ANALYSING COLLISIONS BETWEEN FIBROBLASTS AND FIBROSARCOMA CELLS: FIBROSARCOMA CELLS SHOW AN ACTIVE INVASIONARY RESPONSE

S. W. PADDOCK* AND G. A. DUNN†
M.R.C. Cell Biophysics Unit, 26–29 Drury Lane, London WC2B 5RL, UK

SUMMARY
We describe a direct way of measuring contact inhibition of locomotion by analysing the changes in motion of pairs of colliding cells. This allows values to be assigned to each type of cell in mixed collisions and will enable certain hypotheses about the relationship between contact inhibition and invasion in culture to be tested critically. We find that fibrosarcoma (FS9) cells, on colliding with chick heart fibroblasts, show a reversed contact-inhibition response that we call contact promotion of locomotion. We also describe a measure of the lateral changes in motion that result from collisions between cells and show that this is dependent on the type of colliding cell but, unlike contact inhibition, it does not appear to be dependent on the type of cell with which it collides for the types studied here. Finally, we analyse how the total response is dependent on the dispositions and motions of the cells before collision and we find that FS9 cells, on colliding with fibroblasts, tend to turn towards the point of initial marginal contact. We conclude that the FS9 cells show a pronounced response on colliding with the fibroblasts, which is in contrast to the subjective impression that the FS9 cells do not respond much. These findings support the thesis of Abercrombie and colleagues, that the infiltration of a population of normal cells by a population of invasive cells in culture is dependent on the nature of the response of each cell type to collision with the other and that the invasive cells fail to show contact inhibition in these heterotypic collisions; but the findings further suggest that these invasive cells show an active invasionary response as opposed to merely failing to show contact inhibition.

INTRODUCTION
Abercrombie, Heaysman and their colleagues have for many years been pursuing the idea that contact interactions between moving cells in culture can lead to patterns in cell arrangement that may mimic aspects of certain fundamental biological processes, particularly malignant invasion (Abercrombie, 1979; Heaysman, 1978). By observing collisions between cells in vitro, they have discovered one set of contact interactions that appear to be independent of tissue and species types but to distinguish between whether a cell type is invasive or non-invasive in vivo. If two non-invasive cells collide with each other in culture, whether or not they are of the same type, each cell generally ceases to continue moving in the direction that brought about contact, a response termed 'contact inhibition of locomotion'. If an invasive

*Present address: Department of Cell Biology and Anatomy, Northwestern University, 303 East Chicago Avenue, Chicago, Illinois 60611, USA.
†Author for correspondence.

Key words: quantitative methods, locomotory behaviour of cells, contact inhibition, contact promotion, malignant invasion.
cell collides with a non-invasive cell, it generally fails to show contact inhibition. In the latter case, the non-invasive cell may or may not be inhibited, depending on cell types, which gives rise to the respective qualifying terms, 'non-reciprocal' and 'reciprocal' failure of contact inhibition (Heaysman, 1970). There appears to be no general rule of interaction in the case of two invasive cells colliding with each other; each may or may not show contact inhibition depending on the type or types involved.

While a good deal of research and dispute has been concerned with the possible mechanism of contact inhibition, we believe that a more immediate problem is what significance contact inhibition has for pattern formation in vivo and, in particular, for malignant invasion. The arguments that it is significant, presented by Abercrombie, Heaysman and others, have two distinct aspects. First, there appears to be a strong correlation, over a range of cell types, between the contact responses in collisions, the patterns that form in culture, and the behaviour of the cells in vivo. Secondly, there is evidence for a causal relationship between contact responses and population behaviour – evidence that the patterns are direct consequences of the contact behaviour. Much of this evidence derives from detailed analyses of a cell's behaviour in relation to the number of cells that it contacts (Abercrombie & Heaysman, 1953; Abercrombie & Gitlin, 1965), but it must be added that the arguments for a causal relationship are also based on an appeal to intuition: that the patterns would be expected to result from the known contact responses.

It seems clear to us that the main difficulty in pursuing this problem further has been the lack of adequate methods for analysing the contact responses and for measuring contact inhibition. An example should serve to illustrate this. At present there appears to be one notable exception to the correlation between contact inhibition and population behaviour in culture. Abercrombie & Turner (1978) have found with sarcoma (S180) cells that the level of overlapping is much lower than that expected on the assumption of a random distribution, which generally implies the presence of homotypic contact inhibition, and yet the outward drive of these cells from an explant is no higher than that which would be expected on the assumption of random diffusion, which generally implies the absence of contact inhibition. They suggest that this anomalous behaviour may arise from the fact that, although the cells change their motion on collision (in fact they appear to bounce off each other), their rate of change in direction is so high when moving freely that the extra changes induced by collision do not produce a drive away from areas of high density.

The first question that this poses is whether or not the S180 cells show contact inhibition; in other words, where does the correlation between contact inhibition and behaviour break down? The S180 cells do not show a paralysis of protrusion in single collisions but, in any case, there is evidence that paralysis of protrusion is unreliable as an indicator of contact inhibition (Vesely & Weiss, 1973; Erickson, 1978). Furthermore, it is obvious that the present methods for assessing contact inhibition based on overlapping or outward drive are entirely inappropriate for answering this question. A second question is whether this anomalous behaviour can be explained by taking the free behaviour of the cells into account as well as their contact
Analysing collisions between cells

responses. The direct approach to answering these questions is to analyse how cells change their motion on colliding with each other and to compare this with the spontaneous changes in motion of freely moving cells. We present methods here for doing this.

