquires a long tail at the upper end of the spectrum. This fact suggests that whatever factors are the ones that control production of these granules, they operate on the size parameter rather than on the mass.

I would like to thank the following individuals whose assistance and advice contributed in large part to the completion of this work: Dr. Francois Lamy for drawing my attention to the problem, Dr. William Longphre for making the diameter measurements on the transmission electron micrographs and for the preparation of the zymogen granules, Dr. R. J. Casciato for furnishing the transmission electron micrographs, Mr. Richard Motta for the use of the scanning electron microscope, and to Mrs. Linda Rose for typing and correcting the manuscript.

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## APPENDIX I

### *Relationship between the Parent Three-Dimensional Distribution and the Sectional Two-Dimensional Distribution*

The discussion which follows assumes that an arbitrary thin section cuts through a typical sample of the particle distribution. Although the more general treatment (Wicksell, 1925) includes the probability of encountering any particular size sphere, Krumbein (1938) points out that the simplified treatment fits the experimental data better.

Assume $f(a)$ is a probability function describing the distribution of radii of the population of spheres.

$$\int_0^\infty f(a)\, da = 1. \tag{1}$$

For a sphere of radius $a$, the probability that a cut will result in a circle of radius $\rho$ is derived by assuming that a cut anywhere along the sphere's polar axis is equally likely. After algebraic transformation the result is:

$$dp(\rho) = \frac{\rho}{a} \frac{1}{(a^2 - \rho^2)^{1/2}}\, d\rho. \tag{2}$$

Combining these two functions, the probability that a cut through the population $f$ will give

circles in the range $d\rho$ about $\rho$ is given by:

$$dp(\rho) = \int_{\rho}^{\infty} da \left[ f(a) \frac{\rho}{a(a^2 - \rho^2)^{1/2}} \right] d\rho, \tag{3}$$

where the integration is carried out over the population $f$ starting with spheres whose radii are greater than or equal to $\rho$.

The population of circles may be described by the distribution function $g(\rho)$ which is defined through the relation

$$dp(\rho) = g(\rho)d\rho. \tag{4}$$

One can now proceed to compute the moments of the distribution $g(\rho)$ and establish relationship with those of $f(a)$. This aspect of the problem has been carried out by Krumbein (1935). Here we wish to concentrate on the first two moments of the distributions. These are the mean and standard deviation of the projected distribution.

$$\langle \rho \rangle = \int_0^{\infty} \rho g(\rho)\, d\rho = \int_0^{\infty} \left[ \rho^2\, d\rho \int_{\rho}^{\infty} \frac{f(a)}{(a^2 - \rho^2)^{1/2}} \frac{da}{a} \right], \tag{5}$$

where the use of the symbol $\langle\rangle$ means taking the average. After algebraic manipulation (in which the order of integration is interchanged) we find:

$$\langle \rho \rangle = (\pi/4) \int_0^{\infty} af(a)\, da. \tag{6}$$

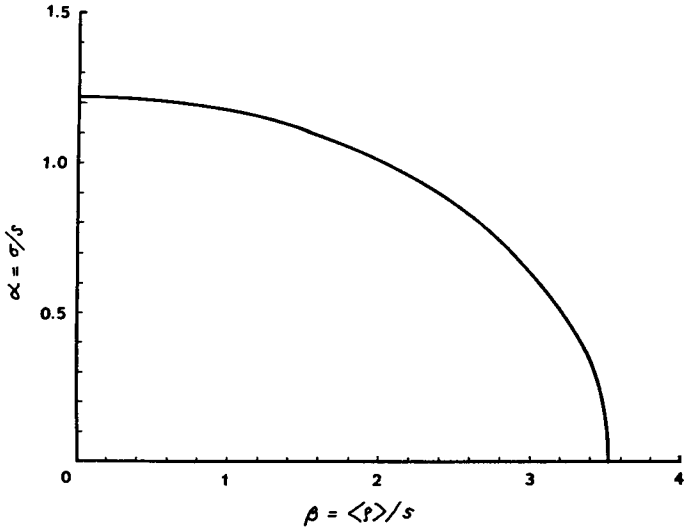


FIGURE 5 Relationship among quantities describing the parent and generated distribution functions as discussed in the test.

