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CONTACT INHIBITION IN TISSUE CULTURE

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INTRODUCTION

The term contact inhibition was coined (1) for a particular form of locomotory behaviour shown by fibroblast-like cells (hereafter called simply fibroblasts) in culture on a plane surface. It was defined as the prohibition, when contact between cells occurred, of continued movement such as would carry one cell over the surface of another. Various problems in the regeneration of peripheral nerves had suggested that there must be some form of short-range cell interaction which limited cell movement; and Paul Weiss (2) had, with his idea of coaptation, formulated the general theory of such interactions. In an endeavour to identify the interactions, cultures were set up in such a way as to bring fibroblast-like cells into collision; and quantitative estimates of the speed and position of cells gave clear evidence that a directional inhibition of movement occurred when one fibroblast made contact with another. At first, the changes in displacement of the whole cell were all the data we had. Not until later (3) were details of the reaction of the individual cells added, making it possible to define contact inhibition in a more elaborate and specific way, as a process involving adhesion, paralysis and contraction. On the whole, however, it seems most useful to continue to define the term in its original broader sense as the directional restriction of displacement on contact, regardless of the way restriction is brought about. As will appear, the detailed reactions of fibroblasts may not be typical of all kinds of cells that undergo such contact inhibition. I should at this point emphasise that the phenomena of contact inhibition, broadly defined in this way, have been known for a very long time. Leo Loeb (4) described its operation amongst the amoebocytes of *Limulus*, and in the analysis of epithelial wound healing it has an even longer history (see 5, 6).

Another use of the term contact inhibition, where what is inhibited is replication, threatened confusion for a time (7). It is now however generally recognised that this kind of inhibition must be treated, at least for the present, as a distinct phenomenon, and that where necessary one should either specify contact inhibition of movement or contact inhibition of replication. The present review is almost entirely about the former.

I shall confine myself to cell behaviour in cultures. The implications of contact inhibition of movement, and of its absence, for cell behaviour in the organism have recently been discussed (8).

CONTACT INHIBITION AMONGST FIBROBLAST-LIKE CELLS

The main discussion of contact inhibition must be limited to fibroblasts; those from embryonic chick heart and neonatal mouse muscle have been the objects of most of the work. I shall only present an outline description of the phenomenon as it is seen in living cells, since there is little to add to previous descriptions.

When a chick heart fibroblast on a plane substrate is approaching another on a collision course, time lapse filming of the leading end (which consists of the ruffled membrane, more appropriately called the leading lamella (9)), with an interval of 2 seconds, and high resolution phase contrast, fail to show any change until after visible contact has been made. The effect of contact is often delayed for 10 to 20 minutes after the cells have apparently met, which allows time for some degree of superimposition of one cell on the other (3). When the collision is not between two lamellae, but is between the front end of one cell and the side of the other, the leading lamella of the former cell may pass some distance between the latter cell and the substratum. It has not been observed yet to extend over what may be called the dorsal surface (i.e. the surface of the cell exposed to the medium) of the cell whose side

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bore the collision (10). When, however, the collision is head-on, if there is an overlap, one leading lamella necessarily extends over the dorsal side of the other. Whether a leading lamella passes dorsally or ventrally depends presumably on the thickness of the edge of the cell with which it collides, and on the closeness of attachment of the two colliding edges to the substratum. The region of the leading lamella seems to be the most firmly adherent to the substratum of any part of the cell, and may be regarded as the locomotory organ of the cell (11, 12).

If contact inhibition is to occur, the first visible reaction of a leading lamella after it has made contact, it now seems to me, is a contraction. Earlier (3) we did not invariably observe it, but with higher resolution it seems to be always present. When well developed it shows itself as a retreat of the front end, drawing out strands of cytoplasm which are attached to the contacted cell, and often the lateral parts of the leading lamella which have not made contact become drawn into a smooth tension curve (3). The contraction, though local, clearly concerns a considerable part of the leading lamella behind the front edge, including part which is not involved in the overlap, and it often spreads sideways to involve a varying part of the leading lamella lateral to the region of contact. Weiss (11) described contact between the expanding pseudopods of two free mesenchyme cells as evoking a shock-like stoppage and contractile response in the colliding ends of the two cells, which deprives these ends suddenly of their motility and of their firm grip on the substratum.

