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# Analysing the Motile Behaviour of Cells: A General Approach with Special Reference to Pairs of Cells in Collision

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# Analysing the motile behaviour of cells: a general approach with special reference to pairs of cells in collision

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[Plate 1]

As a measure of the change in motion of locomoting cells, acceleration has several advantages over more conventional measures such as rate of change in speed and rate of change in direction. In particular the instantaneous acceleration vector associated with a specific event, such as the collision of the cell with another, may be correlated with directional information contained in the structure of the cell and in the geometrical configuration of the event. It is hoped that this will prove to be a generally useful method for examining the relation between cell structure and motion during both normal and aberrant behaviour. Preliminary results obtained with this method to examine the changes in motion of a normal cell and a malignant cell on colliding with each other are already of interest for elucidating the mechanism of contact inhibition of locomotion and its failure during malignant invasion.

## Introduction

A major obstacle to the future development of the study of cell motile behaviour is the lack of sufficiently general and objective quantitative methods. Although there has recently been an increasing interest in techniques for observing cell motile behaviour, notably the phagokinetic method for observing cell tracks of Albrecht-Buehler (1977) and Zigmond's method for observing cells directly in a chemotactic field (Zigmond 1978), the techniques available for its analysis have not changed much in decades. At best these are often arbitrarily quantitative with a narrow field of application or, at worst, they are subjectively qualitative. New general methods are needed, particularly now that the mechanochemical processes of cell translocation are beginning to be understood and we are ready to embark on a description of the behaviour of individual cells in molecular terms. They are equally required for determining how the overall characteristics of the behaviour of cell populations are derived from the movements and interactions of their component cells. Thus quantitative methods for describing cell behaviour may eventually play two key roles in unravelling the molecular basis for the wide variety of processes that are dependent on cell migration *in vivo*.

The general approach we wish to develop here is based on a treatment of each individual cell as if it were a geometric point or particle moving in the plane. This results in a dramatic condensation of the total information presented by a moving and distorting extended body such as a tissue cell in culture; and yet the residual data are applicable to the testing of a wide variety of hypotheses. For example, the reduction in information still allows us to investigate how the distributive behaviour of cell populations is derived from the translocation and interaction of individual cells. By treating the cells as particles and determining a set of rules for how

they interact with each other and with their environment, we can, in principle, predict how large populations of the same particles would behave and compare this with observation of cell populations. The approach is more restrictive when we come to interpret a cell's behaviour in terms of the features of its own structure but, quite often, a little extra information is all that is needed for testing a hypothesis of this nature.

The success of this approach depends on the manner of choosing a particle to represent the whole cell or, in other words, on the manner of defining the cell's position. It is not easy to give general guidelines because the specific hypothesis to be tested is relevant and the method of observing the cell can be a limiting factor. Often the movement of the centre of the nucleus or of a single nucleolus can be representative of the movement of the bulk of the cell. Alternatively, instead of a structural particle, there may be some advantage in selecting the geometrical 'centre of gravity' of the cell, which can be determined fairly readily from the cell outline if a computer with a graphic input facility is available.

Having defined the cell's position satisfactorily, the analysis of its motion is reduced to a problem in kinematics, which is the study of particles in motion. At any instant, the motion of a particle and the rate of change in that motion are completely described by the velocity and the acceleration. These are vector quantities, each having a magnitude and a direction. The particle always has a velocity, provided, of course, that it is not stationary, and it has an acceleration if it is neither stationary nor moving uniformly in a straight line. Thus an instantaneous acceleration is a measure of the magnitude and direction of an instantaneous disturbance to uniform motion.

These disturbances have two sources. Even when moving in a homogeneous environment on a plane surface, cells do not, in general, show uniform motion and it must be concluded that some disturbances to uniform motion have an internal origin within the cell. This is just another way of saying that the locomotory machinery of the cell does not usually exist in a steady state but fluctuates spontaneously as the cell translocates. But we also know that certain heterogeneities of the environment, such as chemotactic gradients or curvatures of the substratum, can influence the speed and direction of locomotion of certain cells. These influences may be thought of as external disturbances to uniform motion that are superimposed on the background of internal disturbances.

The quantities velocity and acceleration are not only a simple and convenient system for describing the instantaneous locomotion of a cell, as we will show later, but they can be converted readily into quantities more familiar to cell biologists, such as speed, persistence in speed and persistence in direction. They therefore provide an alternative way of thinking about cell behaviour and, in the next section, we shall argue that this may have distinct advantages in many circumstances. More importantly, by combining the analysis of accelerations with random sampling techniques, we can identify and quantify those changes in motion that are due to external disturbances caused by interactions with the environment and with other cells. This will be the main theme of the paper and the method will be illustrated by reference to a case study of the analysis of collisions between cells of two types, one malignant and the other not.