Therefore,

$$\langle \rho \rangle = (\pi/4)\langle a \rangle. \tag{7}$$

The standard deviation of the projected distribution is given by

$$s^2 = \langle (\rho - \langle \rho \rangle)^2 \rangle = \langle \rho^2 \rangle - \langle \rho \rangle^2, \tag{8}$$

$$\langle \rho^2 \rangle = \int_0^\infty \rho^2 g(\rho)\, d\rho = \int_0^\infty \rho^3\, d\rho \left[ \int_\rho^\infty \frac{f(a)}{(a^2 - \rho^2)^{1/2}} \frac{da}{a} \right]. \tag{9}$$

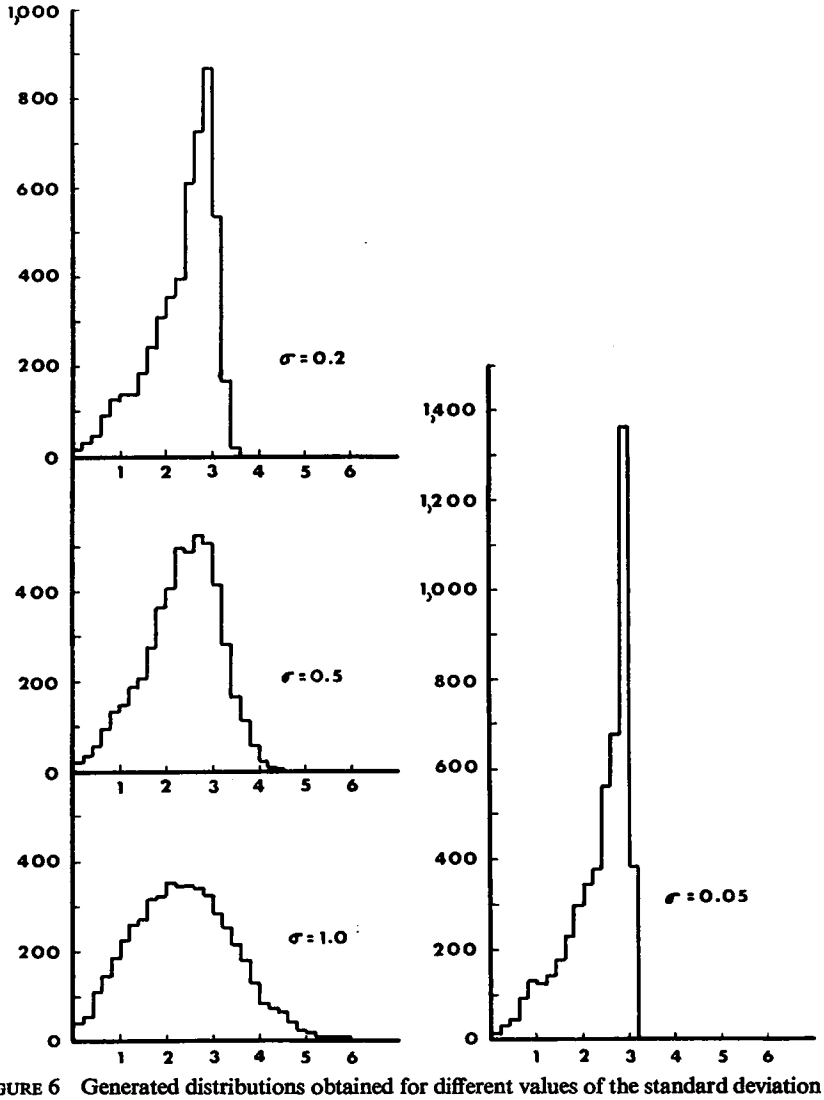


FIGURE 6 Generated distributions obtained for different values of the standard deviation of the parent (normal) distribution function while keeping the mean constant at a value of 3.0. The scale along the abscissa has units of events per bin and the bin width is 0.2. Each histogram contains a total of 1,000 events.