Ruffling of the leading lamella in the region of contact ceases as soon as the contraction sets in. The visible intake of pinocytotic droplets also stops. Ruffling may, however, sometimes cease before the contraction, since it has not been observed while a leading lamella is passing between the substrate and the ventral surface of the cell collided with. Judging by the similar behaviour in the restricted space under an agar overlay, this cessation of ruffling when under the ventral surface of another cell is not part of the subsequent contact inhibition.

Another characteristic of the reaction of contact inhibition is adhesion. Adhesion between the colliding cells has evidently taken place before the contraction occurs. The contraction has never been observed to slide the contacting cells apart without some evidence of stretching of cytoplasm attaching the cells together. Though only one cell may apparently undergo the contraction (in a front to side collision), both may be distorted by it. An analysis with the electron microscope of this adhesion has not yet been made. It is known that tight junctions occur between guinea pig fibroblasts in culture (13), and the electrical coupling between those fibroblasts that have been tested (14) points to specially close junctions. But it is not known whether such adhesions are the ones manifested during the contraction of contact inhibition.

The effects of contact inhibition on cell displacement are of two quite different kinds. Firstly, the contraction may move the cells. Abercrombie and Heaysman (15) recorded that as cells made a contact they underwent a slight acceleration of movement. They suggested that a rapid but limited spreading of the area of contact drew the cells together, but it now seems fairly clear that what was observed was the effect of the contraction. The contraction may at times fail to draw the cells much together before it ruptures their mutual adhesion, so that the cells then jerk apart. This effect of the contraction was emphasised by P. Weiss (11). Differences in adhesion of the cells to substratum and to each other doubtless explain the different results of the contraction. The second effect on displacement, which follows the first effect, is the cessation of locomotion of the cell in the direction which has produced the collision; this of course is the defining characteristic of contact inhibition. There is no total inhibition of the cell's movement: if part of the leading lamella remains unaffected, it may lead the cell in a new direction, or a newly developed leading lamella may do the same. Where contraction has broken the contacting cells apart, the inhibited leading lamella may recover and re-extend, usually to suffer another inhibition.

It is to be noted that the phenomena characteristic of contact inhibition seem to occur from time to time in the leading lamellae of cells in the absence of any collision (3), but this has not been studied in detail.

Assay. Inhibition does not invariably follow contact between two fibroblasts. The possibility that its frequency may vary between cell

types or under different conditions makes it desirable to have a method of quantitative assay. Basically there is only one reliable method, and this is to observe a series of collisions and record their outcome. Clear-cut collisions can be engineered in reasonable numbers by making populations of cells move in a directed way towards a common meeting ground (as by confronted explants). To get adequate data in this way is however very tedious. Indirect methods using the distribution of cells in fixed cultures therefore have attractions, and several of the characteristic distributions that contact inhibition produces can in theory be used. None of these distributions are however known to be uniquely determined by the occurrence of contact inhibition, and so in practice the indices obtained from them cannot be unequivocally interpreted without supporting evidence of various kinds. The most convenient distribution of cells to use is monolayering on a plane substratum; contact inhibition should prevent one cell becoming superimposed on another. An "overlap index" can readily be derived by estimating the number of cell nuclei which may be expected to overlap if the cells in an area were distributed at random and comparing this with the number that do overlap; the overlap index is then the ratio of the observed number of overlaps to this expected number (1, 16, 17). Leaving aside the optical difficulties, cytoplasmic overlaps can be treated in a somewhat similar way: an estimate of the total *area* of cell overlaps, if distribution were random, can be obtained, and compared with the actual, since the total area of overlaps depends on the area of substratum, and the number and area of the cells within it, but not on the shape of the cells. It cannot be too strongly emphasised, however, that overlapping of cells can be produced in other ways than by their failure to become inhibited by contact (18). They may cross each other without making contact, for instance because of the presence of intercellular substances; or they may multilayer through drawing together into clumps (see below) or through mitosis. Conversely, the absence of overlaps does not point unequivocally to contact inhibition; one needs to know that collision has occurred.

