CORRECTION OF POLYRIBOSOME DISTRIBUTIONS AS OBSERVED IN CELL SECTIONS BY ELECTRON MICROSCOPY

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ABSTRACT

Clusters of ribosomes observed by electron microscopy in thin sections of rabbit reticulocytes are of the same order of size as the section thickness of 600 A. Many of the observed clusters must therefore have been transected by the section surfaces and observed as clusters containing fewer ribosomes. A probability method of correcting for this effect is given. Comparison of the results with grid observations of ribosome distributions indicates sufficiently good agreement for application to cell section observations.

THE MODEL

A 600 A thick section of a rabbit reticulocyte shows, under the electron microscope, both individual ribosomes and, depending on the age of the cell, clusters of up to about six ribosomes (1).[1] Because the size of a cluster is of the same order of magnitude as the thickness of the section, many of the clusters which existed before sectioning must have been transected by the section surface. The problem thereupon arises of correcting the observed distribution of ribosomes among clusters of various sizes to the distribution which existed in that intracellular region before sectioning.

A cluster containing j ribosomes is denoted a j-cluster or also, for $j = 1$, a single; for $j = 2$, a double; etc. The observed values of j are 1 to 6, the j ribosomes being arranged in a ladder-like structure (Fig. 1). An occasionally observed 7-cluster, as well as an alternative ring-like structure of the 5-cluster (1), is neglected. The observations are made by counting, in a number of physiologically similar cell sections, a total of N^0 ribosomes, of which N_1^0 exist as singles, N_2^0 exist in 2-clusters, N_n^0 exist in n-clusters. The N_j^0, $j = 1$ to n, define the observed distribution.

A "true" distribution N_j, $j = 1$ to n, is derived from the observed distribution *via* the following model. A region is postulated, containing a large number of non-interacting, randomly distributed and oriented clusters of ribosomes. The total number of ribosomes in this region is N, of which N_1 exist as singles, N_2 exist in 2-clusters, N_n exist in n-clusters. A grid of equally spaced section planes is considered installed in the region, a distribution is derived by counting ribosomes in each section, and corresponding ribosome numbers are added for all sections. The result is a ribosome distribution postulated to equal the observed N_j^0 distribution. This procedure is statistically equivalent to counting ribosomes in a single section, provided the number of ribosomes is sufficiently large. The ribosome distributions are next expressed as cluster distributions:

$$Q_j^0 = N_j^0/j, \qquad Q_j = N_j/j, \qquad i = 1 \cdots n \qquad (1)$$

[1] The observed clusters are identified as the so called polyribosomes (2).

where Q_j^0 is the observed and Q_j the "true" number of j-clusters. To relate the Q_j^0 and the Q_j, consider a given section. Of the total number Q_j of j-clusters, a number $\rho_j Q_j$ intersect or lie within this section and will be observed as clusters, although not necessarily as j-clusters. The fraction ρ_j is not constant but increases with j because a larger cluster has a greater chance of intersecting or lying within a given section than has a smaller cluster. Otherwise expressed, sectioning a region increases the total number of clusters counted in all sections because transecting a cluster in general creates two clusters. Of the $\rho_j Q_j$ j-clusters, a fraction α_{1j} is observed as singles, a fraction α_{2j} as doubles, $\cdots$ and the remaining fraction α_{jj} as j-clusters. The total number of observed i-clusters, Q_i^0, is thus a sum of contributions from i- and higher clusters:

$$\left.\begin{aligned} Q_1^0 &= \alpha_{11}\rho_1 Q_1 + \alpha_{12}\rho_2 Q_2 + \cdots + \alpha_{1n}\rho_n Q_n \\ Q_2^0 &= \alpha_{22}\rho_2 Q_2 + \cdots + \alpha_{2n}\rho_n Q_n \\ &\vdots \\ Q_n^0 &= \alpha_{nn}\rho_n Q_n \end{aligned}\right\} \quad (2)$$

