Quantitative Assessment of the Synchronization of Cell Populations

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INTRODUCTION

The inspection of growth curves does not constitute a precise and reliable method for determining the degree of synchronization of cell cultures. The need for quantification is felt particularly when one is trying to assess the efficacy of a synchronization technique (Helmstetter & Cummings, 1964; Cummings, 1970; Wolosker & Almeida, 1975, 1977) or when comparative studies are made of several techniques (Wolosker, 1976). Such need is certainly at the root of many attempts to provide an index for quantifying the degree of synchronization of cell populations (Mitchison, 1971). An objective appraisal of synchronization should take into account some parameters which characterize the population under study and distinguish it from the asynchronous one. Such factors were described by Scherbaum (1962) and duly considered in his derivation of a synchronization index (S.I.) which has been commonly applied (Scherbaum, 1964; Anagnostopoulos, 1975). However, the essential requirement that any S.I. should have a maximum value (generally 1) for the ideal synchronization and become zero for random exponential growth is not met by that index. We have re-examined Scherbaum's derivation and detected a small but significant inaccuracy which reveals why it fails to fulfil this basic requirement. We propose here the adoption of a new and more correct S.I.

Derivation of the new S.I.

The model growth curves used by Scherbaum for the derivation of the S.I. are of mixed character. While the random exponential curve is drawn to an arithmetical ordinate, variations in cell number for the synchronized multiplication are plotted to a logarithmic ordinate (Scherbaum, 1962, Fig. 1). Our derivation has been based on the model curves in Fig. 1, assuming a logarithmic scale on the ordinate. To allow for easy comparison, we have used the same designations as Scherbaum, S.I. being defined as the ratio S_1/S_2 between areas N_0AB and $N_0N'B$.

According to Scherbaum (1964),

$$S.I. = 1 - \frac{t + gt(2-n)}{1.12 gt}$$

where t is the period during which synchronous multiplication occurs, gt is the generation time and

$$n = 1 + \frac{\text{no. of cells dividing synchronously}}{\text{total no. of cells}}$$

The derivation based on the model curves of Fig. 1 is as follows:

$$S_2 = \frac{gt (\ln 2n_0 - \ln n_0)}{2} = \frac{gt \ln 2}{2} \quad (1)$$

$$S_1 = S_2 - (N_0NA + NN'CA + ACB)$$

Fig. 1. Model growth curves, redrawn from Scherbaum (1962). Curve N_0B (—) is the one used for the present derivation (logarithmic ordinates); curve N_0B (---) is drawn according to Scherbaum's original, which adopted linear ordinates.

$$\begin{aligned}
 S_1 &= S_2 - \frac{(\ln n - \ln n_0) t_1}{2} - (\ln 2n_0 - \ln n) t_1 - \frac{(\ln 2n_0 - \ln n) (gt - t_1)}{2} \\
 &= \frac{1}{2} (gt \ln 2 - t_1 \ln n + t_1 \ln n_0 - 2t_1 \ln 2n_0 + 2t_1 \ln n - gt \ln 2n_0 + t_1 \ln 2n_0 \\
 &\quad + gt \ln n - t_1 \ln n) \\
 &= \frac{1}{2} (gt \ln 2 + gt \ln n - gt \ln 2n_0 + t_1 \ln n_0 - t_1 \ln 2n_0) \\
 &= \frac{1}{2} [gt \ln (2n/2n_0) + t_1 \ln (n_0/2n_0)] \\
 &= \frac{1}{2} [gt \ln (n/n_0) - t_1 \ln 2]
 \end{aligned} \tag{2}$$

Hence,
$$\frac{S_1}{S_2} = \text{S.I.} = \frac{gt \ln (n/n_0) - t_1 \ln 2}{gt \ln 2} \tag{3}$$

For random exponential growth, $n/n_0 = 2$ and $t_1 = gt$, so

$$\text{S.I.} = \frac{gt \ln 2 - gt \ln 2}{gt \ln 2} = 0$$

For the ideal synchronization, $n/n_0 = 2$ and $t_1 = 0$, so

$$\text{S.I.} = \frac{gt \ln 2 - 0}{gt \ln 2} = 1$$

Equation (3) can also be represented as

$$\text{S.I.} = \frac{gt \ln (n/n_0)}{gt \ln 2} - \frac{t_1 \ln 2}{gt \ln 2}$$

leading to
$$\text{S.I.} = \frac{1}{0.693} \ln (n/n_0) - \frac{t_1}{gt} \tag{4}$$

or, for decimal logarithms,
$$\text{S.I.} = \frac{1}{0.301} \log_{10} (n/n_0) - \frac{t_1}{gt}$$

In addition, when $n = 2n_0$,
$$\text{S.I.} = 1 - \frac{t_1}{gt}$$

This new S.I. gives results which are 8 to 10% lower than values obtained with the original S.I. (Scherbaum, 1962), particularly when the index is higher than 0.50. More

importantly, the new index constitutes a fully correct representation of the underlying theory.

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