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A New One-Parameter Model for the Distribution of Chromosome Breaks in Human Cells

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Damage to genetic material can be brought about by chemicals, radioactivity, radiation, etc. This damage can be visualized as chromosome aberrations such as chromosome and chromatid deletions, dicentrics, chromatid exchanges, and others. The study of chromosome aberration induction by chemical and physical agents has evolved over several decades and discrete distribution models have been developed to analyze the data generated in experimental studies. Most of these models have relied on the Poisson, binomial or negative binomial distributions (SAVAGE 1970, KOHLER et al. 1976, YANKOVENKO et al. 1976) to describe the frequency distribution of aberrations in a collection of cells. The intracellular distribution of lesions gives useful information about the mechanism of the interaction of the damaging agent with different cell components. Recently, JANARDAN and SCHAEFFER (1977), JANARDAN et al. (1979), and DuFRAIN et al. (1980) have proposed the use of the Langrangian Poisson Distribution (LPD) as a model of over-, under- or no dispersion relative to the Poisson. (The value of λ_2, the parameter of the LPD, is a quantitative measure of the dispersion while its sign (+, -, 0) indicates the direction.) However, certain asumptions which are used in the development of the LPD are restrictive of the more complex biologic phenomena being studied by cytogeneticists. Further, many workers have questioned the biologic significance of the improvement resulting from the use of two (or more) parameter models over the use of one parameter models, since additional parameters will result in mathematical improvement at the expense of a reduced number of degrees of freedom for the experimental data.

In this paper we develop a new one parameter model, which we call the Poisson-Linear Exponential Distribution (PLED). This distribution is as good as many of the two parameter models at fitting experimental data. The assumptions of the model are developed from biologic observations.

HYPOTHESES:

1. There is a constant rate of aberration for a given cell.

2. The rate of aberrations varies from cell to cell.

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3. The proportion of cells decreases as the rate of aberration per cell increases i.e., there will be a large number of cells with low rate of aberrations and small number of cells with high rate of aberrations. Thus, the resistance by cells for sustaining aberrations decreases over the time as the dose increases.

THE POISSON-LINEAR EXPONENTIAL

Suppose (i) the number of aberrations, x, sustained by a given cell in a period of specified (say 'unit') length follows the Poisson distribution with probability function

$$P(x/\lambda) = e^{-\lambda}\lambda^x/x! \quad (x = 0, 1, 2, \ldots),$$

and (ii) λ (>0) varies from cell to cell according to a probability density function $f(\lambda)$ with parameter β:

$$f(\lambda) = e^{-\lambda/\beta}(\beta^2\lambda+\beta^2)/(\beta+1), \ \lambda>0, \ \beta>0.$$

Then the overall distribution of the observed number of aberrations per cell in a given period of unit length is:

$$(1) \quad P(x) = \int_0^\infty P(x/\lambda)f(\lambda)d\lambda = \int_0^\infty (e^{-\lambda}\lambda^x/x!)(\beta^2(\lambda+1)e^{-\lambda\beta}/(\beta+1))d\lambda$$

$$= \beta^2(\beta + 2 + x)/(\beta + 1)^{x+3}, x = 0,1,2, \ldots, \ \beta > 0.$$

The moment estimator of β is given by:

$$(2) \quad \tilde{\beta} = [-(\bar{x} - 1) + (\bar{x} - 1)^2 + 8\bar{x}]/2\bar{x}$$

MATERIALS AND METHODS

LOESCHEKE and KOHLER (1976) analyzed the intercellular distributions of chromatid aberrations obtained after 24 h treatment of human leukocytes of one male and three female donors in vitro with the bifunctional alkylating agent 2,5-bis-ethyleneimino-p-benzoquinone in concentrations of (0.01-0.04) x 10^{-6} g/ml culture fluid (Table 1).

The experiment was performed with 72-h cultures set up with TC medium 199 complemented with inactivated human serum. Phytohemagglutinin was used to stimulate the leukocytes (KOHLER et al. 1976).

YAKOVENKO et al. (1976) studied the distribution of breaks in human leukocytes induced by 0.5 ml of a 10^{-5}M solution of the bifunctional alkylating agent Phosphemide (P,P-bis(1-aziridinyl)-N-2-pyridimidinyl phosphinic amide (CAS 882-58-6)). The cells were incubated with the phosphemide for 1 h. Then, the phospemide was removed by washing with Hank's solution (2x10 volumes). New medium was substituted for the old one, and the cells were incubated.

RESULTS AND DISCUSSION

The results are presented in Table 1 (modified from JANARDAN and SCHAEFFER 1977) and Table 2 (data from Table 1 of YAKOVENKO et al. 1976). Columns (3)-(7) of Table 1 present the theoretical frequencies expected from the Poisson (PD) (3), Poisson-Linear Exponential (PLED) (4), negative binomial (NBD) (5), Lagrangian (LPD) (6) and modified Poisson (MPD) (7), distributions.

The results in Table 1 are representative of the 68 sets of KOHLER'S data (unpublished) from which the example is taken. Here, the PLED, which is a single parameter distribution, models the frequency distribution as well as do the multiparameter NBD, LPD and MPD distributions. These four are substantially better than the PD as models of biologic processes.

The expected frequencies calculated from the PLED for distributions of breaks resulting from exposure to phosphemide (Table 2) agree with experimental data (YAKOVENKO et al. 1976). In addition to the results for phosphemide (Table 2), the PLED afforded excellent fits (not given here) to their data (YAKOVENKO et al. 1976, Tables 2, 3, 4) on the breaks produced by thiophosphemide, dipine and fotrin, which are tri, tetra-, and pentafunctional alkylating agents.