In terms of ribosomes, Equations 1 and 2 give:

$$N_i^0 = \sum_j' \gamma_{ij} N_j, \qquad i = 1 \cdots n \quad (3)$$

where $\sum_j'$ denotes summation over j from $j = i$ to n and

$$\gamma_{ij} = (i/j)\alpha_{ij}\rho_j \quad (4)$$

Solving the simultaneous equations (3) for the N_j in terms of the N_j^0 (solve the last equation for N_n, substitute into the next-to-last equation and solve for N_{n-1}, etc.) yields:

$$\left.\begin{aligned} N_1 &= \epsilon_{11}N_1^0 + \epsilon_{12}N_2^0 + \cdots + \epsilon_{1n}N_n^0 \\ N_2 &= \epsilon_{22}N_2^0 + \cdots + \epsilon_{2n}N_n^0 \\ &\vdots \\ N_n &= \epsilon_{nn}N_n^0 \end{aligned}\right\} \quad (5)$$

Equation 5 constitutes the scheme for correcting the observed distribution, the N_j^0, to the "true" distribution, the N_j. Some general properties of the equations are:

a. Linearity with respect to ribosome number;

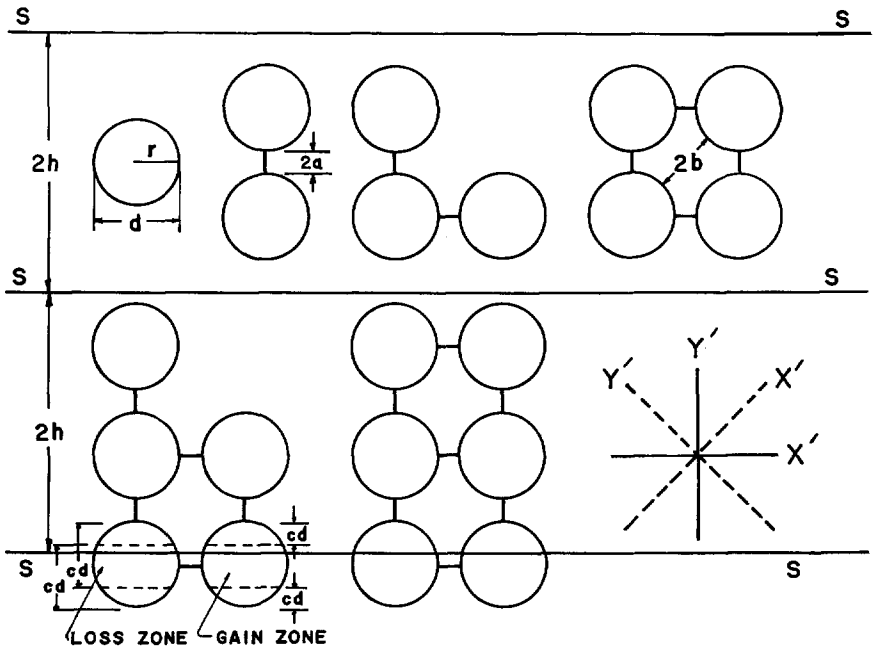

FIGURE 1 Assumed geometry of spherical ribosomes in j-clusters, $j = 1$ to 6, in comparison with section thickness $2h$. Lines connecting spheres denote fixed and equal separation distances. $h = 300$, $r = 100$, $a = 25$, $b = \sqrt{2}a + (\sqrt{2} - 1)r = 76.8$ (Angström units). Each cluster type occurs in randomly different positions with respect to the section surfaces SS. The $X'Y'$ axes are considered fixed with respect to each cluster.

corresponding ribosome numbers of two separate solutions of Equation 5 (N_j^{I}, $N_j^{0\mathrm{I}}$), (N_j^{II}, $N_j^{0\mathrm{II}}$), may be added to give a solution [($N_j^{\mathrm{I}} + N_j^{\mathrm{II}}$), ($N_j^{0\mathrm{I}} + N_j^{0\mathrm{II}}$)]. The linearity results from the assumption of non-interacting clusters, which yields ϵ_{ij} independent of the N_j. This property permits individual sections containing small numbers of ribosomes to be counted, solved by Equation 5 for true distributions, and inspected for various purposes, before combining the solutions by simple addition to yield a statistically more valid solution (1).