From the point of view of malignant invasion, a much more important correlation is between the heterotypic contact inhibition response and the interactions of populations of different cell type. These were reviewed by Abercrombie (1979) and a brief outline must suffice here. If two explants of different cell types are placed some little way from each other in culture, three types of pattern ensue, which correspond to whether there is heterotypic contact inhibition between the two cell types, or whether there is a reciprocal or non-reciprocal failure of heterotypic contact inhibition. In the first case there is no mutual infiltration of the outgrowths from the two explants but infiltration or mixing does occur in the second case; in the third case the non-responsive cells tend to occupy territory held by the responsive cells, but not vice versa, and this is usually accompanied by some infiltration. The second and third cases generally occur only when a cell type that is invasive in vivo is present and the strong implication is that infiltration and territory occupation in culture are representative of invasion in vivo.

The problem with pursuing this correlation is that present methods do not allow contact inhibition to be measured separately for the two participants in a mixed collision and assessments have had to rely on subjective judgement. Measuring the incidence of mixed nuclear overlapping can be quite misleading in the case of a non-reciprocal failure of contact inhibition (Heaysman, 1970). For this reason, we chose an example of non-reciprocal failure of contact inhibition to analyse. The two types that we used are chick heart fibroblasts and cells from the fibrosarcoma FS9. A preliminary analysis of the population behaviour of these two cell types has been published by Abercrombie (1979), who stated that the actual distance invaded by the fibroblasts (into the sarcoma population) was one-third to one-quarter of that of the sarcoma cells (into the fibroblast population). Corresponding to this non-reciprocal invasion, when individual heterotypic collisions were observed, the fibroblasts showed a high level of contact inhibition while the sarcoma cells continued their locomotion. By measuring the contact inhibition of each cell type in heterotypic collisions, we find here that the sarcoma cells actually proceed further in their original direction of travel than they would have done had the collision not occurred. A reversal of the contact inhibition response was discussed as a possible theoretical cause of certain features of cell aggregation patterns by Curtis (1967) but, to our knowledge, this is the first instance of its demonstration.

Finally, there is the question of whether contact inhibition, including its reverse counterpart, is an essentially complete description of the contact responses. By this we mean, is a knowledge of the contact inhibition responses, together with a knowledge of the free behaviour of the cells, sufficient to explain the simple patterns of population behaviour that occur in tissue culture? It is probable that satisfactory answers to this question will not emerge for some time, especially since the contact responses are only easily observed in sparse cultures, whereas many of the patterns of
interest occur in dense cultures where cells are simultaneously in contact with several neighbours. Nevertheless, to demonstrate eventually that a particular type of pattern is a highly probable consequence of a particular type of interaction will require either a formal mathematical treatment or a simulation using some model system. In either case, a prerequisite is that the extent and variability of the changes in motion that occur when two cells collide is known for the types of cells and for their initial dispositions and motions on entering the collision. With this in mind, we have tried here to give a reasonably complete quantitative description of the contact responses and this includes a preliminary exploration of aspects of the contact responses other than contact inhibition.

**MATERIALS AND METHODS**

**Tissue culture**

Primary explants of chick heart ventricle from 7-day-old embryos were cultured at 37°C on acid-cleaned no. 1 glass coverslips in a hanging drop of Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and 100 μg ml⁻¹ each of penicillin and streptomycin (all from Flow Laboratories Ltd). After 24 h the explants were removed to leave a sparse culture of chick heart fibroblast (CHF) cells. These cultures were then seeded with a suspension of cells from the mouse fibrosarcoma FS9, harvested by gentle pipetting from 4-day-old explant cultures in Falcon flasks. The mixed cultures were allowed to settle for 2–4 h before use.

**Time-lapse recording of cell behaviour**

Cell behaviour was recorded at 37°C using Zeiss (W. Germany) phase-contrast microscopy equipment. The use of a silicon-intensified target camera (R.C.A. TC 1030HX) allowed video recordings to be made at very low light levels at time-lapse ratios of 40:1 (National Panasonic NV 8030 video tape recorder) or 150:1 (R.C.A. TC 3550X video cassette recorder). Cell position data were obtained by tracing onto acetate sheets placed directly in contact with a 24-inch monitor screen using a fixed sight to eliminate parallax errors. The selection of cells for analysis was governed by the requirements that they should remain within the imaged area for at least an hour and that either they should be free from contact with other cells or they should collide with just one other cell.

**Data storage**

For each cell, the bulk of the data consisted of a representation of the path of the moving cell drawn onto acetate sheet or paper. Each path consisted of an ordered series of points representing the sequential positions of a prominent nucleolus within the cell's nucleus at 5-min intervals. Additional data included the scale factor of each drawing. For pairs of colliding cells, the time of the onset of collision was determined before drawing the cell paths by noting the frame which contained the first apparent coincidence of the cell margins; the position of this apparent contact was also noted. The position of each cell at this time was taken as one of the recorded points and the
Analysing collisions between cells

Fig. 1. Three successive positions of a hypothetical cell at times $t-\delta t$ (outline on left), $t$ and $t+\delta t$ (outline on right). The angle marked $\theta$ indicates the change in direction of the cell. See the text for further details.

remaining points from both cell paths were subsequently drawn on the same acetate sheet in order to preserve their relative positions. These positional data were input to a PDP 11/44 computer using a digitizing tablet (Summagraphics bitpad).

Analyzing changes in motion

Each cell's change in motion at a particular time $t$, which coincided with the time of initial contact in the case of collisions, was measured by taking the difference between its motion just before $t$ and its motion just after $t$ as illustrated in Fig. 1. The displacement of the cell during the time interval of length $\delta t$ immediately before $t$ is represented by the vector $A$ and its displacement during the time interval of the same length immediately after $t$ is represented by the vector $B$. By shifting vector $A$ to a new position $A'$ so that its origin is the same as that of vector $B$ as shown in the figure, the triangle rule may be used to obtain the vector difference $B-A$ that represents the change in motion of the cell. Inevitably, the choice of magnitude for the time interval $\delta t$ is fairly arbitrary and for this reason we used three values in this study, 10 min, 15 min and 20 min, which range from the smallest value over which reasonably accurate estimates of $B-A$ can be made to a value that is about twice as long as the mean duration of a collision.