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## Velocity and acceleration compared with other measures of locomotory behaviour

In describing a change in motion of a moving object we naturally consider two separate aspects, change in speed and change in direction, and this distinction has been the basis of most systems for describing the movement of cultured cells or free-living microorganisms. However, it is important to bear in mind that, for cells at least, the distinction does not necessarily have a mechanistic foundation. It is quite unlikely that the cell has separate mechanisms, each devoted exclusively to either changing speed or changing direction. The use of vector acceleration to

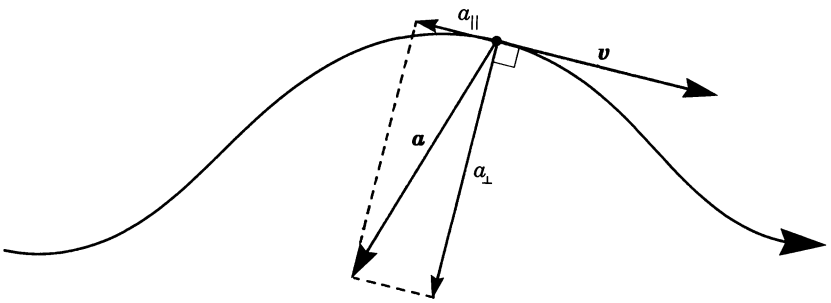


Figure 1. See text for explanation.

describe a change in motion does not introduce this artificial distinction and is therefore less prone to spurious interpretation. Furthermore, it may be a more useful way of appreciating a change in motion caused by a discrete contractile or adhesive event within the cell. By examining the accelerations of an isolated wandering cell, we can quantify the magnitude and direction of its internal perturbations in motion and attempt to relate these to concurrent changes in the configuration of its locomotory machinery.

The relations between acceleration and other measures of change in motion become apparent when acceleration is resolved into two components: one parallel to the direction of travel (the direction of the velocity vector) and one perpendicular to this direction. Consider a particle that traces out a curvilinear path in the plane as shown in figure 1. At any time, its instantaneous vector acceleration ($\boldsymbol{a}$) is the instantaneous rate of change in vector velocity ($\boldsymbol{v}$) with respect to the time variable $t$:

$$\boldsymbol{a} = \mathrm{d}\boldsymbol{v}/\mathrm{d}t. \tag{1}$$

The component of acceleration in the direction of particle travel ($a_{\parallel}$) is a scalar quantity given by

$$a_{\parallel} = \mathrm{d}|\boldsymbol{v}|/\mathrm{d}t, \tag{2}$$

where $|\boldsymbol{v}|$ is the magnitude of the instantaneous velocity or, more familiarly, the speed. Thus $a_{\parallel}$ is the instantaneous rate of change in speed with respect to time. In the example of figure 1, $a_{\parallel}$ is negative (i.e. it is pointing backwards), indicating that the particle is slowing down. The component of acceleration perpendicular to the direction of travel ($a_{\perp}$) is given by:

$$a_{\perp} = \frac{\mathrm{d}\theta}{\mathrm{d}t}|\boldsymbol{v}|, \tag{3}$$

where $\theta$ is the direction of travel in radians. Thus $a_{\perp}$ is the instantaneous rate of change in




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direction with respect to time multiplied by the speed. Alternatively, since

$$\frac{d\theta}{dt} = \frac{d\theta}{ds}\frac{ds}{dt} = \frac{d\theta}{ds}|\boldsymbol{v}|, \tag{4}$$

where $s$ is the variable denoting distance travelled by the particle along its path, (3) may be rewritten

$$a_\perp = \frac{d\theta}{ds}\boldsymbol{v}^2. \tag{5}$$

Thus $a_\perp$ is also the instantaneous rate of change in direction with respect to distance multiplied by the square of the speed. The reader interested in a method of deriving the above results is referred to Troup (1976, chapter 2).

Previously, attention has been directed mainly to change in speed and change in direction, rather than to change in velocity and this has led to a wide range in terminology with its attendant possibilities of confusion. For each of these three variables, speed, direction and velocity, not only is its rate of change with respect to time of interest but its rate of change can also be referred to distance and this has advantages in some cases. For example, the rate of change in direction with respect to distance, $d\theta/ds$, can be measured directly from a cell's path, such as a phagokinetic track, in the absence of any temporal information. In fact, it can be shown that $d\theta/ds$ is equivalent to $1/r$, where $r$ is the instantaneous radius of curvature of the path. Added to this there is the concept of persistence in motion which is often not clearly defined but which, in general, appears to be the reciprocal of the corresponding rate of change. Persistence in velocity, persistence in speed and persistence in direction can each refer to either of the two variables, time and distance.

Thus there are at least twelve valid measures of aspects of change in motion but, by using the above equations, all can be obtained from the one general measure of change in motion, acceleration, together with a knowledge of the velocity. The rates of change with respect to time of the three variables are related to acceleration by (1), (2) and (3), and the rates of change with respect to distance can be derived as shown in (4) and (5) for rate of change in direction with respect to distance. If we define persistence as the reciprocal of the absolute magnitude of the corresponding rate of change, then, for example, time persistence in velocity is simply $1/|\boldsymbol{a}|$ (from (1)) and distance persistence in direction is $\boldsymbol{v}^2/|a_\perp|$ (from (5)) which, as we have seen, is simply the instantaneous radius of curvature of the path.

