MOTILITY OF MOUSE FIBROBLASTS IN TISSUE CULTURE

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ABSTRACT The growth and motion of mouse *L*-cells in vitro have been studied by means of time-lapse photography. In particular, the mitotic period and the motility, defined in terms of $\langle R^2 \rangle$, the mean square displacement of an ensemble of cells, have been measured as a function of temperature. The motility is a function of the phase of the cell cycle. For approximately the first one-eighth of the mitotic period the motility is well described as a random walk with persistence, the duration of the persistence being determined by the time of extension of the filopodic spindle. The temperature dependence of the diffusion constant follows the Arrhenius factor. The mitotic period, which varies exponentially as $(1/T)$, exhibits a large variance, and the time difference in replication of daughter pairs follows approximately a Poisson distribution with a mean difference of 138 min at $T = 37°C$. There is no evidence of mirror symmetry in the motion of daughter pairs for fibroblast cells plated in vitro in Corning tissue culture flasks.

INTRODUCTION

The motility of fibroblast cells in vitro has been a subject of study both with respect to the internal mechanism and in terms of the description of the external motion. The literature on the internal mechanism is extensive; the Yamada conference on Cell Motility (ed. Hatano et al., 1978) and the Cold Spring Harbor Conference on Cell Motility (ed. Goldman et al., 1976) review the progress as of those dates and contain the important references to the earlier literature. A review of the regulation of motility in nonmuscle cells has been given by Hitchcock (1977). The quantitative characterization of the motion, the measurement of the motility, has also been investigated beginning with the pioneering time-lapse photographic studies of Abercrombie and Heaysman (1953). More recently Gail and Boone (1970, 1971) and Gail (1972) have described the motion of an ensemble of cells in terms of a two-dimensional random walk with "persistance." On the other hand Albrecht-Buehler (1978), using his phagokinetic track technique, reports that 40% of 3T3 cell daughter pairs move in mirror image paths, perform directional changes in a mirror symmetrical way, and have mirror symmetry in their actin bundles. He suggests that the relative movement of daughter pairs, rather than being random, has a logic and order that may be predetermined in some way by the mother cell.

Fibroblasts, when plated in vitro, display a fusiform or spindle shape during one phase of the cell cycle. A striking phenomenon is the sometimes extremely rapid extension and contraction of the spindles into enormously long filaments or filopodia. Further, these long filaments, not infrequently longer than thirty body lengths, are observed to cross one over the other with no apparent interaction. Generally the body of the cell moves in the direction of one of the spindles, the spindle being a precursor of the motion. Several interesting questions arise for which the answers are not yet known. Why do fibroblasts move? Do they move in response to external stimuli, are they programmed to move in some particular way, or is the motion purely random? If searching for nutrient, why do two crossing filopodia not react in the same way to what must be the same gradient? What is the source of energy for the rapid growth and contraction?

In attempting to understand the motion, it would be helpful to be able to describe it mathematically.[1] We report here on studies of the mathematical description of the motion, the functional dependence of motility on temperature, and on a study of the relative motion of daughter pairs.

MATERIALS AND METHODS

Mammalian Cells

The cells used throughout these experiments were obtained from American Type Culture Collection (Rockville, MD) and are from the cell line NCTC 2071 (derived from NCTC Clone 929, mouse *L*-cells). The cell repository designation is CCL 1.1. This cell line was chosen because it has been adapted to grow in chemically defined media, and thus confluent growth can be obtained without the necessity of adding complex animal serum. The medium used, Ham's F12M nutrient mixture modified to enhance growth of *L*-cells (Grand Island Biological Co., Grand Island, NY), gave good growth stimulation results in our laboratory. Cells were seeded in tissue culture flasks (Corning Medical, Corning Glass Works, mode 25100, 30 ml disposable polystyrene) and were incubated at 35°C in a moist atmosphere (85–95% humidity) containing 5% CO_2 in air. Confluency was obtained in 7–10 d.

[1]The replication rate and the motility as a function of temperature were needed for understanding a quite different experiment. The results obtained were sufficiently at variance with those reported by others to warrent further investigation.