Failures of inhibition. These occur in a proportion of collisions, and may provide clues as to the nature of the reaction. A new point seems to be emerging concerning failures between normal fibroblasts, though it is not yet securely established. Failures seem to occur predominantly, possibly solely, when the leading lamella of one fibroblast (the collider) runs into the side of another fibroblast (the recipient). In these cases, as already noted, the leading lamella of the collider may pass between the substratum and what may conveniently be called the ventral surface of the recipient cell, but now it is followed by the rest of the collider. Direct observation with the highest resolution phase contrast allows one to establish this by the plane of focus of the two cells. What may be the initial stages of such failures have been examined with the scanning electron microscope (10); in each case, the collider lay between the recipient and the substratum. On this basis it may be suggested that if conditions are such that the main body of a fibroblast adheres poorly to the substratum, the chances of another cell passing between it and the substratum are increased and likewise therefore the overlap index. Carter (19) found increased overlapping on a relatively nonadhesive substrate (methyl cellulose incompleted covered with a layer of palladium). We have found increased overlap index on certain agar substrata. These findings are reasonably explained by an increase in "underlapping." The variations in degree of monolayering between different kinds of fibroblasts, and with different mixtures of fibroblasts (17), may very well also be largely due to different degrees of adhesion to the substrate. The various states of cells and conditions of culture that increase the incidence of failure of contact inhibition will not be discussed at length here. Cells in mitosis or degeneration exert a diminished inhibitory effect on other cells which contact them. High population density increases the overlap index (20). Mixing heterogeneous fibroblasts changes their overlapping behaviour (17). Other conditions are discussed by Curtis (21).

Hypotheses as to mechanism. A rough subdivision of possible mechanisms was suggested in the original paper defining contact inhibition (1), and other varieties have been discussed since (8, 12). A list of those hypotheses that have seemed from time to time to apply to fibroblasts, with some comments on plausibility, is as follows.

(1) It may be that one cell acts simply as a mechanical obstacle to another. A leading

lamella, required to turn suddenly dorsally to surmount another cell, may be unable to bend, or may when so kinked be unable to act as a locomotory organ. This hypothesis has perhaps been too summarily dismissed in the past, and was not included in an early list of possibilities (12). It is true that fibroblasts can cross very rough terrain, but they find it difficult to negotiate a rectangular convexity, as may easily be observed at the edge of a coverslip. They might similarly be obstructed when the substratum bends through a sharply concave angle. A strong count against this hypothesis is that fibroblasts can readily surmount and move across the dorsal surfaces of other fibroblasts which have been fixed in glutaraldehyde with excellent morphological preservation (S. A. Cairns and R. A. Weiss, unpublished). The hypothesis also fails to account for the contraction that seems to be such a regular feature of contact inhibition between fibroblasts, and for the cessation of ruffling that goes with it.

(2) It may be that the firmness of the adhesion between colliding fibroblasts stops the movement. Adhesion is a conspicuous feature of contact inhibition, and it has been invoked as a cause of diminution of speed of movement (15). Curtis (21, 22) developed a theory by which the shear resulting from collision increased adhesion through decreasing surface viscosity. It is conceivable that the mechanism of fibroblast movement is such that tight adhesion of the anterior end of the leading lamella stops it. This hypothesis does not account for the contraction in contact inhibition.

(3) It may be that the dorsal surface of a fibroblast is totally nonadhesive to another fibroblast (in contrast to the edges) so that movement on it is impossible. Placed entirely on such a surface a cell would (gravity apart) round up as it does when suspended in fluid. Its effect would be to make the progress of a leading lamella impossible. Carter (23) adopted this hypothesis, and by postulating that ruffles are normally due to the advancing edge of a leading lamella losing its grip on the substratum, was able to account for the suppression of ruffling, since on another cell there is no grip to lose. The hypothesis does not however account for the contraction. It is also incompatible with the occurrence of typical contact inhibition when one cell is passing ventrally to another. Such a cell may undergo the sudden spasm of contraction even after its leading lamella has begun to emerge at the far side of the cell it is underlying. No change of substratum is involved.