A complication arises, however, when we require a measure of average rate of change in motion over an extended time period. This is quite commonly required for characterizing a long-term pattern of behaviour during, for example, a kinesis response. An obvious solution is to find some way of taking averages of the above instantaneous measures, particularly the instantaneous acceleration vectors, but there are many ways of time-averaging and different ways suit different purposes. Some of these methods will be dealt with in a later paper.

## External disturbances to the locomotory machinery

The analysis of velocity and acceleration is a powerful means of examining external directional influences on locomotion, such as may be due to interaction with other cells or with the environment. An important property of random vectors (each having a random direction and a magnitude that is a random variable independent of direction) is that the vector mean of a




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sample of random vectors has an expected magnitude of zero. At any instant, therefore, the velocities of a sample of cells moving independently and at random tend to cancel each other out so that the magnitude of the vector mean velocity tends to zero as the sample size increases. A vector mean velocity with a magnitude significantly greater than zero indicates that the cells are either not moving independently of each other or that they are responding to some directional property of their environment. These remarks also apply to the instantaneous acceleration vectors of a sample of cells. Whether the velocity, the acceleration or both are affected by the environment depends on the nature of the directional property, on how fast it is changing, if at all, and on whether the cells can adapt to it. In general, a constant directional property of the environment, such as a steady chemotactic gradient, may affect only the mean velocity, whereas a change in that property would be expected to affect mainly the mean acceleration. Dunn (1981) describes a significance test for the magnitude of a vector mean velocity, which may be used to detect a unidirectional or tactic influence, and a method of transforming the data so that a bidirectional influence, such as a structural orientation of the substratum, may be detected. These methods are equally applicable to the vector mean acceleration.

The versatility of this sort of analysis becomes apparent when independent cellular events are to be examined. Consider an imaginary experiment in which observations are made on isolated cells encountering a sharp, straight boundary of change in adhesiveness of the substratum. If the motion of each cell changes in response to colliding with the boundary then the acceleration of the cell at the time of collision will be a measure of this change in motion. In order to get a measure of the mean response and nullify the effects of random change in motion, we must pool the data from a sample of several observations. However, if the data were pooled at random, the accelerations would point in random directions and the expected vector mean acceleration would have a magnitude of zero and be useless as a measure of the response. The data must be pooled with regard to a specific hypothesis about the mechanism of the response. One such hypothesis might be that the direction of acceleration depends entirely on the direction of travel of the cell just before collision. This would be so if the locomotion were generally inhibited at the time of collision so that the cell slows down or reverses but does not turn. Equally we could imagine a mechanism in which the direction of acceleration depends entirely on the direction of the boundary. For example, if the cell were reflected from the boundary without changing speed the acceleration would always be perpendicular to the boundary. By orienting the accelerations with regard to a specific direction, such as the direction of the boundary or of cell travel, and examining the vector mean acceleration, it is possible to determine the relative likelihood of the various hypothetical mechanisms. Of course, in deciding between the above two hypotheses, it is necessary that the angle between the direction of travel and the boundary takes a wide range of values in the sample. A more sophisticated hypothesis might be that the direction of acceleration is some function of the angle of incidence as it would be if the cell were refracted at the boundary, as suggested by Albrecht-Buehler (1979).

## Estimating the instantaneous velocity and acceleration

In practice we cannot obtain instantaneous values of the velocity directly from the data, since these are necessarily collected at discrete time intervals whereas the instantaneous quantities are descriptions of the underlying continuous process. The simplest approach is to obtain biased estimates of $\boldsymbol{a}$ and $\boldsymbol{v}$ from discrete data taken at equal time intervals. These are averages




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over short time intervals, and not true instantaneous values, but this is not usually a disadvantage. Neither is it serious disadvantage that the estimates are biased (that the values obtained would be different if the time interval were changed) provided that all comparisons are based on the same time increment.

Figure 2 depicts the path of a hypothetical cell on which the cell position is marked at the three times: $t-\Delta t$, $t$ and $t+\Delta t$, where $\Delta t$ is the time increment. By constructing the parallelogram on the three positions as shown, we can identify four distinct vectors. $\Delta \boldsymbol{s}$ and $\Delta \boldsymbol{s}'$ are

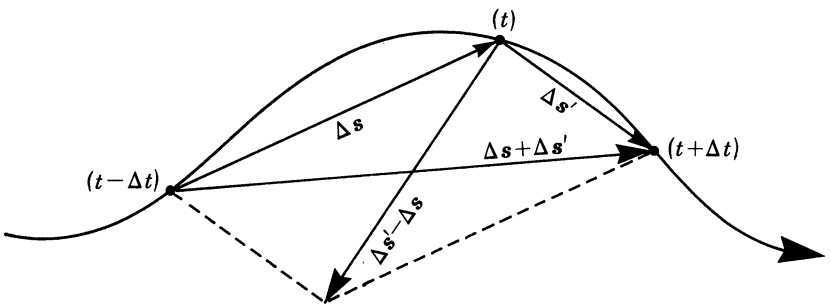


FIGURE 2. See text for explanation.

the vector displacements of the cell over the two time intervals $(t-\Delta t, t)$ and $(t, t+\Delta t)$ respectively. The two diagonals of the parallelogram represent the vector sum and vector difference of $\Delta \boldsymbol{s}$ and $\Delta \boldsymbol{s}'$.