Methods

For both the motility studies and the mirror-image study, cells were subcultured just before confluence by gently scraping part of the seeded cells into suspension in the culture medium with a polyethylene wand, and transferring to a new flask, with a total of 4 ml of Ham's F12M, saturating with 5% CO_2 in air, thus adjusting the pH to ~7.3, and sealing. The density σ of cells seeded per square millimeter is given on the graphs of motility. The flask was then mounted on the microscope stage in a temperature-controlled enclosure and incubated for 24 h at the desired temperature. The temperature was monitored during measurement and remained constant to within 0.02°C. For the motility studies, measurements were made with the flasks held at T = 31.0°C, 33.0°C, 35.0°C, and 37.0°C. The mirror-image studies were made at a temperature of 37.0°C.

Instrumentation

The time-lapse photography studies were carried out using a bright-field inverting microscope (Unitron Instruments, Inc., Woodbury, NY, model BR-MIC-CM). For the motility studies the magnification was 30x, obtained with a 3x objective and 10x eyepiece. Mirror-image studies were made at both 30x and 75x (5x objective and 15x eyepiece). At 75x the field of view was 0.214 mm^2, and at 30x was 0.535 mm^2. The field of view was recorded frame by frame on 16-mm motion picture film (Kodak 7276 plus-x, Eastman Kodak Co., Rochester, NY) using a Bolex H-16M camera fitted with a single-frame drive. In the early measurements the time interval was set at 60.0 s. More recently digital logic circuitry with stepping motor drives has been developed to move the microscope stage automatically in a raster pattern to permit a number of fields of view to be photographed in sequence and thus increase the rate at which data are accumulated. When, for example, five separate fields of view were photographed sequentially and cyclically, the time interval between exposures was set at 120 s, so that the time for one cycle was 10.0 min; thus the position of each cell was recorded every 10.0 min.

The measurement of cell position (x- and y-coordinates as a function of time) was made using a scanning-machine developed by our high-energy physics group for analyzing bubble-chamber pictures of high-energy physics events. It was modified to handle 100-ft rolls of 16-mm film. Briefly, a frame of the film is projected onto a large viewing table. The image of a cell is moved under a fixed fiducial mark by moving the film and projection lenses as a unit. The motion is measured by optical encoders and at the press of a foot pedal is recorded in digital form into a computer memory. Thus the position of each cell in a given frame can be recorded quickly and accurately. At a magnification of 30x the rms reproducibility of measurement of cell position is 3.6 μm. The film can be advanced (or reversed) any selectable number of frames by the touch of a button. Typically, quantities calculated from the stored values of position vs.time are the center of gravity of the ensemble of cells in a given field, the mean-square displacement $\langle R^2 \rangle$ vs. time for the cells in a given ensemble, and various correlation functions. Plots of these data can also be made via the computer. However, one of the more important features of the system is the ability to synchronize the data with respect to the cell cycle.

THEORY AND METHOD OF ANALYSIS

The Random Walk and Brownian Motion

If the motion of fibroblast cells on a substrate can be described either as a random walk or in terms of Brownian motion it is necessary to understand the significance of the parameters used in describing the motion. The theory of Brownian motion and of the random walk are famous problems in physics, and have been the subject of considerable investigation, beginning with Einstein's (1905) paper on Brownian motion together with the problem of random flight (or random walk) first formulated by Pearson (1905).

Einstein showed that for a free Brownian particle the mean square value of the one-dimensional displacement is given by

$$\langle x^2 \rangle = 2Dt = [(2kT)/f]\, t \qquad (1)$$

where D is the diffusion coefficient, f is the viscous friction coefficient, k is Boltzmann's constant, T the absolute temperature, and t is the time. As Einstein pointed out, this is valid only for times that are long compared with m/f, m being the mass of the particle. The generalization of this expression valid for all times was given by Ornstein (1919, see also Uhlenbeck and Ornstein, 1930) and independently by Fürth (1920) in the form

$$\langle x^2 \rangle = [(2kT)/f]\, \tau\, (t/\tau - 1 + e^{-t/\tau}) \qquad (2)$$

where $\tau = m/f$ has the dimension of time and measures the relative importance of the frictional forces to the inertial force. For $t >> \tau$ this reduces to the Einstein formula, (Eq. 1 above), while for $t << \tau$ it reduces to

$$\langle x^2 \rangle = [(2kT)/m]\, t^2, \qquad (3)$$

that is, the displacement is proportional to the time, corresponding to uniform motion.[2] Chandrasekhar (1943) has shown that for $t >> \tau$ the motion of a Brownian particle can be regarded as one of random flight, and therefore as motion governed by the diffusion equation, as given (above) by Einstein.