(4) The dorsal surface may be adhesive enough to another cell to permit movement on it; but it may be less adhesive than the tissue culture substratum. Faced with a choice, a cell may be unable to detach itself from the substratum by means of its adhesions to the less adhesive surface of another cell. This hypothesis has a special attraction because it is closely related to Steinberg's well-known explanation (24) of the sorting out of dissociated cells through relative strengths of adhesion. I have discussed it in these terms in earlier reviews (25, 8). Carter (19) adopted this hypothesis, and he has been followed by Rubin (26). The hypothesis received apparent support from the increased overlap index found when cells are on a relatively non-adhesive substratum (19, 8). But this seems likely to have a different import: it is not that the low adhesion to the substratum makes it easier to climb on to the dorsal surface of another cell, but that it makes it easier to pass between another cell and its substratum. The hypothesis fails to account for the contraction and the suppression of ruffling. It is incompatible with the occurrence of contact inhibition in a cell that is passing ventrally to another. Curtis and Vardé (27) pointed out that it could not explain the monolayering that they found in sheets of fibroblasts suspended free from the substrate.

(5) Hypotheses (1) to (4) all depend on some sort of direct mechanical interference with the movement of one cell by the other. A different kind of hypothesis would make the inhibition and its concomitants a response to some signal received as the result of the collision. The signal may involve a diffusible substance. The approach of two cells towards each other can hardly fail to alter the diffusion gradients around the cells of substances consumed or produced in common by both cells, and hence alter concentrations at the cell surfaces facing each other. Such a concentration change might be the signal. It would of course need to become effective only at separations of no more than a micron or so, if the reaction is to appear to depend so invariably on contact. A point against a diffusible signal is that contact inhibition seems to occur less frequently when

one cell moves through the confined space ventral to another than when a cell undergoes head-on collision and remains freely exposed to the surrounding medium. It is perhaps preferable to suppose that the signal may depend not on the concentration of a diffusible substance, but on the interaction of "fixed" properties of the two contacting cell surfaces, say of their fixed charges, or through other means by which molecules interact.

A signal theory is favored by Gustafson and Wolpert (28), who suggest that contact inhibition is due to contact paralysis, the cessation of activity of an extending pseudopod which they believe occurs when it makes contact with any substratum, in this case with another cell. Wolpert and Gingell (29) suggested that the interaction of the fixed charges at the contacting surfaces, by altering the potential gradient through the membrane, might trigger off the contact paralysis. A signal hypothesis has always seemed the least objectionable of the possible hypotheses, because it makes it easy to explain the complexity of the response. In the light of the discussion so far it appears that the primary effect of the signal may be the spasm of contraction in the leading lamella. This could readily disrupt the mechanism of movement, particularly if it depended on periodic contractions and expansions (28, 30).

It is tempting to connect the signal with the establishment of electrical coupling between cells (31) and the tight junctions that go with them (32). If there is a connection, it seems more likely that the surface changes that produce the coupling are also responsible for the inhibition of movement, rather than that the signal involves the exchange of inhibitory substances through the permeable connections; since it is difficult to see how such a substance could avoid inhibiting the cell that produces it (but see (33)).

Consequences in vitro of contact inhibition. Many features of the behaviour of cell populations in culture can be ascribed to the effects of contact inhibition (12). Monolayering has already been mentioned. Directional movement (34) and the failure of fibroblast foci to scatter into discrete cells (35) have been analysed in terms of contact inhibition. The circular form of fibroblast colonies still awaits full analysis, but it can probably be ascribed to the tendency of contact inhibition to direct fibroblast movement towards cell free space (1, 12). The complete cessation of transposition of a cell may often be due to all round contact, and its resumption, as when a wound is made in a culture (36–39) to release from the inhibition. In this connection, however, the concept of mobilisation, the awakening of a dormant locomotory mechanism, needs to be borne in mind (5). The study of primary explants has suggested that when the replication cycle in vivo is in abeyance, the locomotory mechanism also tends to be sluggish, and the same correlation may perhaps hold in stationary monolayers in vitro. This remains to be explored.

Two correlates of contact inhibition. Amongst chick heart fibroblasts, mutual adhesion and contraction seem to be two phenomena that invariably accompany contact inhibition. I may appropriately here say something about wider implications of these two phenomena for cell behaviour.