The mean velocity, $\bar{\boldsymbol{v}}$, over the double time interval $(t-\Delta t, t+\Delta t)$ is obtained simply from the vector sum

$$\bar{\boldsymbol{v}} = (\Delta \boldsymbol{s}+\Delta \boldsymbol{s}')/2\Delta t.$$

This is an estimator of the instantaneous velocity, $\boldsymbol{v}$, at time $t$ because, in the limit when $\Delta t$ tends to zero, $\bar{\boldsymbol{v}}$ approaches $\boldsymbol{v}$. A quantity, $\boldsymbol{a}'$, which may be obtained simply from the vector difference, we can define as the average acceleration over the same double time interval:

$$\boldsymbol{a}' = (\Delta \boldsymbol{s}'-\Delta \boldsymbol{s})/\Delta t^2.$$

This is not equivalent to the mean acceleration over the time interval because it is obtained from the difference of two mean velocities and not from two instantaneous velocities. But it does serve as an estimator of the instantaneous acceleration, $\boldsymbol{a}$, at time $t$ because, in the limit when $\Delta t$ tends to zero, $\boldsymbol{a}'$ approaches $\boldsymbol{a}$. The directions of $\bar{\boldsymbol{v}}$ and $\boldsymbol{a}'$ are therefore simply the directions of the diagonals in figure 2 and their magnitudes may be obtained by dividing the true lengths (allowing for the scale factor of diagram) of the diagonals by either $2\Delta t$ or $\Delta t^2$ as appropriate.

## ANALYSIS OF COLLISIONS BETWEEN NORMAL FIBROBLASTS AND FIBROSARCOMA CELLS: A CASE STUDY

The study to be described is only at a preliminary stage, but interesting results are beginning to emerge and we present them here to illustrate the method of analysis. The biological problem concerns the mechanism of malignant invasion in so far as it is represented by a tissue culture model. The model consists of two confronted explants, one normal and one malignant, placed




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about 1 mm apart on a coverslip as described by Abercrombie (1979). Invasion is measured as the distance of infiltration of each outgrowth of cells into the opposing outgrowth and is standardized against the distance of outgrowth in regions where there are no opposing cells. Abercrombie drew two major conclusions from these studies: that invasion in the model correlates well with malignancy *in vivo* and that it appears to be due to a defective contact inhibition reaction when a malignant cell meets a normal cell. The single most compelling piece of evidence for this last conclusion is that, with certain cell types, invasion in the model is non-reciprocal, the malignant cells invading the normal cells but not vice versa, and that a non-reciprocity is also observed in individual collisions between the same combinations of cell types; here the normal cells are inhibited in their locomotion but the malignant cells are not, as was first described by Heaysman (1970).

In order to quantify this collision behaviour we chose a combination of cell types that Abercrombie found to be the best example of non-reciprocal invasiveness: primary chick heart fibroblasts (CHF) and mouse fibrosarcoma cells of strain FS9. Dissociated cells of the two types were seeded together at low density on glass coverslips and mounted in chambers suitable for high-resolution microscopy. Time-lapse video recordings of individual collisions between the two types were made using a $\times 40$ phase contrast objective and a recording speed of 1/80 normal. Each collision was recorded from approximately 0.5 h before initial contact of the cell margins to 1 h after initial contact. Part of a typical sequence is shown in figure 3, plate 1. Raw data for analysis were obtained by determining the coordinates of the position of each cell, as defined by a prominent nucleolus, by taking measurements from a video monitor screen and multiplying by the appropriate scale factor. The rectangular coordinate orientation was arbitrarily defined by the edges of the monitor screen and the origin of the coordinate axes, for each collision, was defined as the position of initial contact of the cell margins. The cell position coordinates were taken at intervals of 5 min throughout the course of the collision.

Here we present an analysis of a portion of these raw data. For 15 collisions we shall consider only the time points at 10 min intervals extending from 20 min before initial contact to 20 min after initial contact. All 15 pairs of cells remained in contact during the 20 min after initial contact. Thus for each collision we have 10 pairs of position coordinates, 5 for each cell, with the added information that the origin of these coordinate pairs is the position of initial marginal contact. By using the methods outlined in the previous section, this allows us to calculate, for each cell, three velocity vectors and three acceleration vectors corresponding to the three times at 10 min before initial contact, on initial contact and 10 min after initial contact.

### *Magnitudes of velocities and accelerations*

First we shall compare the absolute magnitudes of these quantities to see if they change significantly with time during the collision and to detect any differences between the CHF and FS9 cells. For this a series of unpaired two-tailed *t*-tests will be used for comparing CHF and FS9 and a series of paired two-tailed *t*-tests for detecting the changes with time in either cell type. The assumption of normality required by the *t* test is probably not justified here (there are more values near to zero than would be expected) but the test is fairly insensitive to distribution for a sample size greater than about 10 (Guenther 1973, p. 254), and so it will suit the requirements of a preliminary survey of the data. The results are shown in table 1.

