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Remarks on the Motility and Thermotactic Response of Fibroblasts*

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Abstract

The motion of mouse L cells (fibroblast) *in vitro* is studied by means of time-lapse photography. In particular, the response of the cells to a temperature gradient of $7{\cdot}22°C\,cm^{-1}$ is studied for several temperatures from 32·6°C to 39·7°C. Three measures of the thermotactic response are used: (1) the motility, defined in terms of the mean-square displacement $\langle R^2 \rangle$ of an ensemble of cells, (2) the displacement of the centre of gravity of an ensemble of cells versus time, and (3) the distribution in the number of cells in an ensemble moving up the gradient compared with the number moving down the gradient. There is no evidence of a thermotactic response as determined by these three measures. The variance in the data can be understood in terms of statistical fluctuations.

1. Introduction

An understanding of how tissue cells move within an organism (*in vivo*) is essential to understanding the mechanisms of morphogenesis and neoplastic invasion. However, *in vivo* studies present considerable technical difficulties, and as a result morphogenesis and locomotion *in vitro* have been the subject of rather intensive study over the past several years. Such studies have been aided considerably by the development of chemically defined culture media, which provide all the necessary nutrients for cell growth, and by the development of suitable substrates to which the cells may attach. Cultures of mammalian fibroblasts cells, and in particular mouse fibroblasts, have been especially amenable to study both with respect to the internal mechanism and in terms of the description of the external motion. (Fibroblasts are cells of connective tissue. The mouse fibroblasts were derived from fibrous tissue below the epithelium, or skin.) The literature on the internal mechanism is extensive; the Yamada conference on cell motility (Hatano *et al.* 1979) reviewed the progress as of that date and contains the important references to the earlier literature.

The quantitative characterization of the motion, i.e. the measurement of motility, has also been investigated, beginning with the pioneering time-lapse photography studies of Abercrombie and Heaysman (1953). More recently Gail and Boone (1970, 1971) have described the motion of an ensemble of cells in terms of a two-dimensional random walk with 'persistence'. On the other hand Albrecht-Buehler (1978) suggested that the relative movement of daughter pairs, rather than being

* Dedicated to the memory of Professor S. T. Butler who died on 15 May 1982.

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random, has a logic and order that may be predetermined in some way by the mother cell. He suggested that daughter pairs move in mirror image paths and perform directional changes in a mirror symmetrical way.