b. Conservation of cluster number: the true number of clusters $\sum_j Q_j$ is increased by sectioning to $\sum_j \rho_j Q_j$, as previously noted. This increased number equals the observed number $\sum_j Q_j^0$. Hence, only $n - 1$ of the n Equations 2 are independent and the α_{ij} satisfy (add all of Equations 2 and note that all but one of the Q_j can be independently made to vanish):

$$1 = \alpha_{11} = \alpha_{12} + \alpha_{22} = \cdots = \alpha_{1n} + \cdots + \alpha_{nn} \tag{6}$$

c. Conservation of ribosome number: when a section surface transects a cluster it may also transect individual ribosomes of the cluster (this is always true for singles). The criterion for observing a ribosome is defined thus: at least a fraction c of a ribosome diameter d must lie within a section for the ribosome to be seen and counted. If the "cut-off parameter" c is between 0 and 0.5, and a transection occurs within the "gain zone" (Fig. 1), the ribosome is counted twice, once from each side (or once from each of two symmetrically situated ribosome positions, from the statistically equivalent single section point of view). The result is a gain in ribosome number by sectioning. Similarly, if c is between 0.5 and 1.0, ribosomes transected in the "loss zone" (Fig. 1) are lost to count. At $c = 0.5$ there is conservation of ribosome number by sectioning. The assumption of conservation of ribosome number by sectioning is made herein, that is, $N^0 = N$. Hence, only $n - 1$ of Equations 3 and 5 are independent and the γ_{ij}, ϵ_{ij} satisfy (compare Equation 6):

$$1 = \gamma_{11} = \gamma_{12} + \gamma_{22} = \cdots = \gamma_{1n} + \cdots + \gamma_{nn} \tag{7}$$

$$1 = \epsilon_{11} = \epsilon_{12} + \epsilon_{22} = \cdots = \epsilon_{1n} + \cdots + \epsilon_{nn} \tag{8}$$

The error produced by this assumption is not thought to be serious because, first, experimental evidence cited later, as well as morphological expectation, indicates that c is not far from 0.5. Second, to the extent that the probability of ribosome loss (or gain) is the same for every ribosome regardless of the size of cluster it is in, the previous formulation is still essentially valid (the N_j^0 in Equations 3 and 5 are multiplied by a constant).

THE PROBABILITY COEFFICIENTS

The coefficient α_{ij} is the probability that a j-cluster which intersects or lies within a section is observed as an i-cluster. Two factors contribute to α_{ij}, overlap and transection. Overlap occurs, for example, when a 2-cluster is oriented with its long axis normal to the section surface. The 2-cluster is then observed as a single. However, a 2-cluster is estimated to be easily distinguishable as such if its long axis is inclined more than approximately 15° with respect to the section surface normal.[2] The solid angle of long-axis orientation within which a 2-cluster is observed as a single is then the fraction $1 - \cos 15° = 0.034$ of the total available solid angle of 4π. The overlap contribution to α_{12}, and by similar considerations, to α_{ij}, should therefore be small, and will be neglected. The error made thereby is on the conservative side; that is, the correction to be derived from observed to true distribution should be somewhat too small.

To obtain the transection contribution to α_{ij}, consider first a 2-cluster (Fig. 2). Fix a Z axis normal to, and with origin O midway between, the two section planes SS. Fix axes $X'Y'Z'$ in the 2-cluster, with origin O' at the center of symmetry and Z' axis normal to the long axis of the cluster. The position of the cluster relative to the section is described by four coordinates[3] (3): the distance z between origins, or equivalently between the initial position S_oS_o and the position SS of the section as it is displaced in the Z direction through the range $-\infty < z < \infty$; the angle θ between Z and Z' axes, ranging from O to π as Z is considered to rotate relative to Z'; the angle ψ_k between initial and final positions of the

[2] The criterion of distinguishability is the circular boundary of each ribosome area, not the (approximately uniform) density distribution within the boundary (1).

[3] The present notation differs slightly from that in (3).

X' axis which rotates with the 2-cluster in the $X'Y'$ plane through a range O to 2π (the simplifying approximation will later be made that ψ_k takes on only a discrete set of values, denoted by $k = 1, 2, \cdots K$); the angle of rotation of the section plane around the Z axis. This coordinate need not be explicitly considered because of symmetry.