As can be seen in the table, this analysis of magnitude does not reveal a great deal. Before collision, the two cell types are fairly well matched in velocity and acceleration, there being no

significant differences. We would expect from Abercrombie's observations that the velocities of the CHF cells would decrease on collision whereas those of FS9 would remain unchanged and, although the data appear to have the appropriate trend, there is no evidence that these changes in velocity are significant. We would further expect that the CHF cells are affected by the collision whereas the FS9 cells are not, and so we should see an increase in the CHF accelerations on collision and no change in the FS9 accelerations. Here again the data trend in the right

TABLE 1. MEAN MAGNITUDES OF VELOCITIES AND ACCELERATIONS

| | $\lvert\bar{v}\rvert$/μm min$^{-1}$ | | | $\lvert a'\rvert$/μm min$^{-2}$ | | |
|---|---|---|---|---|---|---|
| | CHF | | FS9 | CHF | | FS9 |
| 10 min before | 0.9151 | n.s. | 0.7369 | 0.0672 | n.s. | 0.0568 |
| | n.s. | | n.s. | n.s. | | n.s. |
| on contact | 0.6667 | n.s. | 0.9082 | 0.1013 | * | 0.0556 |
| | n.s. | | n.s. | n.s. | | n.s. |
| 10 min after | 0.7551 | n.s. | 0.9268 | 0.0845 | * | 0.0387 |

*, Significant at 5% level; n.s., not significant.

direction but without significance. However, we do find significance when we compare the accelerations of CHF and FS9 on collision; this is barely significant at the 5% level and continues to be significant at this level 10 min after initial contact. We have here, therefore, a test of the non-reciprocal nature of the collision, albeit a not very sensitive one.

*Characterizing movement before collision*

As discussed earlier, the accelerations are far more informative indicators of changes in motion if we consider their directions as well as their magnitudes. For this we must choose a reference direction and, for a freely moving cell before contact, a sensible reference direction is the direction of travel of the cell for which our estimator is the direction of the mean velocity.

In the top panel of figure 4, the accelerations have been oriented so that the directions of their corresponding mean velocities all coincide, the direction of each mean velocity being given by the direction of $\Delta s + \Delta s'$, as shown in figure 2. The upper left-hand cluster in figure 4 represents the 15 accelerations of the CHF cells before collision (some are too small to be seen). The components of acceleration parallel to the mean velocity appear to have greater magnitudes than those perpendicular to the mean velocity. A measure of this difference is the ratio, $F$, of the variance of the parallel components to the variance of the perpendicular components. Whether this ratio is significantly greater than 1 can be determined directly from tables of $F$ by using $N-1$, $N-1$ degrees of freedom, the assumption of normality being more justified for these signed components of the vectors than it was for their absolute magnitudes. For the CHF cells before collision $F = 17.15$, which is highly significantly greater than 1 ($P \ll 0.0005$), and for the FS9 cells before collision $F = 10.95$, which is also highly significant ($P \ll 0.0005$).

We believe that the ratio of the standard deviations of these orthogonal components, $\sqrt{F}$, could be a useful measure of the polarity of cell locomotion and that a $\sqrt{F}$ significantly greater than 1 indicates a distinct polarity. It is a measure of how much greater the changes in motion are in the axis of travel than they are perpendicular to the axis. For the CHF cells $\sqrt{F} = 4.14$ and for the FS9 cells $\sqrt{F} = 3.31$.