The significance of this for describing the motility of mammalian cells in tissue culture has only to do with the form of Eq. 2 and the fact that it does indeed describe the motion, if only during the first phase of the cell cycle. For the two-dimensional motion we write the mean square displacement as

$$\langle R^2 \rangle = 4D\tau\left(\frac{t}{\tau} - 1 + e^{-t/\tau}\right). \qquad (4)$$

It remains then to interpret the two parameters τ and D in terms of physical characteristics of the cells. A possible interpretation is given below.

Analysis of Motion of Daughter Pairs

When a cell divides to form daughter pairs, the daughters must move apart, if they move. Assume the paths followed by the separating daughters are perfect mirror images until either daughter undergoes mitosis or until either cell is inhibited in its locomotion by another cell. For each pair of separating daughter cells, let $t = 0$ be defined as the moment when the cells have become noncontiguous. With each pair of daughter cells at $t = 0$ can be associated a direction vector N that is normal to the line joining the centers of mass of the daughter cells. A mirror plane can be imagined to bisect this segment, as illustrated in Fig. 1 *a*.

A short time interval later, at $t = 1$, daughter cell A will have undergone a displacement $\mathbf{d}_A(1)$ from its initial position at $t = 0$, and similarly cell B a displacement $\mathbf{d}_B(1)$. Each of the vectors $\mathbf{d}_A(1)$ and $\mathbf{d}_B(1)$ can be resolved into components parallel and perpendicular to N. Because by assumption the paths of the daughters are mirror images, it is apparent that the vector sum of $\mathbf{d}_A(1)$ and $\mathbf{d}_B(1)$ is a vector parallel to N.

Let the vector $\mathbf{w}(n)$ be defined as the vector sum of the displacements of the cells A and B from their positions at $t = 1$ to their position at $t = n$, namely

$$\mathbf{w}(n) = \sum_{t=1}^{n} [\mathbf{d}_A(n) + \mathbf{d}_B(n)] = \mathbf{S}_A(n) + \mathbf{S}_B(n).$$

It is clear that if the paths followed by the daughters are mirror images, then $\mathbf{w}(n)$ is parallel to N (Fig. 1 *b*).

[2]Eq. 2 is for one-dimensional motion. Because $\langle r^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle$, the numerical coefficient becomes 4 for two-dimensional motion.

