GROWTH CHARACTERISTICS OF COLONIES IN MONOLAYER CULTURES OF GUINEA PIG SPLEEN

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In two independent series of experiments, in each of which 16 monolayer cultures of guinea pig spleen cells were set up (four at each time), the distribution of the colonies formed by their diameter was studied 6, 7, 10, and 12 days after explanation. At all times studied the distribution of the colonies corresponded to the normal law of distribution; this points to homogeneity of the colony-forming cell population in the hematopoietic organs of guinea pigs as regards their ability to form colonies in monolayer cultures.

KEY WORDS: spleen; colony-forming cells; diameter of colonies.

Modern methods of investigation of stem cells in hematopoietic organs are suitable for quantitative investigations only in mice [10], or alternatively the state of the pool of granulopoietic precursor cells can be assessed by the agar culture method [8]. Meanwhile, experimental data published recently [6, 7] together with the results of the writers' previous investigations [1, 4, 5], indicate that the formation of discrete colonies in monolayer cultures of hematopoietic organs is due to a relatively small number of cells in hematopoietic tissue that are capable of self-support and are stromal cells of the stem type.

It is therefore interesting to study the quantitative, physiological, and other characteristics of colony-forming cells; in particular, it would be useful to determine the quantitative parameters of colony growth in cultures.

The object of this investigation was to study the dynamics of growth of colonies by measuring their diameter in monolayer cultures of guinea pig spleen at different times after explanation.

EXPERIMENTAL

Two series of experiments were carried out, each on 16 monolayer cultures of guinea pig spleen cells (four cultures for each time). The method of preparing the cell suspension and growing the cultures was described previously [5, 7]. The cultures were fixed after 6, 7, 10, and 12 days with absolute alcohol, stained with azure-eosin, and the mean diameters (arithmetic mean of the longitudinal and transverse sections) of the colonies were determined with the MBS-1 microscope. Colonies measuring 0.3 mm or more in diameter were measured. In each series and at each time the diameter of 300 colonies was determined. Altogether 2400 colonies were measured.

RESULTS

The dynamics of the change in the mean diameter of the colonies is shown in Fig. 1 as a function of the duration in culture. The mean diameter of the colonies on the 6th, 7th, 10th, and 12th days in culture was 0.68, 0.76, 1.02, and 1.34 mm, respectively, in series I and 0.65, 0.80, 1.00, and 1.34 mm, respectively, in series II. As is clear from Fig. 1, the increase in size of the colonies in both series of experiments is satisfactorily described by linear regression equations calculated by the method of least squares.

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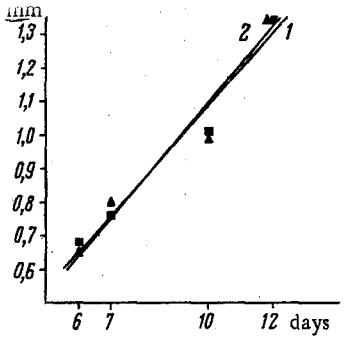

Fig. 1

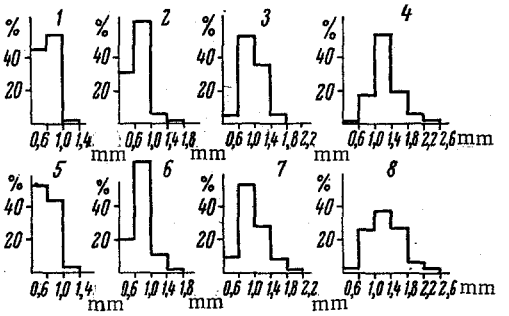

Fig. 2

Fig. 1. Mean diameter of colonies at different times after explantation. Squares denote experiments of series I, triangles series II. Linear regression equations: 1) y = 0.123x − 0.129; 2) y = 0.125x − 0.149. Abscissa, time of culture (in days); ordinate, diameter of colonies (in mm).

Fig. 2. Distribution of colonies by mean diameter at various times after explantation. Series I: 1, 2, 3, 4) 6, 7, 10, and 12 days, respectively, after explantation; series II: 5, 6, 7, 8) 6, 7, 10, and 12 days, respectively, after explantation. Abscissa diameter of colonies (in mm); ordinate, number of colonies (in %).