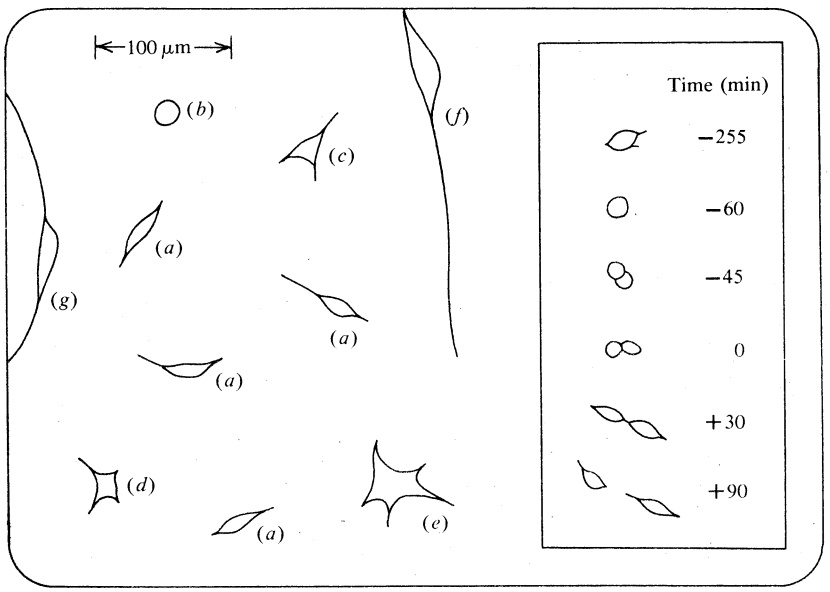

Fig. 1. Typical shapes (morphology) of isolated fibroblast cells plated *in vitro*. The shapes were obtained by tracing cell images from a time-lapse film projected onto a viewing table. The calibration of the field of view is given at the top left. The cells in the montage were selected from several frames to show the variety of shapes observed. Cells labelled (*a*) have typical fusiform or spindle shapes; (*b*) is a cell 'rounded-up' (spherical) and about to divide (i.e. undergo mitosis); (*c*) and (*d*) are shapes often observed in later stages of the mitotic cycle; (*e*) is a 'giant' cell which may be sterile and unlikely to divide; (*f*) and (*g*) are examples of cells exhibiting long filaments which have been measured to grow and contract at rates as high as $0{\cdot}4\ \mu m\,s^{-1}$. The inset follows over relative times a typical cell undergoing mitosis. Again the shapes shown have been traced from a projection of the film. The mitotic period at a temperature of 37°C is about 1550 min so that the time interval for which the shapes are shown is less than a quarter of the mitotic period. A cell invariably rounds-up before dividing. After the daughters separate their shapes are most often fusiform as shown.

Fibroblasts when plated *in vitro* display a variety of shapes (see Fig. 1), but nearly all cells display a fusiform or spindle shape during at least one phase of the cell cycle, the G_1 phase which follows mitosis (the process of a cell dividing to form two daughter cells; see inset to Fig. 1). A striking phenomenon is the sometimes extremely rapid contraction and then extension of the spindles into enormously long filaments or filopodia. Further, these long filaments, occasionally many body lengths, are observed to cross one over the other with no apparent interaction. In general the body of the cell moves in the direction of one of the spindles, the spindle being a precursor of the motion. Just before mitosis the cells 'round-up', the sphere being attached to the substrate through a relatively small area of contact. The volume of a mouse fibroblast cell varies typically from 1000–4000 μm^3.

Several interesting questions arise for which the answers are as yet unknown. Why do fibroblasts move? Do they move in response to external stimuli such as chemical or thermal gradients? Do they exhibit magnetotactic, galvanotactic, or

geotactic responses? 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 rapid growth and contraction?

A study of the description of the motion, the functional dependence of motility on temperature, and the relative motion of daughter pairs has been reported earlier (Parkinson 1983). It was found that the motion can indeed be described in terms of a two-dimensional random walk, but only for approximately the first one-eighth of the mitotic period. While the motion is random each cell exhibits a tendency towards 'persistence' for short periods of time, the persistence being consistent with the formation and contraction of the spindles. No evidence for mirror image motion of daughter cells was found. The mitotic period, the length of the cell cycle from mitosis to mitosis, is an exponential function of temperature (with Arrhenius factor $e^{-1/T}$) and is gaussian in time with a large variance. The growth of a population is logarithmic but with a doubling time longer than the mean of the gaussian indicating that a fraction of the cells in the culture either do not replicate, or replicate in a time much longer than the mean. It was concluded that cell growth and motility at a constant temperature were random processes controlled by the statistical probabilities of chemical thermodynamics. In this paper we give a brief report on measurements of the thermotactic response of fibroblasts.

Information on the response of mammalian cells to thermal gradients is meagre. On the other hand, there is clear evidence of thermotaxis in the cellular slime molds *Dictyostelium discoideum* (Whitaker and Poff 1980) and *Physarum polycephalum* (Tso and Mansour 1975) and in the nematode *Caenorhabditis elegans* (Hedgecock and Russel 1975). Further, the sensitivity is remarkable; the pseudoplasmodium responds to gradients as small as $0{\cdot}04°C\,cm^{-1}$ or 4×10^{-4} °C across its width which typically is 100 μm (Whitaker and Poff 1980) and migrates toward its growth/development temperature. If mammalian cells were to exhibit thermotaxis then the immediate questions would be concerned with the characterization of the thermosensor and the internal mechanism of the thermally sensitive motility.

2. Materials, Methods and Instrumentation

The cells used throughout these experiments were obtained from American Type Culture Collection 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 was Ham's F12M (obtained from Grand Island Biological Co., U.S.A.). Cells were seeded in tissue culture flasks (Corning 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 days. (The fact that the cells were cultured at 35°C rather than the customary 37°C had to do with a different experiment.)