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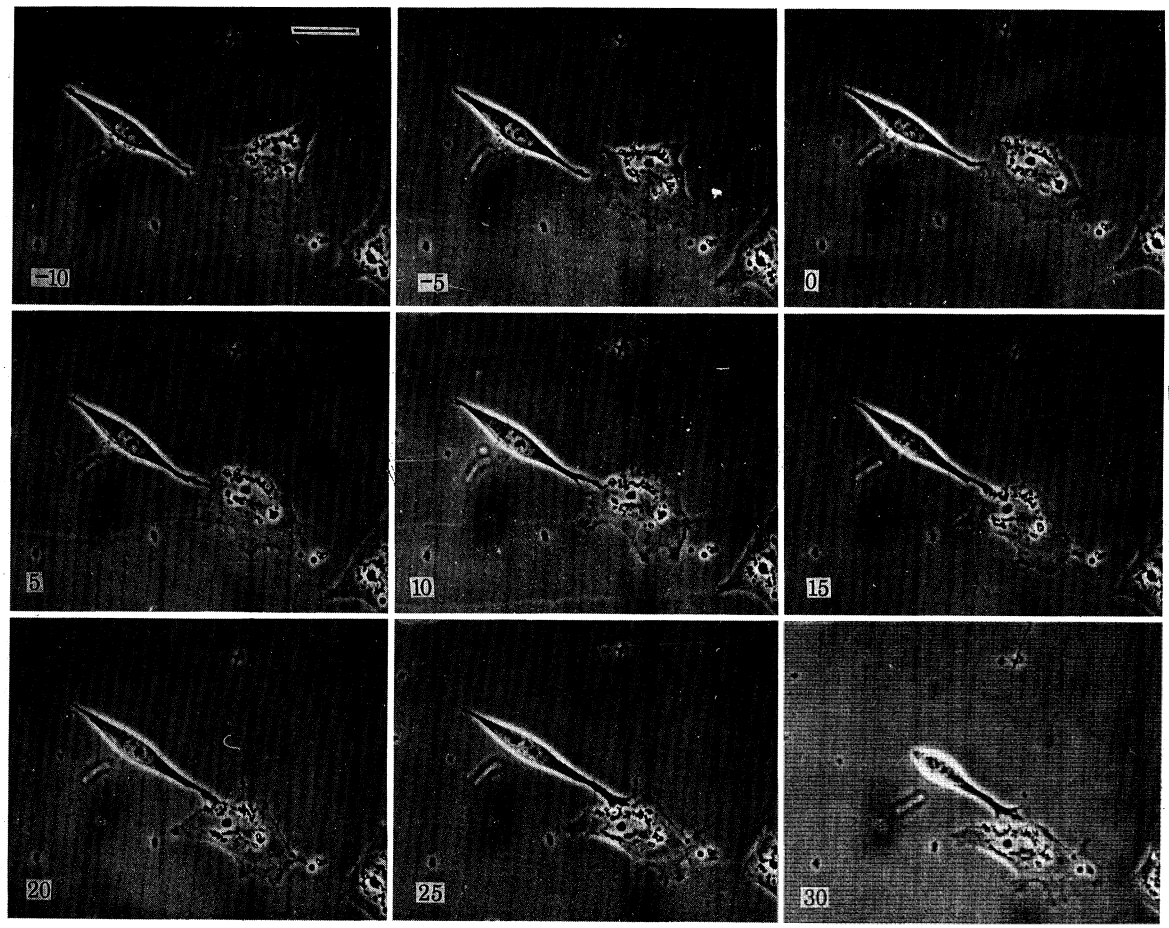


FIGURE 3. Sequence of photographs taken at 5 min intervals showing a collision between a mouse fibrosarcoma FS9 (on the left) and a chick heart fibroblast. Time 0 min is the time of initial marginal contact. Scale bar, 20 μm.

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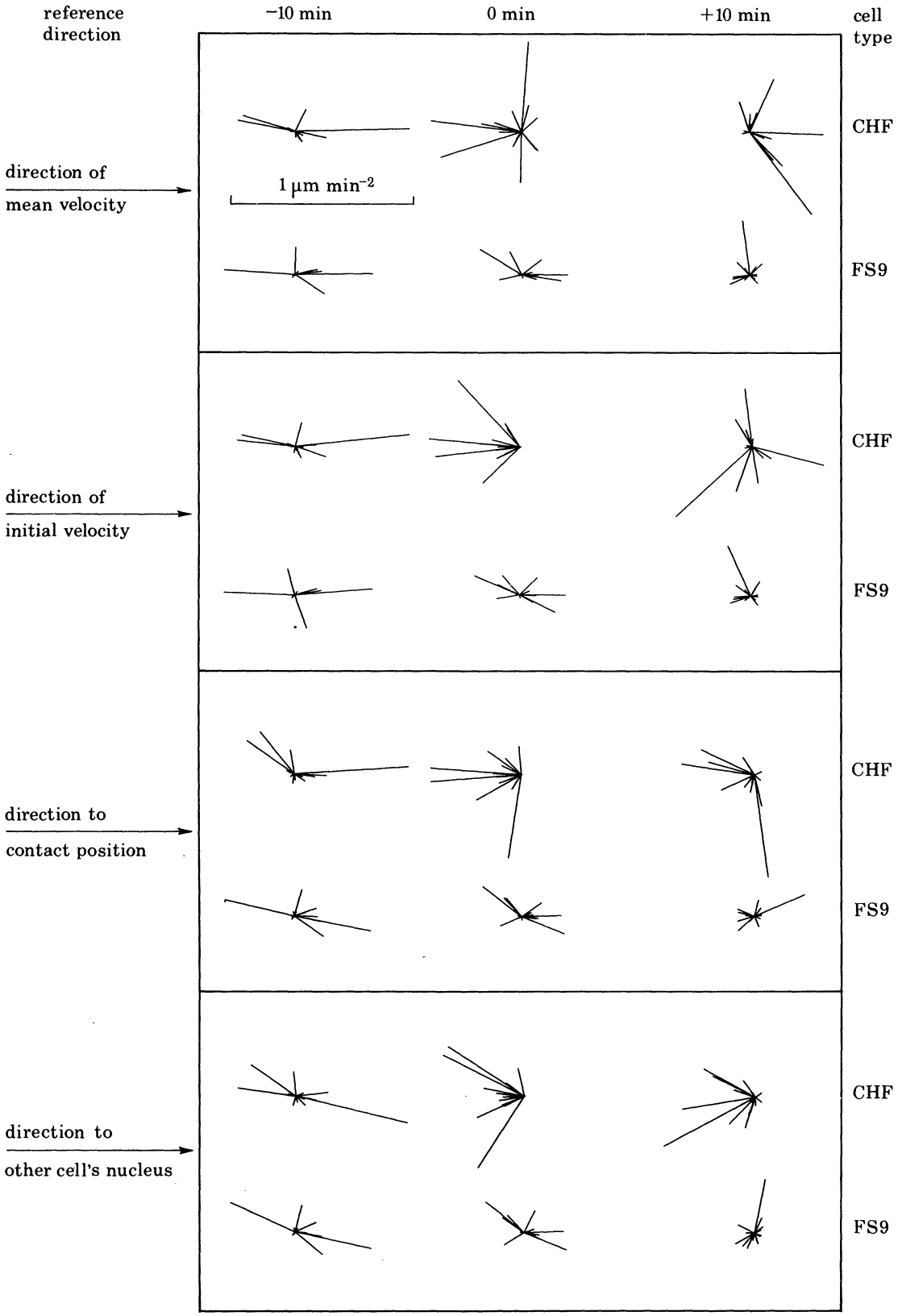