A function $I_{12}(z, \theta, \psi_k)$ is defined, which equals 1 if only 1 ribosome of the 2-cluster is observable within the section at the indicated values of the coordinates, and is zero otherwise; similarly, $I_{22}(z, \theta, \psi_k)$ equals 1 if both ribosomes are observable, and is zero otherwise. These functions are integrated over z and θ and averaged (equivalent to summation) over the K values of ψ_k to yield probabilities P_{i2} for counting $i = 1$ or 2 ribosomes of the 2-cluster, as the cluster assumes all possible positions relative to the section. Thus:

$$P_{i2} = K^{-1} \sum_k \int_0^{\infty} \int_0^{\pi/2} I_{i2}(z, \theta, \psi_k) \sin \theta \, d\theta \, dz \quad (9)$$

in which $\sin \theta$ appears because equal weight is given to equal elements of solid angle and the integrations over z and θ are over half their com-

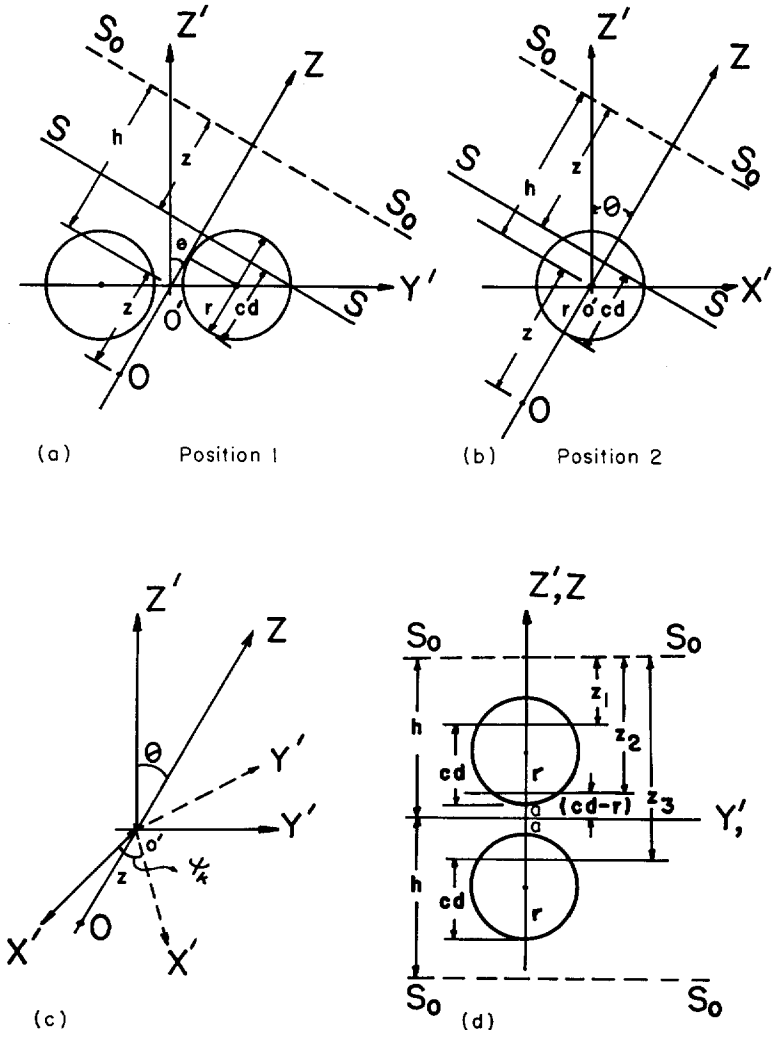

FIGURE 2 Calculation of α_{12} for 2-cluster: (*a*) position $k = 1$; (*b*) position $k = 2$; (*c*) the three coordinates z, θ, ψ_k; (*d*) cut-off values of z, also position of 2-cluster for exact calculation.

plete ranges, by symmetry. The probability α_{i2} is given by:

$$\alpha_{i2} = P_{i2}/(P_{12} + P_{22}), \qquad i = 1, 2 \tag{10}$$

To derive P_{i2}, four ψ_k values 90° apart were taken, of which by symmetry only two need explicit calculation. These are, ψ_1 (position 1, Fig. 2*a*) and ψ_2 (position 2, Fig. 2*b*). For each position, the section surface *SS* moves from $z = 0$ to ∞. Consider first the $k = 1$ position. Three z intervals in the range 0 to ∞ must be separately evaluated:

(*a*) $0 < z < z_1$:

where:

$$z_1 = h - (r + a) - (cd - r) = h - a - 2rc \tag{11}$$

In this interval the section surface *SS* advances from outside the cluster region to the first cut-off position z_1 (Fig. 2*d*). For a fixed z in this interval, two ribosomes are observable within the section for all angular orientations of the cluster or $I_{12} = 0$, $I_{22} = 1$. Hence, by Equation 9:

$$\Delta P^1_{12} = 0 \tag{12}$$

$$\Delta P^1_{22} = \int_0^{z_1}\int_0^{\pi/2} \sin\theta \, d\theta \, dz = z_1 = h - a - 2rc \tag{13}$$

where ΔP^k_{i2} denotes the contribution to P_{i2} from the double integral, for position k, and from this z interval.