For the studies reported here cells were subcultured by gently scraping part of the seeded cells into suspension in the culture medium, transferring them 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 approximately 7·3, and then sealing. The flask (2·45 cm × 5·2 cm) was

turned on its side so that the cells were cultured on the narrow dimensions. The flask was incubated for 24 h at 35·0°C before mounting on the microscope stage between two rectangular aluminium tubes (see Fig. 2). A temperature gradient was established across the narrow 2·45 cm dimension of the flask by water circulating through the bars from two heat reservoirs (insulated aquarium tanks and aquarium circulating pumps). The tanks were maintained at constant temperature so that one bar was at 26·8°C and the other at 44·5°C, producing a gradient of $7{\cdot}22°C\,cm^{-1}$. The temperature of each of the bars was maintained constant to within 0·02°C. The gradient was measured independently and found to be constant across the flask.

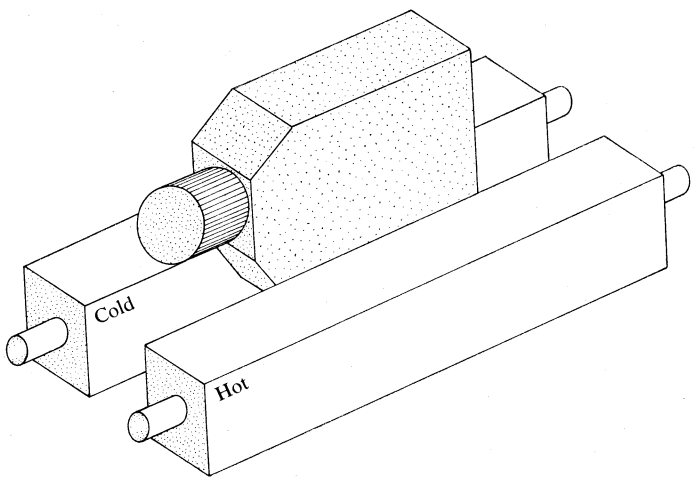

Fig. 2. Arrangement for thermotaxis measurements. The assembly is clamped on and thermally insulated from the microscope stage. Cells are grown on the lower narrow side of the flask.

The time-lapse photography studies were carried out using the bright field Unitron inverting microscope BR-MIC-CM of magnification 30×. The field of view was recorded frame by frame on 16 mm motion picture film (Kodak 7276 plus-x) using a Bolex H-16M camera fitted with a single frame drive. The field of view recorded on the film was $0{\cdot}535\ mm^2$. Digital logic circuitry with stepping motor drives was used to move the microscope stage automatically in a raster pattern, permitting a number of fields of view to be photographed in sequence and thus increasing the rate at which data are accumulated. For the thermotaxis measurements the stage was moved in a raster of ten steps in y (in the direction of the gradient) and two in x (normal to the gradient), so that twenty fields of view were photographed sequentially and cyclically with a time interval between exposures of 20 s. The time for one complete cycle was 6 min 40 s; thus the position and morphology of each cell was recorded every 6 min 40 s.

The measurement of cell position (i.e. x and y coordinates as a function of time) was made using a 'scanning machine' developed for analysing bubble chamber pictures of events in high energy physics, but modified to handle 100 foot (= 30·5 m) rolls of 16 mm film. Briefly, a frame of the film is projected onto a large viewing table and the image of a cell is moved under a fixed fiducial mark by moving as a

unit the film and projection lenses. 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 30× the r.m.s. reproducibility of measurement of cell position is 3·6 μm. The film can be advanced (or reversed) any number of frames selected by the touch of a button. Once the position of each of the cells as a function of time is entered into the computer memory it is relatively easy with simple computer programs to compute the desired quantities. Typical quantities calculated are the centre of gravity of the ensemble of cells in a given field and the mean-square displacements $\langle x\rangle^2$, $\langle y^2\rangle$ and $\langle R^2\rangle = \langle x^2\rangle + \langle y^2\rangle$ versus time averaged over all cells in a given ensemble. Plots of these data can also be made by the computer. One of the more important features of the system is the ability to synchronize the data with respect to the cell cycle.