FIGURE 4. See text for explanation.





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*Characterizing movement during collision*

The CHF accelerations at the time of initial contact, still oriented with respect to the mean velocity, are represented by the upper central cluster in figure 4. These now appear to tend to the left, suggesting a preponderance of negative values in the parallel components, which indicates a general slowing down of the CHF cells on collision. However, a two-tailed $t$-test of the hypothesis that the mean of the parallel components is not zero just fails to show significance at the 10% level ($t = -1.72$, 14 d.f., $0.1 < P < 0.2$). At 10 min after contact the CHF cells appear to speed up and this time the mean parallel component differs significantly from zero ($t = +2.51$, $P < 0.025$). As a control to check that the CHF cells are not changing speed in unison before contact, the same test applied at 10 min before contact shows no significance ($t = +0.602$, $0.5 < P$). The FS9 cells at no stage before or during collision show a significantly non-zero mean parallel component. Also, no cells of either type show a significantly non-zero mean perpendicular component, indicating that there is no tendency to turn preferentially either to the left or to the right. In summary, there is a slight indication that the CHF cells slow down on collision and a stronger indication that they speed up again when the collision has progressed a further 10 min, whereas there is no indication that the mean speed of the FS9 cells changes.

Just as with analysing the magnitudes of the velocities, there is still no good evidence here that locomotion is generally inhibited in the CHF cells on collision. So what aspect of locomotion is inhibited during contact inhibition of locomotion? Abercrombie (1979) suggests that it is continued movement in the direction that brought about contact that is inhibited. To test this hypothesis we examined the accelerations oriented with respect to a new reference direction: the direction of the initial velocity corresponding to each acceleration, which is shown as the direction of $\Delta s$ in figure 2. These accelerations are shown in the second panel in figure 4 and, for the CHF cells on contact, the parallel components now have a mean that differs very significantly from zero ($t = -4.43$, $P < 0.001$). We also tried two alternative hypotheses of a localized inhibition of the locomotory machinery, and the results are shown in the third and fourth panels of figure 4. The first of these is that locomotion is inhibited on collision in a direction from the cell's position (as defined by the chosen nucleolus) towards the point of initial marginal contact. Again the mean parallel component of acceleration is significantly non-zero ($t = -3.89$, $P < 0.005$). The other alternative is that locomotion is inhibited towards the opposing cell's position and, in this case, the mean parallel component shows the highest significance of all ($t = -4.91$, $P < 0.001$). All three hypotheses therefore show a significantly negative acceleration in the CHF cells on initial contact, and it is probably not worth trying to distinguish between them by using these data, but 10 min after the onset of collision there are interesting differences. Only the last two orientations illustrated in the third and fourth panels of figure 4 show a continued inhibition of CHF cell locomotion in the reference direction, although they are somewhat less significant (for the direction to the initial contact position $t = -2.34$, $P < 0.05$, and for the direction to the FS9 cell's position $t = -3.00$, $P < 0.01$). Apart from the above and from one exception, there is no significantly non-zero mean of either parallel or perpendicular components of acceleration in either cell type. The exception is the mean perpendicular component of the CHF cells 10 min before contact referred to the direction to the initial contact position; this, however, is only significant at the 10% level and is reasonably attributable to random chance ($t = +1.99$, $0.05 < P < 0.1$).