If β in equation (1) is replaced by its estimator (equation (2)), it is seen that the probability $P(x)$ is a function of only the mean number of aberrations, $\bar{x}$, and the number of aberrations in a given cell, x. Since β decreases as x increases, β is inversely related to the survival fraction (f_0/n). The empirical exposure (D)-response function for β appears to be.

$$(3)\quad \hat{\beta} = b_0 + b_1 \ln(D+1) + b_2 (\ln(D+1))^2.$$

Equation (3) is the same as the theoretically derived relationship for λ_2 of the LPD (SCHAEFFER et al. 1980). Thus, we can estimate the threshold for the response using the method described by us previously (SCHAEFFER et al. 1980). For the data in Table 2, b_0=40.68, b_1=21.49, and b_2=2.92. From these data, the threshold estimated by extrapolation from the linear term (b_1) is 0.02, where b_0, the β value at zero dose, and b_1 and b_2, the slopes of the linear and quadratic terms are obtained by regression. The left hand side of Eq. 3 is the net effect due to exposure.

TABLE 1

Distribution of Number of Chromatid Aberrations in 400 Cells

Aberrations	Obs.	Frequency PD	PLED	NBD	LPD	MPD
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	268	226.2	252.6	271.5	273.04	271.51
1	87	128.9	94.7	74.7	77.04	81.62
2	26	36.7	34.3	29.6	28.09	35.52
3	9	7.0	12.1	12.9	11.66	9.40
4	4	1.0	4.2	5.9	5.23	1.69
5	2	0.1	1.4	2.8	2.46	0.22
6	1	0	.5	1.3	1.21	0.02
7 or more	3	0	.3	1.3	1.27	0.02
chi-square		26.7	5.28	4.9	4.89	7.79
degress of freedom		3	6	5	5	1
probability		$<10^{-5}$	0.47	0.44	0.44	<0.01

TABLE 2

Analysis of the distribution of the number of breaks after treatment with different phosphemide concentrations

Number of breaks in a cell	Concentration of phosphamide, M · 10^{-5}					
	4.4	PLED	8.8	PLED	13.2	PLED
0	417	411.9	384	386.3	355	353.0
1	28	32.3	58	54.7	72	76.5
2	5	2.8	8	8.0	19	16.3
3	0	0	0	0	3	3.4
4	0	0	0	0	1	0.8
5 or more	0	0	0	0	0	0
mean	0.084		0.164		0.273	
Beta	12.71		6.86		4.34	
Chi-square	0.137		0.213		0.58	
Degrees of freedom	1		2		2	
Probability	0.75		0.9		0.75	

Number of breaks in a cell	Concentration of phosphemide, M · 10^{-5}					
	17.6	PLED	22.0	PLED	26.4	PLED
0	290	286.5	291	285.2	220	217.6
1	102	105.9	88	106.3	109	117.0
2	38	37.7	56	38.2	68	59.6
3	10	13.1	8	13.4	21	29.4
4	7	4.5	5	4.6	15	14.1
5 or more	3	2.3	2	2.3	17	12.3
mean	0.518		0.567		1.062	
Beta	2.32		2.31		1.41	
Chi-square	2.43		11.45		6.0105	
Degrees of freedom	4		4		5	
Probability	0.55		0.02		0.03	

MATHEMATICAL APPENDIX

Properties: Moments

The rth crude moment, μ_r of the linear exponential distribution is given by

$$\mu_r' = \int_0^\infty \lambda^r f(\lambda)d\lambda = \frac{\beta^2}{\beta + 1} \int_0^\infty \lambda^r(\lambda+1)e^{-\lambda\beta}d\lambda.$$

$$= \frac{\beta^2}{(\beta + 1)} \int_0^\infty \lambda^{r+1}e^{-\lambda\beta}d\lambda + \int_0^\infty \lambda^r e^{-\lambda\beta}d\lambda$$

Using the gamma integral identity:

$$\int_0^\infty e^{-ax}x^{n-1}dx = \Gamma(n)/a^n$$

$$\mu_r' = \frac{\beta^2}{\beta + 1} \frac{\Gamma(r+2)}{\beta^{(r+2)}} + \frac{\Gamma(r+1)}{\beta^{r+1}} = \frac{\Gamma(r+1)\ (\beta+r+1)}{\beta^r\ (\beta+1)} = \mu'(r)$$

Here, $m'_{(r)}$, the rth factorial moment of the compound Poisson distribution, equals the rth crude moment μ'_r of the compounding distribution (in our case the distribution of λ)(ORD 1972, p. 125).

Thus, the first and second factorial moments and the variance of the Poisson-Linear Exponential Distribution are

$$m'_{(1)} = \beta^2/(\beta^2+\beta),\ m'_{(2)} = (2\beta+6)/(\beta^2+\beta^2),$$

$$\sigma^2 = m'_{(2)} + m'_{(1)} - m'^2_{(1)} = \frac{(\beta^3+4\beta^2+6\beta+2)}{\beta^2(\beta+1)^2}.$$

The probability generating function (p.g.f.) of the PLED is

$$G(Z) = \sum_{x=0}^{x} Z^x P(x) = \frac{\beta^2}{\beta+1} \frac{\beta + 2 - Z}{(\beta+1-Z)^2},\ Z < \beta+1.$$

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