3. Method of Analysis: The Random Walk and Brownian Motion

In describing the motion of an ensemble of fibroblast cells on a substrate in terms of Brownian motion or of a random walk, the interest is to relate the parameters used for the motion to physical processes occurring in the cell. For the measurements described here the aim is to determine whether the parameters change in response to temperature gradients. The theory of Brownian motion and of the random walk are of course 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

$$\langle y^2\rangle = 2Dt = (2kT/f)t, \tag{1}$$

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

$$\langle y^2\rangle = 2D\tau\{t/\tau - 1 + \exp(-t/\tau)\}, \tag{2}$$

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

$$\langle y^2\rangle = (kT/m)t^2, \tag{3}$$

that is, the displacement is proportional to the time, corresponding to uniform motion. Chandrasekhar (1943) has shown that for $t \gg \tau$ the motion of a Brownian particle can be regarded as one of random walk and, therefore, as motion governed by the diffusion equation (1) given by Einstein. For the two-dimensional motion we write*

$$\langle R^2\rangle = 4D\tau\{t/\tau - 1 + \exp(-t/\tau)\}, \tag{4}$$

* Equation (2) is for one-dimensional motion. Since $\langle r^2\rangle = \langle x^2\rangle + \langle y^2\rangle$, the numerical coefficient becomes 4 for two-dimensional motion.

where $\langle R^2 \rangle$ is 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.

To interpret the two parameters τ and D in terms of physical characteristics of the cells it has been suggested (Parkinson 1983) that τ is a measure of the time of extension of the filopodia immediately following mitosis and that D is a diffusion constant associated with the ensemble and is a function of temperature. If fibroblasts exhibit a thermotactic response then the mean-square displacement versus time along the gradient $\langle y^2 \rangle$ might be expected to differ from that normal to the gradient $\langle x^2 \rangle$. In particular, if τ is related to the time of extension of the filopodia, and the filopodia are precursors of the motion, then one might expect $\tau_y > \tau_x$.

A second measure of thermotactic response is given by the displacement of the centre of gravity of the ensemble as a function of time. The component of the displacement $\langle x \rangle$ normal to the direction of the gradient would be expected to be zero while a nonzero component for $\langle y \rangle$ (in the direction of the gradient) would indicate a thermotactic response. Further, the sign of the $\langle y \rangle$ component should indicate whether the response corresponds to positive or negative thermotaxis.

A third measure is given by the distribution in the number of cells in an ensemble moving in the $+y$ direction compared with the number moving in the $-y$ direction. In the absence of a thermotactic response one would expect on the average an equal distribution.