If one type of cell generally moves more slowly than another then, other things being equal, its changes in motion will be smaller. In order to make a comparison of the two cell types that ignores any difference in speed, we measured the changes in motion relative to the speed by dividing each vector $B-A$ by an estimate of the cell's speed, obtained by summing the magnitudes of $A$ and $B$. This is equivalent to scaling the diagram for each cell so that the sum of the lengths of $A$ and $B$ is equal to unity. This procedure had another important advantage in that, after scaling, the horizontal and vertical components of $B-A$ had distributions that closely approximated the normal distribution. Without scaling, the distributions for the cells in this study showed a significant positive excess of kurtosis. A consequence of this was that the
distributions had 'straggling tails' which, strictly speaking, invalidate the use of the $t$ test.

The data on changes in motion from each sample of cells were summarized by means of a diagram. We call such diagrams **accelograms** because the change in motion vectors may be thought of, loosely, as acceleration vectors (see Dunn & Paddock, 1982, for discussion); and for convenience we will refer to the scaled change in motion vectors as **accelerations**. These diagrams form useful 'fingerprints' of the characteristic behaviour of different cells under different circumstances, particularly when they are synchronized with events such as collisions.

Each accelogram was constructed from a sample of accelerations, all obtained using the same value of $\Delta t$, by gathering them together so that they had a common origin at the centre of a circle of radius 1. Each sample consisted either of the successive accelerations of a group of freely moving cells of one type or of the accelerations synchronized with a particular type of collision. In the latter case, only one acceleration was provided by each collision and the samples were therefore very much smaller than those from freely moving cells. A circle served to contain each accelogram since no acceleration can have a magnitude of greater than 1. (This is a result of scaling but note that these accelerations are not comparable with those described by Dunn & Paddock (1982), which were not scaled.) Each acceleration was oriented so that its corresponding displacement vector $\mathbf{A}$ would have pointed horizontally to the right had it been included in the diagram and so the accelograms show the change in motion in relation to the initial direction of motion. Fig. 2 shows

![Fig. 2. The accelogram of the hypothetical cell shown in Fig. 1.](image)
Analysing collisions between cells

an accelogram containing only the one acceleration from Fig. 1. The system of coordinates superimposed on the accelogram allows the original behaviour of any cell to be reconstructed from reading the position of the arrowhead of its acceleration vector. Thus the cell in Fig. 1 has changed in direction by 60° to the left (read from the straight line coordinates) and has increased its speed by a factor of 1.5 (read from the circular coordinates).

Measuring the free behaviour of cells

The accelograms of freely moving cells of both types were used to compare aspects of the free behaviour between types but, because they are compensated for speed differences, they are not very suitable for this purpose and were constructed for different reasons as will be described in the next section. A more meaningful comparison of free locomotion was obtained by fitting a theoretical model of random motion to data consisting of the mean square displacements of the cells over time intervals of variable length T according to the method of Dunn (1983). This method yields estimates of two parameters of motion: speed (S) and persistence in direction with time (P); and these can be used to derive an estimate of a third interdependent parameter (rate of diffusion (R)), which is a simple multiple of the augmented diffusion constant of Gail & Boone (1970). The fitted function is given by:

Expected square displacement (during time $T$) = $2S^2[PT - P^2(1 - \exp(-T/P))]$

and $R$ is obtained as:

$R = 2S^2P$.

Measuring contact inhibition

Contact inhibition occurs when a cell's continued progress in its original direction is less than it would have been had the collision not occurred. Of course, it is impossible, for any individual colliding cell, to know how it would have moved if there had been no collision and this can be estimated only by observing a sample of similar cells that do not collide. Strictly speaking, therefore, 'contact inhibition' is undefined for a single event, since it describes a statistical phenomenon that can be measured only by sampling.

It will be apparent from Fig. 1 that the component $C_x$ of the vector $B - A$ represents the scalar difference between how far the cell has actually progressed in the direction of $A$ and how far it would have progressed had there been no change in motion. In the particular example shown in Fig. 1, the value of this component is negative (i.e. it lies in a direction opposite to that of $A$), indicating that after collision the cell makes worse progress in the direction of $A$ than it did before collision, even though its speed is increased after the collision. For measuring contact inhibition we used the horizontal components of the scaled accelerations instead of the raw $C_x$ components since these are distributed much more nearly normally.

In order to measure contact inhibition, we subtracted the mean scaled value of $C_x$ obtained from a sample of freely moving cells, from its mean scaled value from a
sample of equivalent colliding cells. The significance of this difference and its 90% confidence interval were obtained using a two-tailed unpaired *t* test of the null hypothesis that the difference is zero. This measure may be described as the mean longitudinal component of acceleration due to collision and we will refer to it as the *contact acceleration index*. For each cell type and each type of collision, we obtained three values corresponding to the three different values of \( \Delta t \). A significantly negative value indicates the presence of contact inhibition (i.e. a colliding cell shows an excess of longitudinal deceleration over a free cell) and the meaning of a significantly positive value will be discussed later.

**Measuring the lateral acceleration due to collision**

The scaled acceleration vectors contain more information about the locomotory response to collision than is necessary for determining contact inhibition. By considering only the component in the direction of vector \( \mathbf{A} \), we are discarding half of this information. The component at right angles to this, \( C_y \) in Fig. 1, contains information about the lateral displacement of the cell, relative to its speed, that occurs during collision and it is probable that this is of further significance in determining the overall characteristics of population behaviour. The mean of this new component does not, however, indicate how much lateral displacement has occurred in a sample of accelerations, only whether there is a bias in displacing more to one side than the other. To assess how much lateral displacement has occurred in a sample of collisions, it is necessary to measure the statistical dispersion of these components and, to assess how much is due to collision, the collisions must be compared with free cells of the same type.