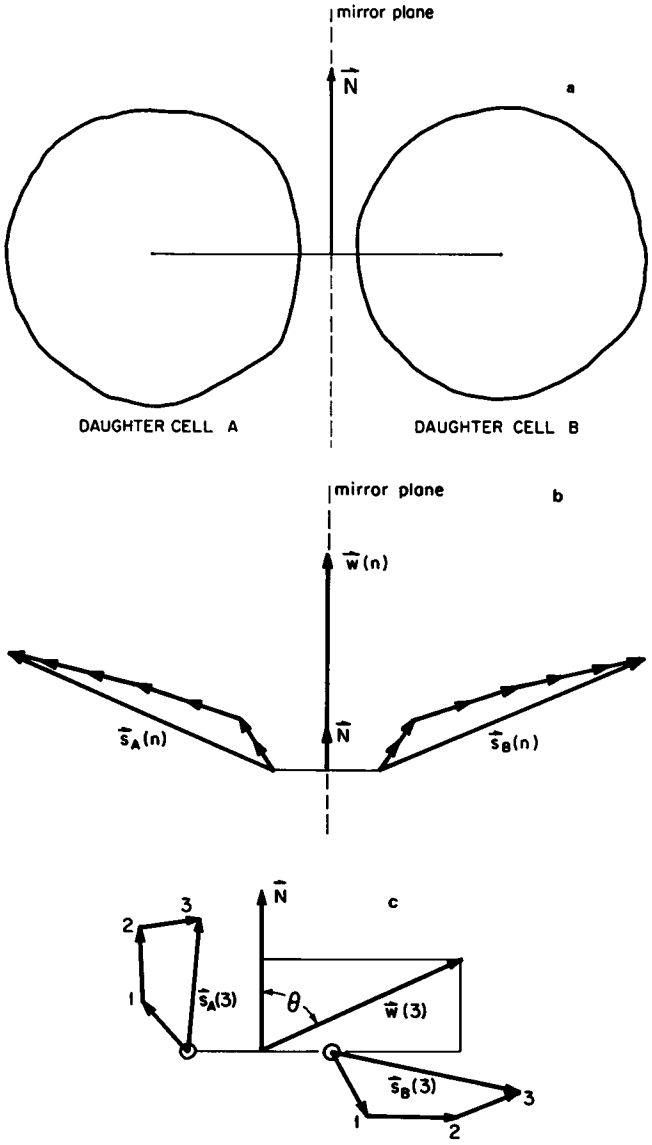

FIGURE 1 Geometry used in the analysis of the relative motion of daughter-pairs. See text for description.

Let $|\Delta\theta|$ be defined as the absolute value of the angular deviation between N and w(n) for $\theta \leq 90°$. If the angular deviation is $>90°$, $|\Delta\theta|$ is defined as the angular deviation between $-$N and $w(n)$. For the case $S_A(n) = -S_B(n)$, then w(n) = 0. For this special case, w(n) is defined to be a vector perpendicular to the line joining daughter cells A and B at $t = n$. The value of $|\Delta\theta|$ can then be obtained as for the general case (Fig. 1 *c*.).

If the tracks of daughter cells are perfect mirror images up to $t = n$, then $|\Delta\theta| = 0°$. If the paths are slightly distorted mirror images, then $|\Delta\theta|$ is either 0° or a relatively small value. However, if the paths are traced out in a random way then $|\Delta\theta|$ would be expected to take on with equal probability any value from 0° to 90°.

RESULTS

Motion of Daughter Pairs

A qualitative inspection of the paths of many pairs of daughter cells showed no evidence of mirror symmetry about the perpendicular bisector of a line joining the centers of the daughter pair. The results obtained from calculating $|\Delta\theta|$ for pairs of daughter cells at time intervals of 37.5, 75, 300, and 675 min are summarized in Fig. 2. At no time interval does there appear to be a skewed distribution at small values of $|\Delta\theta|$. None of the values of χ^2 calculated for each time interval on the assumption that the distribution of $|\Delta\theta|$ values is completely random, was significant. We conclude therefore, that the paths followed by the daughter pairs of mouse *L*-cells plated in Corning 25100 tissue culture flasks are not mirror images. Possible reasons for the lack of symmetry are discussed below.

Cell Growth

A plot of the total number of cells counted in three fields of view, each of 0.535 mm^2, as a function of time and at temperature 37.0°C is given in Fig. 3. In general, the length of the cycle, the mitotic period P, as deduced from such cell density–time plots will, over the relative short time interval plotted, be longer than the true value due to counting nonreproducing cells and artifacts. The doubling time determined from a least-squares fit to the data is P = 2,188 min. However, if many cells are followed from birth to mitosis by time-lapse photography (see below) an average value of P = 1,548 min with a standard deviation of 285 min is obtained for 84 events. Using this value of P in the equation $n = n_0(2^{t/P})+k$, where k represents the number of nonreproducing cells and artifacts, and searching for n_0 and k to give the best fit to the data, the values n_0 = 78.1 and k = 86.6 are obtained with χ^2 = 24. The deviation for a straight-line relationship at low values of t is consistent with "conditioning of the medium." The large difference between doubling time (2,188 min) and generation time (1,548 min) is consistent with the data of

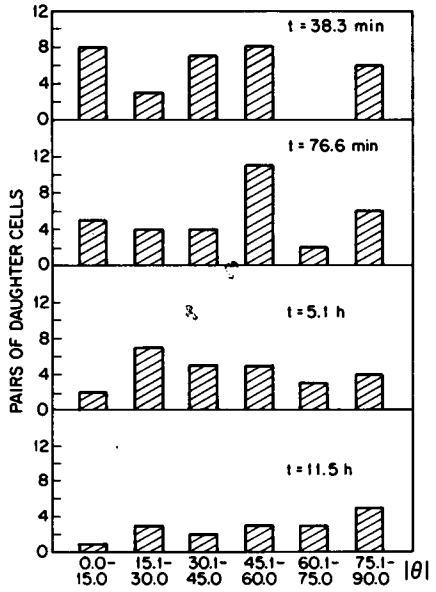

FIGURE 2 Histogram plots of pairs of daughter cells vs. $|\theta|$ for four different time intervals.