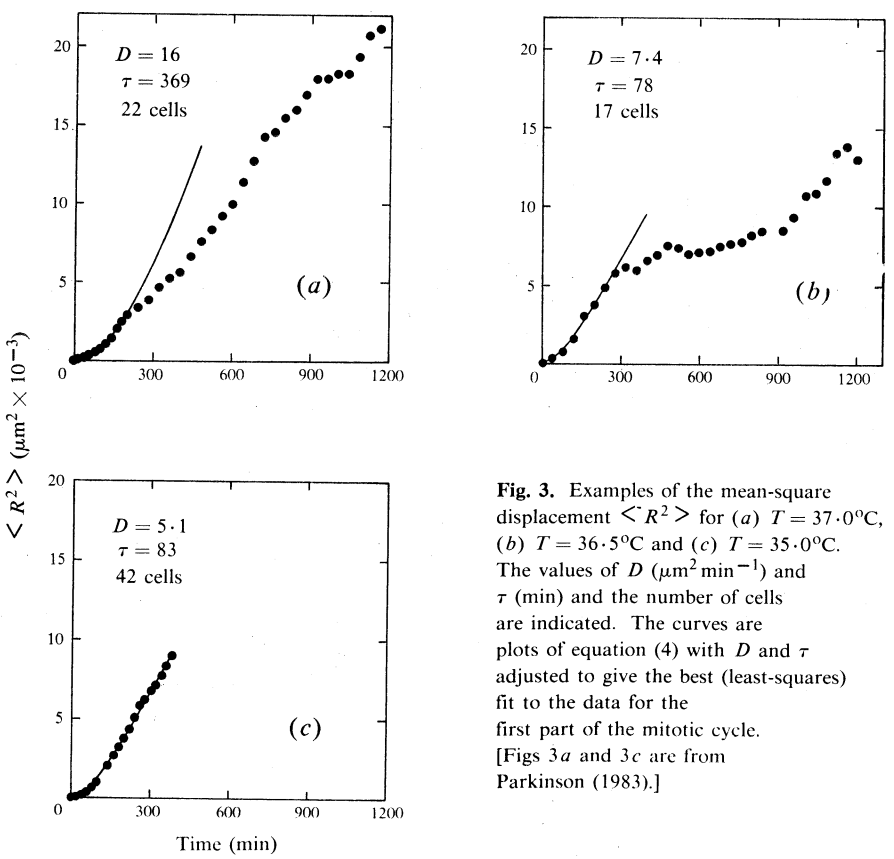

Fig. 3. Examples of the mean-square displacement $\langle R^2 \rangle$ for (*a*) $T = 37{\cdot}0^{\circ}$C, (*b*) $T = 36{\cdot}5^{\circ}$C and (*c*) $T = 35{\cdot}0^{\circ}$C. The values of D (μm^2 min^{-1}) and τ (min) and the number of cells are indicated. The curves are plots of equation (4) with D and τ adjusted to give the best (least-squares) fit to the data for the first part of the mitotic cycle. [Figs 3*a* and 3*c* are from Parkinson (1983).]

4. Results

In analysing the data the cell cycles were first 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 non-contiguous (see Fig. 1). The criteria for selection of a cell as a candidate for measurement were that it be born in the course of the filming and that it did not come in contact with another cell after $t = 0$. These criteria severely limit the number of candidates so that in the interpretation of the data we are dealing with small numbers.

Examples of the dependence of motility on the phase of the cell cycle in the absence of a gradient are given in Fig. 3 which are plots of $\langle R^2 \rangle$ versus time for cells synchronized in mitotic phase and at uniform temperatures of (*a*) 37·0°C, (*b*) 36·5°C and (*c*) 35·0°C. The curves are plots of equation (4) with the two parameters D and τ adjusted by means of a computer program to give the best (least-squares) fit to

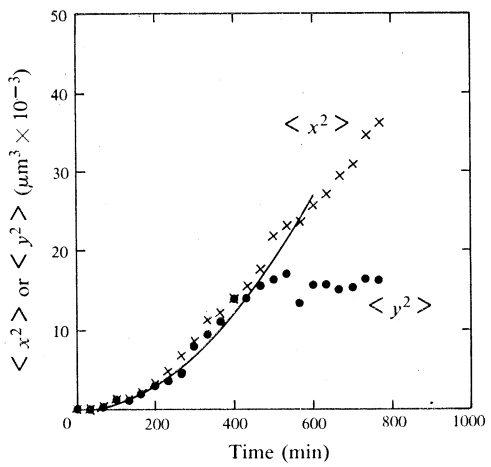

Fig. 4. Plots of $< x^2 >$ normal to and $< y^2 >$ along the gradient of $7 \cdot 22°C\,cm^{-1}$ at a mean temperature of 36·67°C. The curve is a plot of equation (2) with D and τ adjusted for the best fit to the $< y^2 >$ data for the first 366 min.

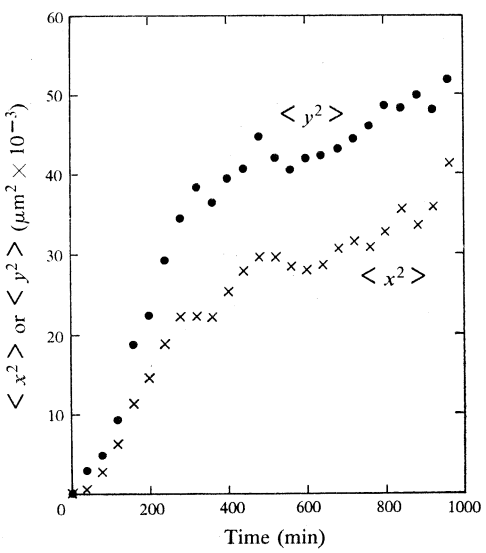

Fig. 5. Plots of $< x^2 >$ and $< y^2 >$ against time and at $T = 36 \cdot 5°C$ for the data of Fig. 3*b*.