By dividing the variance in the scaled \( C_y \) components from a sample of colliding cells by their variance from a sample of freely moving cells, we arrived at a measure, which we call the *lateral displacement ratio*. The significance of this measure was determined by testing the null hypothesis that the variance ratio is equal to unity using Fisher's *F* distribution. A variance ratio significantly greater than unity indicates that collision increases the lateral displacement, whereas a value significantly less than unity indicates that the lateral displacement is decreased by collision. For each cell type and each type of collision, we obtained three values corresponding to the three different values of \( \Delta t \).

**Analysing the relations between colliding cells**

The above analysis takes account only of what happens to a colliding cell in relation to its own movement before the collision and to the type of cell with which it collides, but takes no account of the speed and direction of this other cell or of which part of each cell first makes contact with the other. Of course, these features of a collision could all have a bearing on the overall behaviour of populations of cells and any relationship that we find between these features and the outcome of a collision could tell us more about the mechanisms of the contact responses.

The geometrical relations between two colliding cells up to the moment of collision were quantified as a set of variables, which we will call the *predictor* variables. The
outcome of the collision was quantified as another set of variables, the outcome variables. In order to discover how well these relations between cells before collision determine the behaviour of each cell subsequent to collision we used a multivariate statistical technique known as canonical correlation analysis. This technique is too complicated to describe here in detail but any good textbook on multivariate statistics, including Harris (1975) or Mardia, Kent & Bibby (1979), should give a good account. Briefly, different weights are tried for each variable until the maximum Pearson correlation (called the maximum canonical correlation) is obtained between the two sets of sums of weighted variables. The weights are then called the canonical coefficients for the predictor and outcome measures, respectively. The procedure generates as many canonical correlations and sets of canonical coefficients as there are variables in the smaller of the two sets. Each of the subsequent canonical correlations represents a combination of predictor variables and a combination of outcome variables that are as highly correlated as possible, subject to the condition that they are uncorrelated with any of the previous combinations. And so the set of canonical correlations describes several uncorrelated ways in which the variables relate to each other and each may be tested separately for significance by referring to tables (Harris, 1975) or charts (Morrison, 1978). In the case of the maximum canonical correlation, this is a test of the null hypothesis that the set of outcome variables is independent of the set of predictor variables.

By considering the outcome for only one cell of a colliding pair at a time (which we will call the principal cell), and for one value of $\delta t$, the geometrical data available from a single collision may be summarized as the six positions marked by dots in Fig. 3. Taking $t$ as the time when the margins of the cells first coincide, the positions of the principal cell are marked for times $t - \delta t$, $t$ and $t + \delta t$; the positions of the other cell are marked for times $t - \delta t$ and $t$. The remaining position marked is the marginal contact point, where the margins of the cells first coincide. We took the final displacement vector (B) of the principal cell as the outcome of the collision and the three vectors $P_1$, $P_2$ and $P_3$ as possible predictors of the outcome. These three predictor vectors describe aspects of the relationship between the cells up to the moment of collision and contain information about the position of the marginal contact point ($P_1$), the position of the other cell at time $t$ ($P_2$) and the initial displacement of the other cell ($P_3$). All vector directions were referred to the direction of initial displacement of the principal cell, which was not itself used as a predictor. In order to convert these vectors into variables for canonical correlation analysis, one variable was formed by the magnitude of each vector and two more variables consisted of the sine and cosine of the direction of each vector. By thus using two variables to describe the direction of each vector, we avoided the problem of a sharp discontinuity, which would have occurred if we had used an angular measurement. The large quantity of data generated by the canonical correlation analysis is presented as a very condensed summary of some of the more interesting relations between variables that were discovered.
Fig. 3. The three successive positions of the cell shown in Fig. 1 together with two successive positions, at times $t-\Delta t$ and $t$, of the cell with which it collides. Collision starts at time $t$. See the text for further details.

RESULTS

Cell behaviour in mixed cultures

Despite the different morphologies of CHF and FS9 cells (Fig. 4), the two cell types show quite similar locomotory behaviour when moving freely across a planar glass substratum. Collisions between pairs of CHF cells (CHF/CHF collisions) showed the classical pattern of behaviour associated with the contact inhibition response as described by Abercrombie & Heaysman (1953). Collisions between pairs of FS9 cells (FS9/FS9 collisions) also showed some contact paralysis and contact retraction, features normally associated with contact inhibition. These collisions were rather rare, perhaps because there is a much reduced spread area of leading lamella in these spindle-shaped cells and so they tend to pass each other more often without collision.

As we discussed in the Introduction, it is not these homotypic collisions but the heterotypic collisions between pairs of cells of different type that Abercrombie and colleagues have proposed are crucial in determining whether a population of cells of one type invades another of different type. Consequently, we will examine the collisions between CHF and FS9 cells in greater detail. We will distinguish between a CHF/FS9 collision, where we are describing the behaviour of a CHF cell in heterotypic collision, and an FS9/CHF collision, where we are describing the behaviour of the FS9 cell.