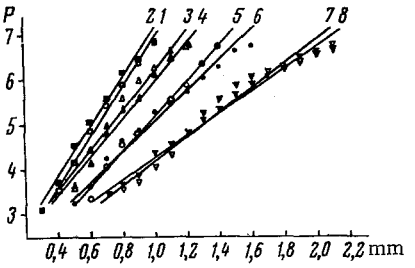

Fig. 3. Integral curves of distribution of colonies by diameter in cultures of guinea pig spleen cells at various times after explantation. Series I: 1, 3, 5, 7) 6, 7, 10, and 12 days, respectively, after explantation. Linear regression equations:

1 — $y = 5.40x + 1.69$. 2 — $y = 5.57x + 1.42$.
3 — $y = 4.45x + 1.75$. 4 — $y = 4.23x + 1.77$.
5 — $y = 3.54x + 1.48$. 6 — $y = 3.36x + 1.73$.
7 — $y = 2.67x + 1.50$. 8 — $y = 2.16x + 2.18$.

Abscissa, mean diameter of colonies (in mm); ordinate, cumulative frequency (in probits).

The distribution of the colonies by diameter at each time for both series of experiments is given in the histograms in Fig. 2. Clearly, the maximal diameter of the colonies on the 6th, 7th, 10th, and 12th days after explantation was 1.4, 1.8, 2.2, and 2.6 mm, respectively. The number of small colonies (under 0.6 mm in diameter) fell from 45-53 % on the 6th day to 1-2 % on the 12th day of culture.

Analysis of the histograms shows that 12 days after explantation the distribution of the colonies by diameter was close to normal; the character of the distribution later was not so evident. However, by probit transformation of the frequencies, the distribution curves straightened out, indicating that the distribution of the diameter of the colonies at all times of the investigation was satisfactorily described by the normal law of distribution (Fig. 3). With an increase in the duration of cultivation, the increase in the mean diameter of the colonies was accompanied by an increase in the value of dispersion. Because of the importance of estimating precisely the degree to which the experimental data for the diameters of the cell colonies corresponded to the normal law of distribution, a special mathematical analysis was made of the results. To test the normality of the distribution of a series of independent random values (d_i) the well-known Kolmogorov test of goodness of fit can be used. However, very large and very small values of d are not sufficiently accounted for by this test, yet their behavior under certain conditions may be of decisive importance when deviations from normality are judged [2].

To analyze the data, the method of moments was therefore used, for it makes rather better allowance for changes in the values of dispersion [3]. The values of $AS = M_3/(M_2 \cdot {}^3/_2)$ and $EX = (M_4/M_2^2) - 3$, i.e., the asymmetry and excess, are both zero in the case of the normal distribution. The random values $g_1 = m_3/(m_2 \cdot {}^3/_2)$ and $g_2 = m_4/(m_2^2) - 3$ can be used as estimates of AS and EX, where $m_k = \frac{1}{n}\sum (d - \bar{d})^k$; (k = 1, 2...) are the central sampling moments.

The values of g_1 and g_2, calculated from the data on the distribution of diameters of the cell colonies on the 6th day (at the moment of least conformity, according to the histograms, to the normal law of distribution), are 0.07 and 0.39, respectively.

It can thus be concluded that the distribution of the cell colonies by diameter corresponds to the normal law of distribution, except for defective flat-toppedness. The explanation of this feature is that in each series at each time of investigation, colonies were measured in four culture flasks; cultures even if growing from the same cell suspension differ very slightly from each other both in the efficiency of colony formation and in colony diameter.

The character of distribution of the colonies by mean diameter is regarded as important in principle, for it allows the heterogeneity of the population of colony-forming cells to be judged. Although in the present experiments the dispersion of the colonies by diameter reached a high value (from 0.5 to 2.6 mm), on the 12th day of cultivation this phenomenon can be explained both by differences in the time when individual colony-forming cells start the first mitosis (from 28 to 60 h after the moment of explantation [6]) and on deviations of the mean duration of the mitotic cycle of individual cells. According to some workers this second factor may be of decisive importance to the explanation of the increasing dispersion of colony diameter.

Merr and Ross [9], for instance, observed considerable variations in the size of cell clones in human diploid cell cultures of strain Wi-38, which increased with an increase in the time after explantation; they explained this finding by the marked variation in the duration of the mitotic cycle of individual cells within the clone.

Previous investigations showed that the distribution of the diameters of the cell colonies characteristically corresponds to the normal law of distribution for colony-forming stem cells, not only of healthy guinea pigs but also of those exposed to pathogenic or extremal factors, with a significant effect on the colony-forming cell population (x-ray irradiation, administration of polyanions). Although these factors induced considerable changes in the number of colony-forming cells in the hematopoietic organs, the diameter of the colonies, and the degree of their dispersion by size, the character of the distribution remained normal. At the same time it can be expected that the appearance of cells differing from normal in their physiological properties (for example, the duration of the mitotic cycle) in the hematopoietic tissue will be accompanied, if grown in monolayer cultures, by the formation of colonies of unusual sizes. The distribution of the colonies by their size in this case will not correspond to the normal law of distribution.

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