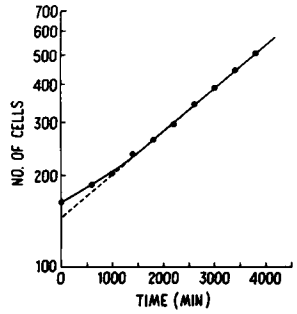

FIGURE 3 Cell growth as a function of time. The ordinate, on a logarithmic scale, gives the number of cells counted in an area of 1.60 mm^2 as a function of time. The doubling time determined from a least squares fit is 2,188 min. See text for further discussion.

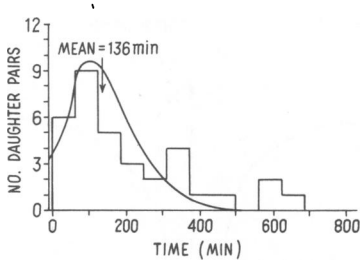

FIGURE 5 Histogram of the frequency distribution of replication time differences between siblings for 35 events. The mean (and median) value is 136 min; thus if one member of a daughter-pair undergoes mitosis, then there is a 50% probability its sibling will undergo mitosis within 136 min. The solid curve is the Poisson distribution $P = a^n e^{-a}/n!$ for $a = 2.27$ corresponding to the mean value of $2.27 \times 60 = 136$ min.

McQuilkin and Earle (1962) who find for cells of strain NCTC-929 at 37.5°C an average doubling time of 56 h but an average generation time of 28 h (for 24 events; the range of times was from 17 to 41 h). The reasons for the difference are not understood, but contributing factors may be cell death and long-term survival without division, that is, so-called sleeper cells. Further investigation is warranted.

In Fig. 4 we plot the distribution of the 84 events at $T = 37.0$°C, with the division time measured to the closest 10 min but combined into channel widths of 80 min. Over the 108-h interval photographed, some 44 cells underwent mitosis. Of these 35 daughter pairs, nine single cells and seven granddaughters could be followed to mitosis. Thus, of the original 44 daughter pairs (88 cells), nine siblings either left the field of view or did not replicate in the interval filmed, and eight were observed to produce granddaughters of which seven could be followed through mitosis. Two cells were excluded from the plot because they deviated from the mean, one above and one below by more than 2.5 standard deviations. The solid curve is a Gaussian distribution adjusted to give the best (least-squares) fit to the data. The mean mitotic period for the 84 events is 1,548 min (25 h, 48 min), with a standard deviation from the mean of 285 min (4 h, 45 min).

Another quantity of interest is the variation in mitotic period between siblings. The histogram of Fig. 5 shows the distribution in time differences for mitosis between the 35 daughter pairs of Fig. 4. The most probable value is 136 min; that is, if one member of a daughter pair undergoes mitosis, then there is a 50% probability its sibling will undergo mitosis within 136 min. The histogram approximates the Poisson frequency distribution $a^n e^{-a}/n!$ shown by the solid curve for $a = 2.27$ corresponding to the mean of 136 min for the 35 events (each data bin is 60 min).

Figure 6 is a plot of the log of the mitotic period as a function of the reciprocal of the absolute temperature. The variation of the period with temperature is as expected in view of the well-known temperature dependence of chemical reaction rates, as given by the Arrhenius equation.