With the CHF/FS9 collisions, the response was generally more marked and less variable than with CHF/CHF collisions and showed a remarkably consistent series of events. Ruffling in the leading lamella of the CHF cell ceased immediately on
contact with the FS9 cell. This inhibition of ruffling and protrusion was very localized to the region of contact and the region of paralysed leading lamella eventually retracted towards the CHF nucleus (Fig. 4). Even a transient contact with a fine filopodial protrusion of the FS9 cell was usually sufficient to initiate this response in the CHF cell. This response occurred in 77 of the 79 collisions observed (not all were analysed). The mean time from the onset of collision to the end of the retraction in 22 collisions was $10.1 \pm 0.7$ min (standard error of mean). The

Fig. 4. Time-lapse film sequence of a chick heart fibroblast (lower cell) colliding with an FS9 cell. The elapsed time (min) is given. Bar, 10 $\mu$m.
overall impression given by these collisions is that the CHF cell moves out of the way
to make room for the encroaching FS9 cell. In nine of the 79 collisions the CHF cell
entered mitosis soon after the first contact and this seemed much too frequent to be a
random event, although its significance was not tested.

In the case of FS9/CHF collisions, the response was again generally less variable
than with the homotypic equivalent (FS9/FS9 collisions) but in this case the
response was also less marked. Indeed, the only obvious response of the FS9 cells to
collision was an apparent increase in the amount of protrusion of the leading lamella
in the region of contact (Fig. 4) which was not obvious in the homotypic collisions.
This occurred in 75 of the 79 collisions observed and in 65 of these it was
accompanied by a rapid spreading of the leading lamella of the FS9 cell between the
CHF cell and the substratum. In the remaining 10 collisions, the leading lamella
continued to spread, even though the CHF cell had moved away. When the initial
contact was made via a filopodial process of the FS9 cell, the increased protrusion
was seen as a rapid thickening of the filopodium, which passed from the cell body
outwards.

The sample of heterotypic collisions studied form a classical example of non-
reciprocal contact inhibition as described by Heaysman (1970). Of the 79 collisions,
only two departures from non-reciprocal contact inhibition occurred. In each of
these two cases the FS9 cell rounded up on collision and was transported onto the
dorsal surface of the CHF cell, where it remained until it apparently pulled itself
back onto the substratum by contracting a tenuous retraction fibre.

Analysis of freely moving cells

Fig. 5 is an accelogram of 213 accelerations from 18 freely moving CHF cells and
Fig. 6 of 412 accelerations from 31 freely moving FS9 cells, both using a $\delta t$ of
15 min. In these two figures the acceleration arrowheads are represented by small
crosses, their common origin being the + symbol in the centre of the accelogram.
The open circle in each figure represents the arrowhead of the mean acceleration
vector. Allowing for the fact that there are fewer accelerations from the CHF cells,
the two distributions look remarkably similar. They are both symmetrical about the
central horizontal axis (the vertical or $C_y$ components of the accelerations have means
that are not significantly different from zero: CHF, $-0.003 \pm 0.002$ s.e.m.; FS9,
$-0.005 \pm 0.013$ s.e.m.). This is not surprising since any significance here would
indicate that cells tend to turn predominantly either to the left or to the right.

They are not symmetrical, however, about the central vertical axis. (The
horizontal or $C_x$ components of the accelerations have means that are nearly the same
and both are significantly less than zero at the 0.1 % level on a two-tailed $t$ test: CHF,
$-0.124 \pm 0.023$ s.e.m.; FS9, $-0.127 \pm 0.016$ s.e.m.) This bias does not indicate that
the cells are slowing down significantly; it indicates only that further progress in the
original direction of travel is reduced in freely moving cells because of random
changes in direction. Despite this asymmetry, the $C_x$ and $C_y$ acceleration components
for both cell types show no significant deviations from normal distributions in either
skewness or kurtosis (see chapter 3, Snedecor & Cochran, 1967, for tests used). The
Analysing collisions between cells

Analysing collisions between cells

accelograms of the two cell types also match each other very closely in the standard deviations of the $C_x$ and $C_y$ acceleration components, which have the following values:

<table>
<thead>
<tr>
<th></th>
<th>CHF</th>
<th>FS9</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_x$ components</td>
<td>0.338</td>
<td>0.324</td>
</tr>
<tr>
<td>$C_y$ components</td>
<td>0.285</td>
<td>0.262</td>
</tr>
</tbody>
</table>

The dispersion of the components is summarized in each accelogram as a broken ellipse that encloses the 90% confidence interval for new single values from the hypothetical parent bivariate distribution.

In order to obtain measures of the absolute motility of the two cell types, the mean square displacements of the two cell types are plotted against time in Fig. 7, together with least-squares fits of the theoretical random motion model. The two cell types again appear to be remarkably similar in their free behaviour as is shown by the values of the three parameters of motility:

<table>
<thead>
<tr>
<th></th>
<th>CHF</th>
<th>FS9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed ($S$) ($\mu$m min$^{-1}$)</td>
<td>1.94 ± 0.29</td>
<td>1.75 ± 0.20</td>
</tr>
<tr>
<td>Persistence in direction ($P$) (min)</td>
<td>24.5 ± 11.8</td>
<td>24.2 ± 7.7</td>
</tr>
<tr>
<td>Diffusion rate ($R$) ($\mu$m$^2$ min$^{-1}$)</td>
<td>187 ± 74</td>
<td>149 ± 51</td>
</tr>
</tbody>
</table>

Homotypic contact acceleration

Fig. 8A is the accelogram of 40 CHF cells colliding with each other in pairs and Fig. 8B is the same for 28 FS9 cells, both accelograms using a value for $\delta t$ of 15 min. The 90% confidence ellipse for the equivalent freely moving cells is marked on each diagram and the arrow at the bottom of each diagram shows how the horizontal component of the mean of each sample of accelerations differs from that of the equivalent freely moving cells. In other words, the arrows are graphical representations of the contact acceleration indices.