the data for approximately the first one-eighth of the mitotic period. Note that τ is slightly larger at $T = 35{\cdot}0°C$ (Fig. 3*c*) than at $36{\cdot}5°C$ (Fig. 3*b*). This suggests that τ is not a good measure of motility. The particular value of τ depends in a marked way on the substrate and varies from flask to flask. While the characteristics of the substrate can vary across a given flask, a comparison of τ_x and τ_y for cells in the same flask should have more significance. Plots of $\langle x^2 \rangle$ and $\langle y^2 \rangle$ for cells in a gradient of $7{\cdot}22°C\,cm^{-1}$ and at a mean temperature of $36{\cdot}67°C$ are given in Fig. 4. The curve on the $\langle y^2 \rangle$ plot is fitted by least squares for the first 366 min and yields the values $D = 298\ \mu m^2\,min^{-1}$ and $\tau = 769$ min. The identical curve is redrawn on the $\langle x^2 \rangle$ plot and the agreement is good for the early part of the cycle. For comparison the values of $\langle x^2 \rangle$ and $\langle y^2 \rangle$ for the plot of Fig. 3*b* (at $T = 36{\cdot}5°C$) are given in Fig. 5.

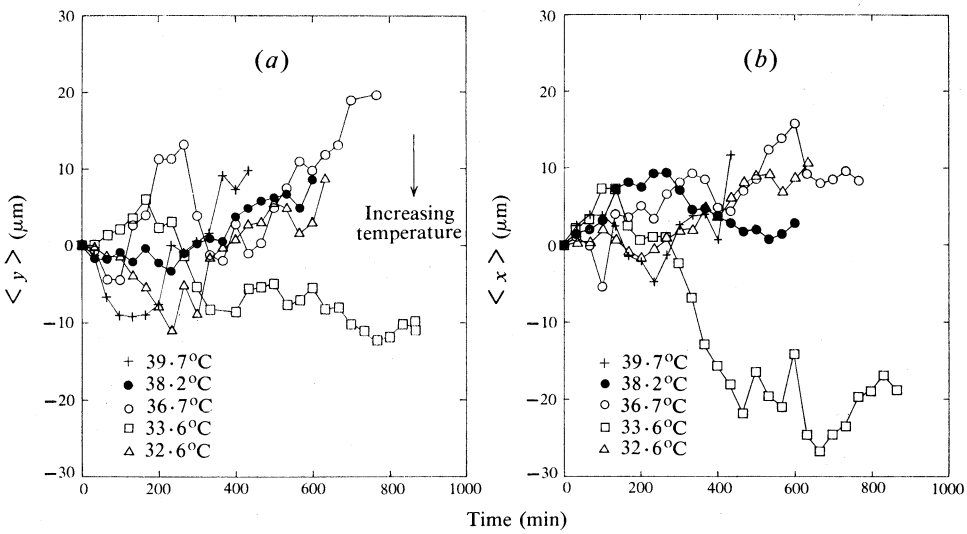

Fig. 6. Components of the centre of gravity (*a*) $\langle y \rangle$ and (*b*) $\langle x \rangle$ as a function of time in a gradient of $7{\cdot}22°C\,cm^{-1}$ for the mean temperatures indicated. The temperature increases in the $-y$ direction.