Cell Motility

For the measurements reported here, the cell densities were sufficiently low that density inhibition of motility (Stoker and Rubin, 1967) was not a factor; any cell making contact with another was excluded. In addition the culture medium was exchanged for 4–5 ml of fresh medium 24 h

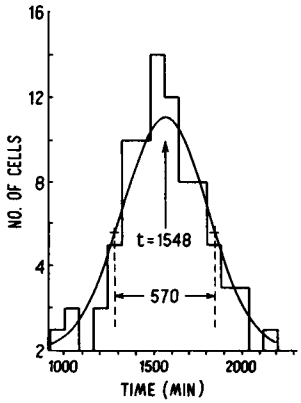

FIGURE 4 Frequency distribution of replication times. For the 84 events plotted the mean mitotic period is 1,548 min with a standard deviation from the mean of 285 min.

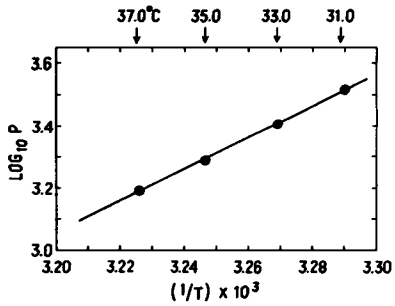

FIGURE 6 Logarithmic plot of the mitotic period vs. $1/T$, where T is the temperature in degrees Kelvin. Expressing the reaction rates as the reciprocal of the periods, it follows that the empirical activation energy μ has the value 35 kcal/mol as determined from the expression $(k_1/k_0) = (P_0/P_1) = \exp\{(\mu/2)[(T_1 - T_0)/T_0T_1]\}$ (e.g., Bronk, 1978).

before measurement to avoid depletion of a "locomotion factor" (Gail and Boone, 1971).

We express cell motility in terms of $\langle R^2 \rangle$, the square of the displacement of each cell at time t from its position at $t = 0$ averaged over all the cells in the ensemble. In earlier measurements the ensemble consisted of all those cells, generally 15 to 30, in a field of view that could be followed for a suitably long time without undergoing mitosis or contacting another cell. The results were not reproducable even though conditions appeared to be identical. The cell cycles were then synchronized by shifting the data in the computer file so that each cell division was brought to a common $t = 0$, corresponding to the time the two daughters became noncontiguous. Graphs of $\langle R^2 \rangle$ vs. time for cells synchronized in mitotic phase are given in Fig. 7. Clearly the motility is a function of the phase of the cell cycle, thus it is not surprising that earlier results were inconsistent; the measured $\langle R^2 \rangle$ will depend on the relative mix of cell phases in the small sample of cells in the ensemble. In the graphs of Fig. 7, the solid curves are plots of Eq. 4 with the two parameters D and τ adjusted by means of a computer program to give the best (least-squares) fit to the data for approximately the first one-eighth of the mitotic period.

In Fig. 8 the logarithm of the diffusion constant D is plotted against the reciprocal of the absolute temperature. The data against suggest that D has an Arrhenius dependence on T. However, there remains a large variation in the motility data, even though the cell cycles are synchronized. The values of D measured for two ensembles are omitted from Fig. 8. One ensemble of 34 cells, at $T = 37.0$°C yielded a value of log $D = 2.52$ (large motility), and another, also of 34 cells, at $T = 33.0$°C gave a value of log $D = -0.41$ (low motility). We believe this lack of reproducibility has to do with the nature of the substrate. In Fig. 9 we plot $\langle R^2 \rangle$ for the first one-eighth of the mitotic period

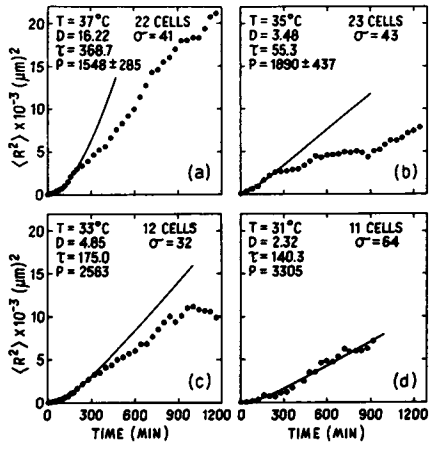

FIGURE 7 The motility $\langle R^2 \rangle$ in square micrometers vs. time in minutes for four temperatures. The number of cells in each ensemble, synchronized in phase, is noted on the graphs together with the cell density σ (cells/mm^2) at the beginning of the measurements. The solid curves are Eq. 4 of the text with the parameters D and τ adjusted to give the best least-squares fit to the data for the first one-eighth of the mitotic period.