These homotypic collisions again show some similarity in the behaviour of the two cell types. The upper half of Table 1 shows the contact acceleration indices for the homotypic collisions together with their 90% confidence intervals and significance levels. There is a significant indication of contact inhibition in the CHF/CHF collisions. The values for FS9/FS9 collisions indicate that they have about half of the contact inhibition of the CHF/CHF collisions but this is not quite significantly different from zero at the 10% level for all three values of $\delta t$. The lack of significance here could well be due to the small size of the sample of FS9/FS9 collisions, especially since all three values approach the 10% significance level. Note that the value of the index does not appear to be very sensitive to the value of $\delta t$ for both types of collision.
Heterotypic contact acceleration

Accelograms for 44 heterotypic collisions, with a $\delta t$ value of 15 min, are shown in Fig. 9 and the longitudinal contact acceleration indices for heterotypic collisions are given in the lower half of Table 1. Note that the CHF cells are very strongly contact inhibited when colliding with FS9 cells and that, as with the homotypic contact inhibitions, their indices are remarkably constant for different values of $\delta t$. The FS9 cells, on the other hand, are not contact inhibited at all when colliding with CHF cells. In fact, their index is significantly positive at the 5% level for $\delta t = 15$ min and significantly positive at the 1% level (almost reaching the 0.1% level) for $\delta t = 20$ min, indicating the presence of a different phenomenon, which, following Curtis (1967), we will call contact promotion of locomotion. In the case of contact promotion, the value of the index is not stable for different values of $\delta t$, as it is with the three contact inhibitions, but approximately doubles in magnitude for each increase in $\delta t$ value. This suggests that contact promotion is not simply a reversal of the mechanism of contact inhibition, but that the promotion reaction develops over a longer time scale.

Differences between homotypic and heterotypic collisions

In order to establish whether a particular cell type behaves differently according to the type of cell it collides with, we examined the differences between homotypic and
heterotypic collisions by comparing the samples of $C_3$ components in unpaired two-tailed $t$ tests. For CHF cells at $\delta t = 15$ min, the difference between the indices for homotypic and heterotypic collisions is $-0.183 \pm 0.123$, which is almost significant at the 1% level ($t = 2.48$, d.f. 86) and for FS9 cells the difference is $+0.196 \pm 0.116$, which is significant at the 1% level ($t = 2.81$, d.f. 70).

**Lateral displacement ratios**

The lateral displacement ratios for the four types of collision and the three values of $\delta t$ are given in Table 2. Without exception the variance ratios for CHF cells are greater than 1.0 whereas those for FS9 cells are less than 1.0. With the CHF/CHF collisions none of these ratios is significant, however, and with the CHF/FS9 collisions only two are significant at the 10% level. This indicates rather surprisingly that when a CHF cell collides with another, its lateral displacement is not significantly greater than would have been the case in the absence of collision; even when it collides with an FS9 cell, the increase in lateral displacement is only barely significant, although with these collisions the contact inhibition response is very marked. The significances of these ratios for FS9 cells are all greater than any for CHF cells and the FS9 cells show a considerable reduction in lateral displacement as a result of collision. It is interesting to note that this reduction in lateral displacement is exceptionally significant for the FS9/CHF collisions that demonstrate contact promotion. Although these lateral displacements are measured relative to the speed
of the cells, differences between the mean speeds of the samples of freely moving cells and colliding cells are small and do not nearly account for these significant differences in lateral displacement.

**Relations between cells as predictors of the outcome of a collision**

The results of the canonical analysis are quite complicated since with three outcome variables and nine predictor variables the analysis generates three canonical correlations each with nine canonical coefficients for the predictor and three for the outcome variables. One problem is interpreting what the coefficients mean. In fact with a large number of intercorrelated predictor variables, as we have, interpretation of the individual coefficients is practically impossible since their values are unstable and change considerably as a result of deleting random small sections of the data set. We can, however, look at the significance of the canonical correlations. Table 3 shows the maximum canonical correlation for each of the four types of...
Analysing collisions between cells

Diagram A

Diagram B
Table 1. Contact acceleration index

<table>
<thead>
<tr>
<th></th>
<th>$\delta t$</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homotypic collisions</td>
<td>CHF/CHF</td>
<td>-0.152 ± 0.091**</td>
<td>-0.200 ± 0.096***</td>
<td>-0.216 ± 0.111***</td>
</tr>
<tr>
<td></td>
<td>FS9/FS9</td>
<td>-0.091 ± 0.097</td>
<td>-0.091 ± 0.104</td>
<td>-0.101 ± 0.109</td>
</tr>
<tr>
<td>Heterotypic collisions</td>
<td>CHF/FS9</td>
<td>-0.318 ± 0.083***</td>
<td>-0.383 ± 0.093***</td>
<td>-0.387 ± 0.107***</td>
</tr>
<tr>
<td></td>
<td>FS9/CHF</td>
<td>+0.048 ± 0.076</td>
<td>+0.105 ± 0.084*</td>
<td>+0.199 ± 0.099**</td>
</tr>
</tbody>
</table>

Values are given ± 90% confidence limits; significance: *$P<0.1$; **$P<0.01$; ***$P<0.001$.

collision and each of the three values of $\delta t$. The apparent discrepancies in the assignment of significances arise because different sizes of samples are available for different values of $\delta t$ and different types of collision, the actual number of collisions in each sample being given in the table.

These canonical correlations are largely significant (even the two entries not assigned significance are significant at the 10% level). This confirms that the details of the relations between cells up to the time of collision do have a significant bearing on the outcome of collision in all these cases. Note also that the canonical correlations are not trivially due to a correlation between the initial and final displacements of the cell of interest since its initial displacement, although used to orient the data, is effectively ignored as a predictor vector. In fact the initial magnitude of displacement is positively correlated (Pearson’s $r$ value) with the final magnitude of displacement in all 12 cases, indicating that the colliding cells show some persistence in speed, but this correlation is excluded from the canonical analysis. We cannot draw any further firm conclusions from this overall canonical analysis but it is interesting to note that the outcome for the FS9/CHF collisions, the ones in which we detected contact promotion, appears to be the most predictable.