(b) $z_1 < z < z_2$:

where

$$z_2 = h - (cd - r) = h + r - 2rc \tag{14}$$

$$z_2 - z_1 = r + a \tag{15}$$

For a fixed z in this interval, two ribosomes are observable in the angular interval $0 < \theta < \theta_1$ and one ribosome is observable in $\theta_1 < \theta < \pi/2$. The angle θ_1, at which *SS* cuts one ribosome at the cut-off distance (Fig. 2 *a*), is a function of z given by

$$\sin\theta_1 = (z_2 - z)/(z_2 - z_1) \tag{16}$$

Hence, by Equations 9 and 16:

$$\Delta P^1_{12} = \int_{z_1}^{z_2}\int_{\theta_1}^{\pi/2} \sin\theta \, d\theta \, dz = (z_2 - z_1)\,\pi/4 = (r + a)\,\pi/4 \tag{17}$$

$$\Delta P^1_{22} = \int_{z_1}^{z_2}\int_0^{\theta_1} \sin\theta \, d\theta \, dz = (z_2 - z_1)(1 - \pi/4) = (r + a)(1 - \pi/4) \tag{18}$$

(c) $z_2 < z < z_3$:

where

$$z_3 = h + (r + a) - (cd - r) = h + 2r + a - 2rc \tag{19}$$

$$z_3 - z_2 = r + a \tag{20}$$

In this interval one ribosome is observable for $\theta_2 < \theta < \pi/2$ and no ribosomes otherwise, where:

$$\sin\theta_2 = (z - z_2)/(z_3 - z_2) \tag{21}$$

Hence, by Equations 9 and 21:

$$\Delta P^1_{12} = \int_{z_2}^{z_3}\int_{\theta_2}^{\pi/2} \sin\theta \, d\theta \, dz = (z_3 - z_2)\pi/4 = (r + a)\pi/4 \tag{22}$$

$$\Delta P^1_{22} = 0 \tag{23}$$

The sum of Equations 12, 17, and 22 is:

$$P^1_{12} = (r + a)\pi/2 \tag{24}$$

The sum of Equations 13, 18, and 23 is:

$$P^1_{22} = h - a + (r + a)(1 - \pi/4) - 2rc \tag{25}$$

where P^k_{i2} is the contribution to P_{i2}, Equation 9, from the double integral, and position $k = 1$.

For position $k = 2$ (Fig. 2 *b*), ribosomes are observable in only one z interval, $0 < z < z_1$, where:

$$z_1 = h - (cd - r) = h + r - 2rc \tag{26}$$

In this interval two ribosomes are observable for $0 < \theta < \pi/2$. Hence:

$$P^2_{12} = 0 \tag{27}$$

$$P^2_{22} = \int_0^{z_1}\int_0^{\pi/2} \sin\theta \, d\theta \, dz = z_1 = h + r - 2rc \tag{28}$$