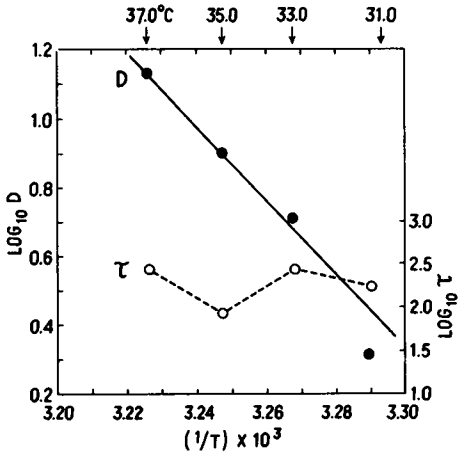

FIGURE 8 The diffusion constant D and the time constant τ vs. $1/T$°K.

for cells at 37.0°C but plated in Corning flasks from three different lots. In each case the fit to the theoretical form is equally good as measured by χ^2 but the values of D and τ as given in Fig. 9 show a wide variation.

The data of Fig. 7 suggest that the motility is a function of the phase of the cell cycle. The motion may, however, remain random, and with a persistence. The data plotted in Fig. 7*b*, 23 cells at $T = 35.0$°C, were used to recalculate $\langle R^2 \rangle$ for times beginning later in the mitotic cycle. Fig. 10 *a* is calculated with $t = 0$ beginning 280 min after mitosis, Fig. 10 *b* with $t = 0$ beginning 520 min after mitosis, and Fig. 10 *c* for 800 min after mitosis. There would appear to be little significance to the values of D and τ obtained in fitting the theoretical curve to the data.

If the motion of cells in an ensemble is random, then in a sufficiently large sample the center of mass of the ensemble should remain fixed. The coordinates of the center of mass as a function of time were calculated for two ensembles, one of 34 cells and a second of 40 cells. The standard deviations from the mean positions were 5.1 and 1.4 μm, respectively. These are to be compared with the root mean square displacements in the same time intervals of 85 and 55 μm, respectively.

Other tests for randomness exist, such as the various correlation functions. However, in view of the finite size of the ensembles they are not theoretically significant and are not reported here.

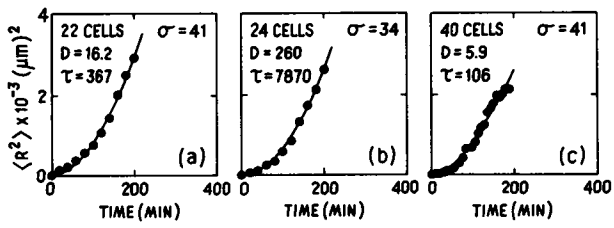

FIGURE 9 Graphs of $\langle R^2 \rangle$ measured for the first one-eighth of the mitotic period for synchronized cells at 37°C in flasks from three different lots.

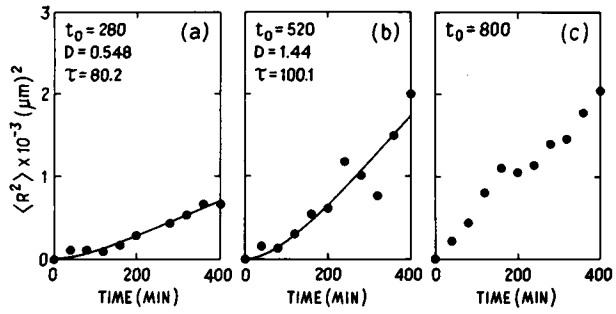

FIGURE 10 Graphs of $\langle R^2 \rangle$ for 23 cells at T = 35°C calculated using the data of Fig. 7*b* for initial times beginning later in the mitotic cycle. *a*, calculated with t = 0 taken as 280 min after mitosis; *b*, with t = 0 taken as 520 min after mitosis; and *c*, for 800 min.