In the rest of this paper we will summarize briefly an attempt to use the technique of canonical correlation analysis to explore the data in more detail in order to find out which relations between cells are important. The problem of interpreting individual canonical coefficients can be overcome to some extent by considering much smaller sets of variables. Here we will consider only one predictor vector at a time. This means that the technique is no longer able to detect whether combinations of predictor vectors are relevant to the outcome but the canonical coefficients are much more stable.

**CHF/CHF collisions**

With the CHF/CHF collisions, the three canonical correlations for predictor $P_1$ are all very significant ($P<0.01$) and in two of the three cases ($\delta t = 10$ min, $\delta t = 15$ min) $P_1$ is the best of the three predictors. Therefore it seems likely that the...
Analysing collisions between cells
position and direction of the marginal collision point, in relation to the direction of movement of the principal cell on approaching collision, is of prime importance in determining the outcome of the collision. Predictor $P_2$ also gives very significant maximum canonical correlations in all three cases but this could well be due to the high Pearson correlations between $P_1$ and $P_2$. Predictor $P_3$ appears to be relatively ineffective for these collisions, reaching only the 5% level of significance in one case.

**FS9/FS9 collisions**

These collisions are unique in that $P_3$ is the most effective predictor in two of the three cases and reaches the 1% level of significance for $\delta t = 20$ min, which suggests that the behaviour of the principal cell after collision is determined to some extent by the displacement vector of the other cell on entering collision. The other two predictors appear to be less effective; although $P_1$ is the best predictor for $\delta t = 10$ min, its significance reaches only to the 5% level.

**CHF/FS9 collisions**

The CHF/FS9 collisions give very significant maximum canonical correlations with all three predictors but $P_2$ is the best in two cases ($\delta t = 15$ min, $\delta t = 20$ min). $P_1$
being the best in the third. It seems, therefore, that the behaviour of the CHF cell is determined to some extent by the position of the FS9 cell, although other relationships are probably present.

**FS9/CHF collisions**

With predictor $P_1$, the FS9/CHF collisions give the largest and most significant of all the canonical correlations for each value of $\delta t$. So the final velocity vector of the FS9 cell appears to be determined by the position of the marginal contact point. It is interesting, since these are the only collisions that do not show contact inhibition, that the relationship is so significant here.

**Interpreting the canonical relationships**

So far we have found that with all four types of collision the relations between the two cells up to the time of collision determine to some extent the outcome of the collision. In some cases there is a more or less clear indication of which relations between the two cells are the most important. Finding out what these relations are entails interpreting the canonical coefficients. Unfortunately, even with the reduced numbers of variables these coefficients are not easy to interpret and only one simple relationship emerges. Not surprisingly, this is given by the strongest canonical correlation shown by the FS9/CHF collisions using predictor $P_1$. The first canonical relationship is highly significant and indicates mainly that the sines of the directions of $P_1$ and $B$ are positively correlated. The second is also highly significant and indicates that their cosines are also positively correlated. In fact the two respective Pearson correlations are $+0.63$ and $+0.52$. The third canonical relationship is not significant, which indicates that these relationships between the directions of $P_1$ and $B$ are the only ones present in the total canonical relationship. Nevertheless, the relationship is strong and it can be calculated that the canonical correlation accounts for 62% of the variation in the data.

The scattergram of angles in Fig. 10 shows exactly how these two directions are related. It is clear from the figure that the two angles tend to be equal, but note that the angles are generally quite small and so their relationship might easily be missed in a qualitative study. This indicates that, after collision with a CHF cell, the FS9 cell tends to move towards the marginal contact point. Assuming a causal relationship, we can speculate that the change in direction of the FS9 cell is largely determined by the position of its point of first contact with the CHF cell.

Although $P_1$ is the best overall predictor for the three other types of collision, which all show contact inhibition responses, it is perhaps surprising that no clear relationships emerge even from the strong contact inhibition shown by the CHF/FS9 collisions. However, there is a pattern revealed by the Pearson correlations between $P_1$ and $B$. Examining just the three correlations between magnitudes, between sines and between cosines of the two vectors, at three different values for $\delta t$, gives nine correlations in all for each type of collision. In the cases of both the CHF/FS9 and the FS9/FS9 collisions, all 18 correlations are negative, with
values around $-0.25$, indicating that the cells tend to move away from the marginal contact point, but these negative correlations are not nearly as strong as the positive ones shown by FS9/CHF collisions. The CHF/CHF collisions still show no clear relationship, the correlations being very small except for those between sines, which are around $+0.20$. So with these CHF/CHF collisions, although the canonical correlations reveal that the direction of movement after collision is very significantly related to the position of the marginal contact point, the exact nature of this relationship is not easily discernible.

**DISCUSSION**

In this paper we have concentrated on the locomotory aspects of cell behaviour, that is on how cells change their positions with time. Other aspects of cell motility, such as the paralysis of ruffling activity, which often accompanies cell collision, are relevant to the understanding of pattern formation by populations only insofar as they may be relevant to understanding the mechanisms of the locomotory responses. Our long-term aim is to describe the free locomotion of cells and their locomotory response to collision in such a way that it ought to be possible, eventually, to understand the mechanisms of pattern formation in mixed cultures in terms of the local interactions between cells.