TABLE I

Calculation of the P_{ij} and γ_{ij} Coefficients

Symbol*	Formula‡	Evaluation‡	γ_{ij}§
P_{11}	z_2	300	1.000
P_{12}	$r + a$	125	0.204
P_{22}	$z_2 - (r + a)/2$	238	0.796
P_{13}	$(7r + 3b + 4a)\ \pi/32$	101	0.112
P_{23}	$(7r + 3b + 4a)\ \pi/32$	101	0.224
P_{33}	$z_2 - (7r + 3b + 4a)\ \pi/32$	199	0.664
P_{14}	$(r + b)\ \pi/8$	69.5	0.0580
P_{24}	$(r + a)\ \pi/4$	98.2	0.164
P_{34}	$(r + b)\ \pi/8$	69.5	0.174
P_{44}	$z_2 - (2r + b + a)\ \pi/8$	182	0.604
P_{15}	$(5r + 3b + 2a)\ \pi/32$	76.7	0.0511
P_{25}	$(3r + b + 2a)\ \pi/16$	83.9	0.112
P_{35}	$(3r + b + 2a)\ \pi/16$	83.9	0.168
P_{45}	$(5r + 3b + 2a)\ \pi/32$	76.7	0.205
P_{55}	$z_2 - (11r + 5b + 6a)\ \pi/32$	140	0.464
P_{16}	$(r + b)\ \pi/8$	69.5	0.0385
P_{26}	$(r + a)\ \pi/8$	49.1	0.0545
P_{36}	$(2r + b + a)\ \pi/8$	119	0.198
P_{46}	$(r + a)\ \pi/8$	49.1	0.109
P_{56}	$(r + b)\ \pi/8$	69.5	0.193
P_{66}	$z_2 - (2r + b + a)3\pi/16$	122	0.407

* Equation 9, subscript 2 replaced by j.

‡ $z_2 = h + r - 2rc$, $c = 0.5$, see legend for Fig. 1.

§ Equation 37.

Averaging Equations 24 and 27 and Equations 25 and 28 in accordance with Equation 9, and evaluating numerically (Fig. 1), gives:

$$P_{12} = (r + a)\pi/4 = 98.2, \text{ A} \tag{29}$$

$$P_{22} = h + r - (r + a)\pi/8 - 2rc = 350.9 - 200c, \text{ A} \tag{30}$$

$$P_{12} + P_{22} = h + r + (r + a)\pi/8 - 2rc = 449.1 - 200c, \text{ A} \tag{31}$$

Hence the probability α_{12}, Equation 10 is:

$$\alpha_{12} = 1 - \alpha_{22} = 98.2/(449.1 - 200c) \tag{32}$$

The present method is approximate because the number K of ψ_k positions is finite. It becomes exact as $K \to \infty$. In the special case of 2-clusters, however, an exact result is obtained by considering just the one ψ_k position shown in Fig. 2 *d*, as here all other ψ_k positions are the same by symmetry. The exact result for α_{12} is:

$$\alpha_{12} = 1 - \alpha_{22} = 125/(462.5 - 200c) \tag{33}$$

The approximate result Equation 32 is about 22 per cent less than the exact result Equation 33 and is on the conservative side; that is, requires a smaller correctional shift from singles to doubles than the exact result.

For the higher j-clusters, eight ψ_k positions were considered (X' rotated successively by 45°, Fig. 1). Because of the increased number of positions, the results should be more exact than for $j = 2$, Equation 32, but still on the conservative side because of generally similar geometry. The derived P_{ij}, defined by Equation 9 with sub-

TABLE II

Coefficients for Correcting Ribosome Distributions

j	ϵ_{ij}*	ϵ_{2j}	ϵ_{3j}	ϵ_{4j}	ϵ_{5j}	ϵ_{6j}
1	1.000					
2	−0.264	1.264				
3	−0.080	−0.429	1.509			
4	−0.0015	−0.219	−0.434	1.654		
5	−0.0169	−0.0532	−0.355	−0.728	2.153	
6	−0.0122	+0.122	−0.447	−0.0973	−1.02	2.454

* Each column contains the ϵ_{ij} of one of Equations 5.

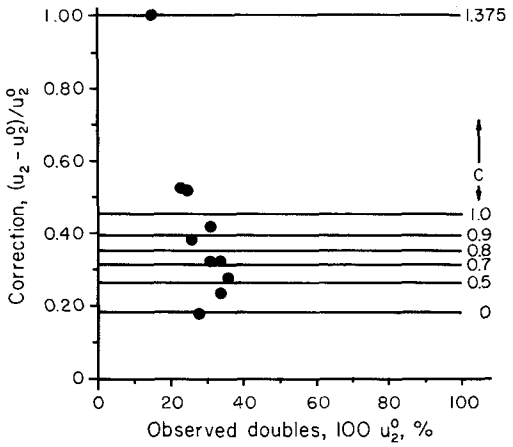

FIG. 3 Calculated and grid-observed corrections in populations of singles and doubles. Circles, grid observations (1); lines, Equation 42 for various values of cut-off parameter c. The value $c = 1.375$ does not have physical significance on the present model.

script 2 replaced by j, were evaluated for cut-off parameter $c = 0.5$ (Table I). The exact results for P_{i2}, Equation 33, and for P_{11} determined by the same method, are included in Table I.