Proceeding as for the case of the mean, we find that

$$\langle \rho^2 \rangle = \frac{2}{3} \langle a^2 \rangle. \tag{10}$$

As pointed out by Krumbein (1935) these results are independent of the parent distribution $f$. That is to say that the moments about the origin of the projected distribution $g$ are related to the corresponding moments for the parent distribution $f$ through a simple constant which depends only upon the order of the moment. Collecting the results we have:

$$\langle \rho \rangle = (\pi/4) a_0 \tag{11 a}$$

$$s^2 = \frac{2}{3} \sigma^2 + a_0 \left[ \frac{2}{3} - (\pi^2/16) \right] \tag{11 b}$$

where $a_0 = \langle a \rangle$ and $\sigma^2 = \langle (a - \langle a \rangle)^2 \rangle$. Transform Eq. 11 $b$ by expressing $a_0$ in terms of $\langle \rho \rangle$ and defining the universal variables $\alpha = \sigma/s$, $\beta = \langle \rho \rangle / s$.

$$1 = 0.667\, \alpha^2 + 0.808\, \beta^2. \tag{12}$$

This is the equation of an ellipse and is shown in Fig. 5, which depicts graphically the relationships among the relevant parameters. This figure can be used to read the value for $\sigma$ from the quantities $\langle \rho \rangle$ and $s$ obtained for the observed projected distribution. The special case of a normal parent distribution function is shown in Fig. 6. Here are depicted a progression of generated distributions obtained in each case from a parent distribution whose mean is 3.0, but whose standard deviation was changed as indicated. As can be seen, the generated distribution approaches a $J$ curve as the parent function is made narrower (smaller $\sigma$), but the mean of the generated data is, in all cases, the same as is predicted by Eq. 11 $a$.

## APPENDIX II

### *Monte Carlo Method for Producing the Generated Data Distribution*

The objective is to perform a mock experiment simulating in detail the processes which take place when the raw data are obtained. For this purpose, one takes an assumed parent distribution function and chooses spheres to be cut from it. The method for picking spheres out of this distribution is designed to maintain the relative frequency with which a sphere of any given diameter occurs within the distribution and is outlined below. Then one chooses a plane to cut the aforechosen sphere at a random distance from the sphere's center. This set of operations results in a circle which belongs to the generated data distribution. In this procedure the distribution of chosen spheres must be a representative sample of the parent distribution and the cutting plane must be chosen anywhere from the center to tangent to the sphere with equal probability. To do this a random number generator is required. Such a routine where random numbers are chosen uniformly over the range 0–1 is a normal component of most digital computer systems. For each circle produced, then, two random numbers are needed.

Described first is the way in which a sphere belonging to a random sample of the parent distribution is chosen. Let $f(r)$ be this parent distribution function. $f(r)dr$ is the probability that a sphere has a radius between $r$ and $r + dr$. Assume that $r$ is bounded, i.e., that there are no spheres greater than $R$. Divide the range $(0, R)$ into $N$ equal parts each of size $\Delta N =$

$R/N$. The $k$th bin then is defined by the limits $(k - 1)\cdot\Delta N$ to $k\cdot\Delta N$. Generate a new set of $N$ bins where the $k$th one has the width

$$\Delta N_k = f\left(\frac{2k-1}{2}\cdot\Delta N\right)\cdot\Delta N$$

i.e., each new bin has a width proportional to the old bin multiplied by the value of $f$ at its midpoint. Thus the new bin widths reflect the value of the parent distribution function.

Now choose random numbers uniformly along the line from 0 to 1, determine into which new bins they fall, and plot them over the corresponding original bins. They will form a random sample of the given function $f$. Using this procedure the outline of the Monte Carlo calculation is as follows:

(*a*) Choose a sphere at random from the parent distribution function. Let its radius be $a$.

(*b*) Choose a distance $0 < x < a$ at random to represent the distance at which the plane will cut the chosen sphere.

(*c*) Compute $\rho$ the radius of the circle obtained from the cut.

(*d*) Repeat operations 1–3 until the desired number of events has been generated.