Much remains to be done before this ambition can be realised but, in the meantime, we have introduced here some quantitative methods that will enable the
current hypotheses of the relationships between contact responses and population behaviour, notably those of Abercrombie, Heaysman and their colleagues, to be tested more critically than previously. These new methods, applied here to mixed cultures of two cell types, have already detected that malignant cells can show a reversal of the contact inhibition response when colliding with normal cells. Furthermore, we have discovered aspects of the contact responses, other than contact inhibition, that seem likely to have an additional relevance for pattern formation. Taken overall, the most important thing to emerge from this study is that, despite the fact that these malignant cells do not appear to respond very much when colliding with the normal cells, the measurements reveal a very significant response. When more malignant cell types have been observed, measurements of their responses may lead to a revision of the current view that malignant cells generally fail to respond to collision with normal cells.

The first conclusion to be drawn from the analysis is that the two cell types studied here showed a remarkable similarity in their measured locomotory behaviour when moving freely. This was apparent not only in the accelograms of freely moving cells but also in the analysis of cell displacements over different time intervals. The two parameters of locomotion obtained by the last method, speed and persistence in direction, together form a reasonably complete probabilistic description of the pattern of translocation of freely moving cells (Dunn, 1983) and we feel it is safe to conclude, therefore, that any differences in population behaviour between the two cell types are caused entirely by their different responses to collision, at least in the simple cultures studied here.

The homotypic contact acceleration responses of the two cell types also appear to be similar in character; both cell types have negative indices, indicating contact inhibition, although the response is only about half as strong with the malignant cells and does not reach an acceptable level of significance in this small sample of homotypic malignant collisions. Abercrombie (1979) makes the general point that, although much has been made of the assessment of homotypic contact inhibition as an indication of invasiveness, the relationship between the two is by no means clear. It seems evident that the variation in homotypic contact inhibition among malignant cell types is much wider and has a lower median than the variation found in normal cells. Our measured values are consistent with the hypothesis of Abercrombie and his colleagues that contact inhibition leads to a general migration from regions of high cell density to regions of lower density. Abercrombie (1979) reports that CHF cells migrate radially from an explant with a high outward velocity whereas the outward drive is not so powerful with FS9 cells. It should be noted that our method for measuring contact inhibition could form the basis of a critical test of this hypothesis since it incorporates a comparison of the collision behaviour with the free behaviour. In this respect it can be considered to be a measure of effective contact inhibition in the sense in which Abercrombie & Turner (1978) considered that the homotypic contact inhibition response of S180 cells is ineffective in producing an outward drive.

When the contact acceleration index was measured in heterotypic collisions, each cell type showed a significantly different behaviour from its own homotypic response.
with the result that there was a very marked difference between the two heterotypic responses. The contact inhibition response of CHF cells becomes much stronger when colliding with FS9 cells, whereas the heterotypic response of FS9 cells appears to be a reversal of their homotypic response, with the proviso that it also appears to have a different time course in relation to the time of initial contact. Abercrombie (1979) reported that, with confronted populations of the two cell types, the CHF cells are almost totally obstructed on encountering the FS9 population, whereas the FS9 cells are only partially obstructed by the CHF cells. Our measured values are therefore consistent with the hypothesis that a low invasion is associated with a high level of heterotypic contact inhibition, and vice versa, with these two cell types. But what is interesting is that the FS9 cells show contact promotion on colliding individually with CHF cells, whereas the FS9 population is somewhat obstructed by the fibroblasts. Therefore, the consequences of contact promotion for behaviour in dense culture, whatever they may be, do not seem to include a facility for FS9 cells to move through CHF populations more readily than they can occupy free space on the substratum.

Perhaps the most significant conclusion to be drawn from our analysis of heterotypic collisions, with regard to the mechanism of malignant invasion, is that the behaviour of these fibrosarcoma cells can no longer be regarded merely as a suppression or failure of the contact inhibition response. Taken as a whole, the data indicate that the FS9 cells consistently show an active response in these collisions that is quite different from their behaviour in the absence of collisions. In fact, the significance of this difference is greater than with any of the three other combinations of cell types in the cases of the lateral displacement and canonical correlation analyses: a surprising result in view of the subjective impression that the fibrosarcoma cells do not appear to respond very much to collision with normal fibroblasts. An intriguing possibility is that all three aspects of this FS9/CHF response that have been discovered here – the contact promotion, the reduced lateral displacement and the tendency to turn towards the marginal collision point – could function to increase the efficiency of infiltration of FS9 cells into CHF territory over what would be expected from a simple failure of the FS9 cells to respond to collision. Furthermore, the behaviour of the CHF cells in collisions with FS9, as opposed to homotypic collisions, appears to have changed in a manner that would tend to increase the invasiveness of the FS9 cells: CHF/FS9 collisions show a higher level of contact inhibition and a greater lateral displacement than CHF/CHF collisions. It will now be of interest to find how generally dispersed these properties are among other examples of collisions between invasive and normal cells.

In isolation, the measurements of lateral displacement index and the canonical correlation analysis do not suggest any new hypotheses of malignant behaviour to us. When more cell types have been studied, it may become clear, for example, that a reduced lateral displacement index is a characteristic feature of invasive cells in both homotypic and heterotypic collision or that the malignant heterotypic response generally shows an active movement towards the marginal contact point in contrast with the normal heterotypic response. These measurements do not contradict the
Analysing collisions between cells

187

general hypothesis of Abercrombie, Heaysman and colleagues that changes to the normal heterotypic contact inhibition response are the most important single factor in determining invasiveness, at least in culture, but there is little doubt that measurements of contact inhibition alone do not present a complete picture of the general collision response for the purposes of predicting population behaviour or for clarifying the nature of the biological mechanism of the response.

G.A.D. is a member of the scientific staff of the Medical Research Council. S.W.P. was supported by a Cancer Research Campaign grant to G.A.D.

REFERENCES


(Received 18 July 1985 – Accepted 6 September 1985)