DISCUSSION

The observation and the analysis of the paths of many daughter pairs suggests that their relative motion is random. Following mitosis, at the moment the daughters become noncontiguous, each cell is essentially spherical in shape. After a short but finite time, as contact points on the substrate develop, the morphology of the daughters suddenly changes to a flattened irregular shape with characteristic lamellipodia developing in a random direction. This is not surprising, since the orientation of the cell, and hence the microtubule bundles, is free to change before contact points develop. Filopodia then develop, and each cell moves with essentially uniform motion in the direction of an extended filopodium for a small fraction of the mitotic period, characterized by the quantity τ in the expression for the mean square displacement. The filopodia may then retract and develop again in an arbitrary or random direction with movement in this new direction again determined by the time the filopodia are extended. These measurements are consistent with the observation on microfilament bundles summarized in the review by Hitchcock (1977). For longer times the motion is characterized as diffusion. After a time on the order of an eighth of the mitotic period, there is a change in the average velocity, which suggests a resting phase. The data hint at a correlation of the motility with the four phases of the cell cycle, but any stronger statement would have to await more corroborative data.

A disturbing characteristic of the data reported here is the lack of reproducibility for what appears to be essentially identical conditions. The data plotted in Fig. 8 exclude two measurements, one at 37.0° and one at 33.0°C, which fall off the graph. The data were taken in three different lots of culture flasks, but even for the same batch of flasks, the same culture medium, constant temperature, and similar cell density, there is considerable variation in the response. From strictly visual observation of the motion, and of the change in morphology it appears that the dominant factor may be local differences in the character of the substrate. On occasion, within one field of view the cell attachment appears to vary. This would have a marked influence on the motility, since the motility as noted above depends on the formation and attachment of the filopodia. In a small sample of cells, much of $\langle R^2 \rangle$ may be carried by a single cell or a few cells.

In spite of the lack of quantitative reproducibility, certain features stand out. The motility appears to be a function of the cell cycle. The first phase, lasting approximately one-eighth cycle, is characterized in all cases by a random walk with persistence, the persistence being a measure of the tendency of the individual cells to move in straight lines for short periods of time. Surprisingly, the persistence as characterized by τ does not appear to be a function of temperature (log τ vs. $1/T$ is plotted in Fig. 8). Because the motion is in the direction of a filopodium, and depends therefore on the time the filopodium is extended, one might expect τ to be related to the mitotic period, and therefore be a function of temperature.

The mitotic period is a function of temperature as shown by the data in Fig. 6. It does not appear to be influenced by the substrate. The variance in the mitotic period for an ensemble of cells at a given temperature is large, a fact known for some time (McQuilkin and Earle, 1962; Dawson et al. 1965; Bronk et al., 1974). The standard deviation from the mean, for 84 events at T = 37.0°C is ±285 min for a mean period of 1,548 min. This is a measure of the variation in the time required for absorption through the cell membrane and for synthesis of the cell material. A simple calculation shows that all the molecules required for cell growth are available at the cell surface in more than adequate supply. For example, for each of the amino acid of the lowest concentration in Ham's F12M nutrient mixture, the number of collisions with the cell membrane is on the order of 10^8/s. The large variance is consistent with the current thinking that the membrane is the regulating factor in cell growth.

CONCLUSIONS

We conclude from these studies that results of measurements using cell motility as a measure of effect must be treated with caution. The measurements would have to be carried out under well-controlled conditions, and only after standardized substrates have been developed. Even then, such studies will be significant only if the cells in the ensemble are synchronized in mitotic phase.

The growth rate, and thus the mitotic period, exhibits an Arrhenius factor ($e^{-1/T}$) dependence with temperature as expected since chemical reaction rates are proportional to temperature.

The motion of mouse *L*-cells plated in vitro is random, but each cell exhibits a tendency toward persistence in its motion for short periods of time. Such persistence is consistent with the formation and contraction of microfilament bundles, which are believed to be the structure responsible for cell motion. The time constant τ associated

with the presistence does not appear to be a function of temperature nor is it a reproducible characteristic of cell ensembles. The amount of motion is a function of the phase of the cell cycle.

The data are consistent with the relative motion of daughter pairs being random. There is no evidence for mirror-image motion of mouse *L*-cells plated in Corning 25100 tissue culture flasks.

Nothing in these data suggests that cell growth and motility are other than random processes controlled by statistical probabilities of chemical thermodynamics.

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