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## Conclusion

The data show that a hypothesis of a general inhibition of CHF locomotion during collision is no longer tenable in relation to hypotheses of localized inhibition. This is perhaps not surprising in view of the fact that collision between two normal cells usually shows a localized inhibition of protrusive activity such as ruffling (Trinkaus *et al.* 1971). However, the analysis does not distinguish between three more specific hypotheses that we tried. This is doubtless because the most frequently occurring collisions are those where the two cells meet each other head on, when the three chosen reference directions tend to coincide. In order to distinguish between these and similar hypotheses, a sample of collisions is needed in which head to head collisions are largely excluded, and we intend to publish an analysis of such collisions in a later paper. There is also the problem of which aspects of the change in motion are responsible for the invasiveness of different cell types. This can, of course, be approached by looking for common features in the acceleration patterns of different invasive types when they collide with normal cells. But an alternative approach might be to construct models that simulate population behaviour from the known features of individual behaviour and to experiment with the variation of different parameters in these models.

We envisage that the analysis of accelerations is by no means limited to testing hypotheses based on simple configurations of the cells as discussed in this case study. As techniques become available for observing in more detail the structure of the locomotory machinery in living moving cells, methods will be needed for relating changes in motion to the changes in structure of the locomotory machinery in order to understand the molecular dynamics of locomotion. General methods for observing these changes in motion will inevitably involve the analysis of acceleration.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

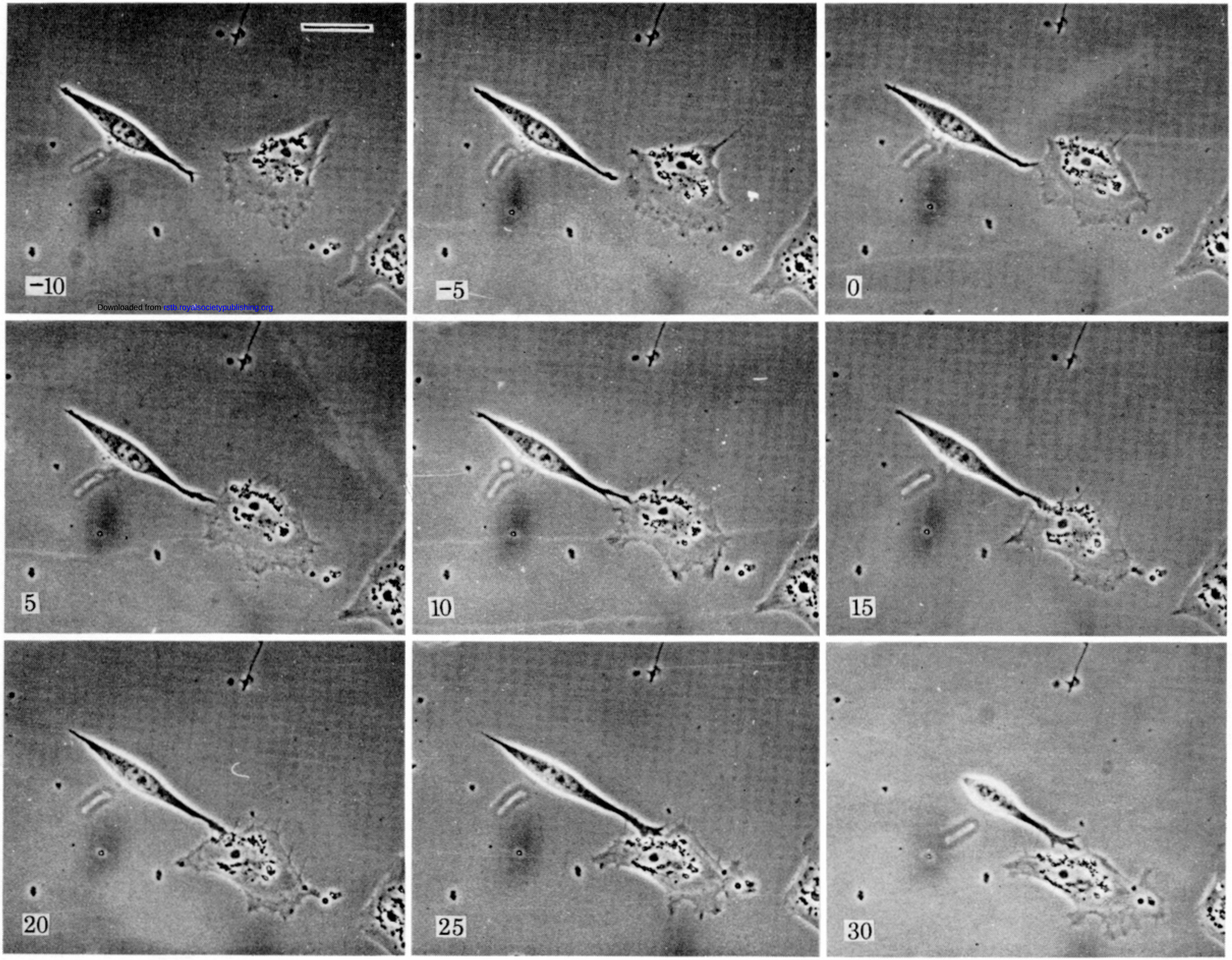


FIGURE 3. Sequence of photographs taken at 5 min intervals showing a collision between a mouse fibrosarcoma FS9 (on the left) and a chick heart fibroblast. Time 0 min is the time of initial marginal contact. Scale bar, 20 μm.