The other probability coefficient ρ_j, that a j-cluster from the presectioned population will intersect or fall within a given section, is proportional to the total z interval in which any of the ribosomes of the j cluster are observable within the section. Since P_{ij} is essentially the z interval over which a j cluster is observable as an i cluster, the total z interval in question is simply the sum over $i = 1$ to j of P_{ij}, or

$$\rho_j = C \sum_i P_{ij} \tag{34}$$

Hence, the generalization of Equation 10:

$$\alpha_{ij} = P_{ij} / \sum_i P_{ij} \tag{35}$$

becomes, upon substituting Equation 34:

$$\alpha_{ij}\rho_j = CP_{ij} \tag{36}$$

The constant C is evaluated by Equations 4 and 7 as $1 = \gamma_{11} = \alpha_{11}\rho_1 = CP_{11}$. Hence $C = 1/P_{11}$ and Equations 4 and 36 yield:

$$\gamma_{ij} = (i/j)P_{ij}/P_{11} \tag{37}$$

From the γ_{ij} (Table I) the ϵ_{ij} were derived (Table II) as described preceding Equation 5. The ρ_j, Equation 34, have the relative values for $j = 1$ to 6 of 1, 1.21, 1.34, 1.40, 1.54, 1.59. These values show the expected progressive increase as previously noted.

APPLICATIONS TO EXPERIMENT

The validity of the preceding analysis will be assessed by comparison with grid observations (1) made on two types of cluster populations: singles and doubles, and 1- to 6-clusters. The observations consist of ribosome distributions measured on electron micrographs, with and without a superimposed set of grid lines to simulate the transection effect (see paragraph preceding Equation 1). The distribution without grid is expressed as the set of numbers u_1, u_2, etc., where u_1 is the fraction of the total number N of observed ribosomes existing as singles, u_2 is the fraction of the total number N of observed ribosomes existing in the 2-cluster configuration, etc. The distribution with grid is expressed similarly as u_1^0, u_2^0, etc. Thus:

$$u_j = N_j/N, \qquad u_j^0 = N_j^0/N^0 \tag{38}$$

where N, the total number of observed ribosomes without grid, can be equated to N^0, the total number of observed ribosomes with grid (negligible ribosome loss due to grid line masking ef-

fects). Consider first a cluster population of singles and doubles. Equations 3 are, after dividing by $N = N^0$,

$$u_1^0 = \gamma_{11}u_1 + \gamma_{12}u_2 \quad (39)$$

$$u_2^0 = \gamma_{22}u_2 \quad (40)$$

Equation 39 is equivalent to Equation 40 since $u_1^0 = 1 - u_2^0$, $u_1 = 1 - u_2$ (see Equation 7). Equation 37 and Table I yield:

$$\gamma_{22} = P_{22}/P_{11} = [(h + r - 2rc) - \tfrac{1}{2}(r + a)]/(h + r - 2rc) \quad (41)$$

Equations 40 and 41, and the pertinent dimensions (Fig. 1) yield:

$$(u_2 - u_2^0)/u_2^0 = 62.5/(337.5 - 200c) \quad (42)$$

Equation 42 states, for example, that if the cut-off parameter c for observability of a ribosome is 0.5, then any value of doubles fraction u_2^0 observed with grid should be multiplied by 1.26 to yield the value of doubles fraction u_2 without grid. The corrections given by Equation 42 for various values of c are indicated by the horizontal lines (Fig. 3). Also plotted are the grid observations on various populations of singles and doubles (1). The scatter in the grid observations indicates small number fluctuations (only several hundred ribosomes were counted for each distribution) or also "grid experimental" error (such as ambiguity in assigning a ribosome when it is intersected by a grid line of finite width). Although the scatter precludes determination of a precise value for c, the observed corrections indicate compatibility with the model in that they mostly correspond to physically allowed values of c (0 to 1). The observations give a median value $c = 0.8$. The value chosen, mainly on morphological grounds, for use with the higher j-cluster distributions, was $c = 0.5$. The two values do not yield greatly different corrections (0.35 *versus* 0.26). Moreover, the $c = 0.5$ correction is on the conservative (smaller) side.