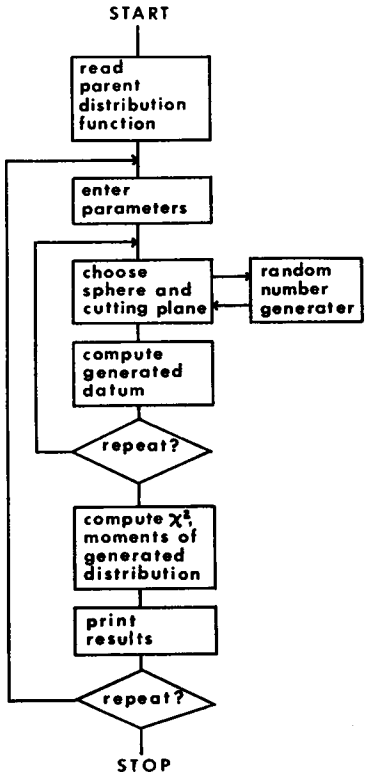


FIGURE 7 Computer flow chart showing the process of creating a generated distribution from a given parent distribution function, as well as its comparison with raw data using the chi-square function as a measure of the goodness-of-fit.

(*e*) For the distribution of calculated circles, compute the chi-square and other relevant statistical measures.

(*f*) Printout the results. The operator now has the option of returning to the beginning and inserting new parameters, or of terminating the calculation.

The sequence of operations involved in obtaining the generated data distribution and in comparing this with the raw data is blocked out in the flow chart shown in Fig. 7.

## APPENDIX III

### *The Fitting Program*

The quantity chi-squared is the parameter used to determine the best fit of the Monte Carlo generated data to the raw data. Chi-square is computed according to the following equation.

$$\chi^2(p_1, p_2, \cdots) = \sum_{j=1}^{M} \frac{[G_j(p_1, p_2, \cdots) - D_j]^2}{w_j},$$

where, $D_j$ is the raw data in the $j$th bin, $G_j$ is the generated data, $w_j$ is the weighting factor, and $p_1$, $p_2$, etc. are the parameters of the parent distribution. The weights $w_j$ are constructed from a combination of errors from the raw data and the Monte Carlo generated data.

$$w_j = g_j^2 + d_j^2$$

where $g_j$ and $d_j$ are errors associated with the generated and raw data, respectively.

The parameter set $\{p_i\}$ is intrinsic to the parent distribution. For instance in the case of the normal distribution there would be only the two parameters: mean and standard deviation. The value of $\chi^2$ will depend upon these parameters and indeed the essence of the fitting process is to first choose a set of $p_i$'s and then to vary only one of them until a minimal value of $\chi^2$ is obtained. After this other $p_i$'s are varied one at a time always searching for a

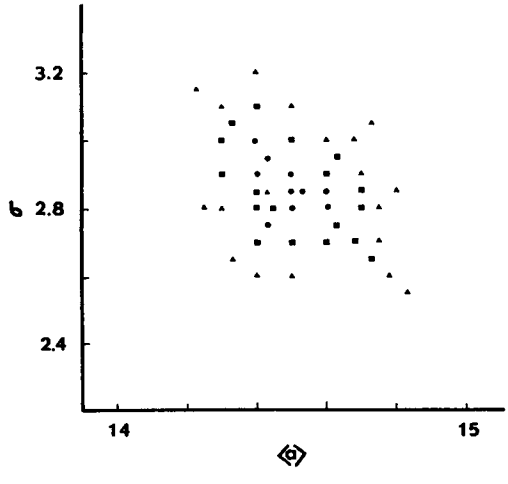


FIGURE 8 Contour map of the chi-square function in the region of the minimum value of chi-square. Filled circles represent $22 < \chi^2 \leq 24$; squares, $25 < \chi^2 \leq 30$; and triangles, $30 < \chi^2 \leq 40$.

minimum. The problem becomes difficult as the number of parameters to be adjusted increases beyond three, and the question of whether the global minimum has been found or just a local pocket is always present. However, if the initial guess for the parameter set is close to the best fit, the convergence is rapid and the later problem is not troublesome.

Fig. 8 shows the result of applying the fitting program to some raw data. The plot is essentially a contour map of the region about the minimum of the chi-square function ($\chi^2_{min}$ = 22.0). The most minimal region is the oblong area spotted with filled circles. This region contains points whose $\chi^2 \leq 25$. Around this valley are slopes defined by $25 \leq \chi^2 \leq 30$ (filled squares) and higher regions where $30 < \chi^2 \leq 40$ (filled triangles). These data were used to determine errors on the values of mean and standard deviation of the parent distribution used to fit the raw data (see Orear [1958] or Cramer [1955] for detailed methods).

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