The components of the centre of gravity $\langle y \rangle$ and $\langle x \rangle$ for ensembles of cells in a temperature gradient of $7{\cdot}22°C\,cm^{-1}$ and for various mean temperatures are plotted against time in Fig. 6. The gradient increases in the negative y direction as indicated. These plots are to be compared with that corresponding to an ensemble of cells in a uniform temperature of $36{\cdot}5°C$ (no gradient) shown in Fig. 7. The displacement of the component of the centre of gravity in the direction of the temperature gradient (Fig. 6*a*) does not appear to differ significantly from the component normal to the gradient (Fig. 6*b*). Further, there is no correlation of the motion in $\langle y \rangle$ with either the direction of the gradient or with the mean temperature. In each case the mean displacement is of the order of the cell size, being on the average less than two body lengths, and is small compared with $\langle R^2 \rangle^{\frac{1}{2}}$. The corresponding plots of $\langle x \rangle$ and $\langle y \rangle$ in the absence of a gradient (Fig. 7) show a larger displacement.

The third criterion, the frequency distribution within an ensemble of cells moving either up or down the gradient as a function of time, again indicates no thermotactic response. At each mean temperature the distribution is well within the expectations

of equal probability. In the absence of a thermotactic effect, for N cells in an ensemble one would expect on the average one-half to be moving in the direction of the gradient and one-half opposite the gradient. The probability of any one cell having a displacement in the direction of the gradient is $p = \frac{1}{2}$ and in a direction opposite is $q = \frac{1}{2}$.

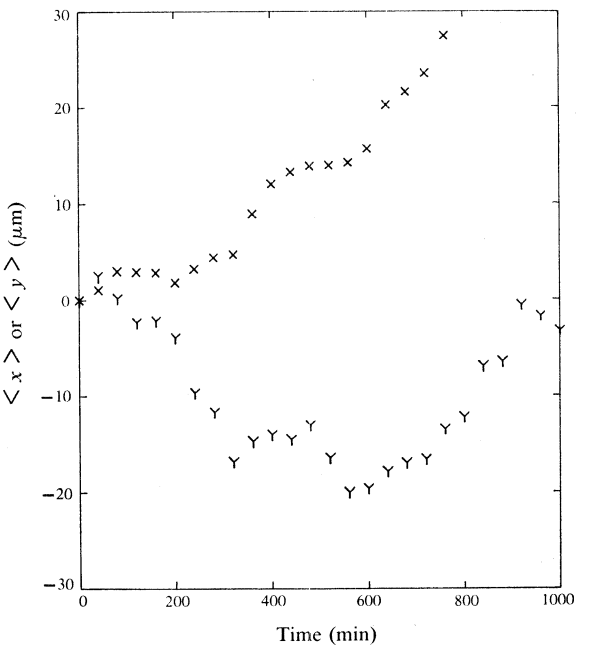

Fig. 7. Plots of $< x >$ and $< y >$ against time for an ensemble of cells at a uniform temperature of 36·5°C.

For N cells the most probable value of the number of cells in one direction is Np with a standard deviation of the mean of $\sigma = (Npq)^{\frac{1}{2}}$. For the five sets of data at the mean temperatures given in Fig. 6, the number of cells with a displacement in either direction after $5\frac{1}{2}$ hours is within one standard deviation of the most probable. Earlier in time, after $2\frac{1}{2}$ hours, the deviation for $T = 39{\cdot}7°C$ is $\frac{4}{3}\sigma$ towards higher temperatures, for $T = 36{\cdot}7°C$ it is $\frac{5}{3}\sigma$ towards lower temperatures, and for $T = 33{\cdot}6°C$ it is 2σ towards lower temperatures. For $T = 38{\cdot}2$ and $32{\cdot}6°C$ the deviations are within σ. By way of comparison, for the ensemble at a uniform temperature (no gradient) of $T = 36{\cdot}5°C$ after $2\frac{1}{2}$ hours, the deviation is within σ and after $5\frac{1}{2}$ hours it is within $\frac{3}{2}\sigma$.

5. Conclusions

There is no evidence for a thermotactic response of mouse fibroblast cells as determined by (1) differences in the motility parameters as measured in the direction of and normal to the temperature gradient; (2) the motion of the components of the centre of gravity of an ensemble of cells (the displacements of the centre of gravity are small compared with the r.m.s. displacements $\langle R^2 \rangle$); (3) the frequency distribution of the number of cells in an ensemble with displacements in the direction of (or opposite to) the temperature gradient. Any variance in the data can be understood in terms of statistical fluctuations.

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