TABLE III

Correction of Observed Ribosome Distributions

j	$u_{j\,\text{grid}}^{0}$*		u_1‡	u_2‡	u_3‡	u_4‡	u_5‡	u_6‡
1	18.3		18.30					
2	27.8		−7.34	35.18				
3	29.3		−2.34	−12.58	44.15			
4	16.3		−0.02	−3.57	−7.07	27.00		
5	7.6		−0.13	−0.40	−2.70	−5.53	16.36	
6	0.7		−0.01	+0.10	−0.31	−0.07	−0.72	1.72
(*a*)	$u_{j\,\text{theor}}$§	=	8.5	18.7	34.1	21.4	15.6	1.7
(*b*)	$u_{j\,\text{true}}$*	=	7.7	18.7	28.3	27.0	14.2	4.1
(*c*)	$u_{j\,\text{grid}}^{0}$*	=	18.3	27.8	29.3	16.3	7.6	0.7
(*d*)	u_j^0	=	7.7	18.7	28.3	27.0	14.2	4.1
(*e*)	u_j§	=	0.2	5.3	24.1	33.9	26.4	10.1

* From grid observations (1).
‡ Add numbers in this column to give $u_{j\,\text{theor}}$.
§ From Equations 5 and 38, and Table II.

Grid observations were made (1) on a population of up to 6-clusters, containing 1720 ribosomes from 5 cells. The distribution observed with grid $u_{j\,\text{grid}}^{0}$, was corrected by Equation 5 and Table II. The result, $u_{j\,\text{theor}}$, is compared with the distribution observed without grid, $u_{j\,\text{true}}$ (Table III, lines *a* and *b*. All ribosome fractions are expressed in per cent). The $u_{j\,\text{grid}}^{0}$ column is repeated as a row (Table III; line *c*) for convenience in judging the magnitude of the correction. Agreement is good for $j = 1$, 2, and 5 but only fair for $j = 3$, 4, and 6. One reason for discrepancy, aside from "grid experimental" error or small number statistical effects, is that the ϵ_{ij}

for 3-, 4-, 5-, and 6-clusters were calculated approximately. The expected direction of approximation, too small a correction of u_6^0, appears to be verified. Approximation in the calculated ϵ_{ij} is also indicated by ϵ_{26} which is positive (Table II), whereas all the off-diagonal ϵ_{ij} should be negative, by the nature of the correction. However. ϵ_{26} itself contributes little to $u_{2\,\text{theor}}$ (Table III).

Application of the correction scheme, Equations (5) and Table II, to the $u_{j\,\text{true}}$ distribution (Table III, line *b*), now regarded as a u_j^0 distribution (Table III, line *d*), gives an example of the use of the correction scheme in (1). The most notable result (Table III, line *e*) is that $u_1^0 = 7.7$ per cent is corrected to $u_1 = 0.2$ per cent; *i.e.*, the cellular distribution before sectioning contained essentially no single ribosomes. This result is also an additional check on the validity of the correction scheme because the corrected ribosome distribution numbers must all be positive. If the off-diagonal ϵ_{ij} (Table II) are too greatly in error in the negative direction, some u_j and especially u_1, might turn out negative. This, incidentally, is what happens if the grid-observed $j = 1$ correction, $18.3 - 8.5 = 9.8$ per cent (Table III, lines *a* and *c*), is applied to $u_1^0 = 7.7$ per cent (Table 3, line *d*). The result is $u_1 = 7.7 - 9.8 = -2.1$ per cent, which is an example of the error made if a correction from electron micrograph-to-electron micrograph + grid is applied from electron micrograph-to-cell.

It is concluded that the present scheme represents a satisfactory first approximation for correcting specifically the ribosome cluster distributions observed in (1). The method used should also be applicable more generally to problems of this type.

I thank Dr. D. Danon and Dr. R. A. Rifkind for suggesting, discussing and communicating results relevant to the present problem, and Mrs. Leonora LaForte for typing the manuscript.

This work was supported in part by the United States Public Health Service under Grant HE-07482 (CV) and Grant GM-10810, and in part by the Health Research Council of the City of New York under Contract U-1089.

Received for publication, December 5, 1963.

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