Mutual adhesion, apart from perhaps initiating the reaction of contact inhibition, has other distinguishable consequences for cell movement. A population of fibroblasts tends to become linked into a meshwork by these adhesions. The possibility that a fibroblast in such a meshwork can change its position by infiltrating between its fellows is thereby much reduced, because the cross-linking increases the chance of collision and consequent contact inhibition. This at least seems to be so on a plane substratum, which incidentally is the condition of many fibroblasts in vivo. Another effect of mutual adhesion is that it obviously hinders cells from moving apart (which contact inhibition encourages). This hindrance may be reflected in speed of fibroblast locomotion (15). The implications of this hindrance are probably far wider for other kinds of cells, embracing such phenomena as the sorting out of mixed cells by differential adhesion (24), or total immobilisation of Schwann cells by axons (40).

It is striking that a meshwork of fibroblasts, when the cells are not adhering to a rigid substratum, has an internal tension (41, 39) which James and Taylor (42) have recently measured and found to be about 3.4×10^4 dynes/cm^2. It is tempting to relate this tension to the contraction found in contact inhibition, mediated through the adhesions between the fibroblasts.

The tensions normally found in tissues in vivo may have a similar origin; and in the remarkable phenomenon of wound contraction, the population of fibroblasts is a very good candidate for the source of the tension that moves the wound edges (43). It is I think important from the point of view of cell behaviour to distinguish the cell movement produced by this ability of cells to draw themselves together ("associative movement" (8)) from the locomotion across a solid substrate that is so conspicuous in culture, and that I have so far been discussing. The underlying cell physiology may have close similarities, but the former is not contact inhibited (in fact contact with other cells is a prerequisite), while the latter is. It should be added that cell contraction may not be the only source of associative movement: cells may also draw together by maximising their areas of contact through surface forces (see for instance (44)). Associative movement may produce important spatial rearrangements of cells in culture, for instance in the sorting out within aggregates of mixed dissociated cells (45), and in the clumping of cells in monolayer cultures. It can clearly involve the dragging of one cell over the surface of another without the intervention of contact inhibition.

Contact Inhibition amongst Other Cell Types

The previous section has been concerned with the phenomenon of contact inhibition as it occurs in the most closely studied kinds of cells, the embryonic chick heart fibroblast and the neonatal mouse muscle fibroblast. The rest of this review is concerned with the question as to how widespread the phenomenon is amongst the different kinds of vertebrate cells. The evidence is somewhat miscellaneous, and some preliminary discussion is necessary as to how the occurrence or absence of contact inhibition can be recognised. The most reliable methods test whether or not directed movement is obstructed by collision, observing either mass populations or individual cells. These require as a rule special experiments. Simple observation of cultures may however provide important indications. A significant degree of monolayering on a plane substratum strongly suggests that contact inhibition is occurring, provided it can be taken that the cells are colliding or have collided, and that they are not achieving their monolayering by moving much faster across each other than on the substratum itself. Multilayering is not such a good indication that contact inhibition has failed (18). It can arise by clumping; or through mitosis, if for some reason the population cannot spread on the substratum sufficiently fast to accommodate the extra cells; or from insulation of the cells from each other by secreted intercellular substance. Another easily observed form of behaviour that is often assumed to indicate contact inhibition is the parallel alignment of elongated cells; and conversely a criss-crossed arrangement is taken to be due to failure of contact inhibition. This may well be correct. Directional clues would be expected to be lost if contact inhibition is diminished (34); and fibroblasts migrating from a focus, and aligned by directional movement produced by contact inhibition, criss-cross when they move on to a relatively non-adhesive agar substrate which much diminishes the occurrence of contact inhibition. The exact cell behaviour that produces alignment has not yet however been analysed (see 21) though Elsdale (46) has made some interesting advances with confluent cultures. It seems difficult to ascribe the alignment found in some small isolated colonies of cells (47) entirely to contact inhibition, which should direct the peripheral cells radially; contact guidance and associative movement by lateral adhesion may be concerned here.

The data from other cell types will be surveyed under two main headings: firstly, homologous interaction, that between like cells; secondly, heterologous interaction, that between cells of different types. The points to be particularly considered are: how widespread is the ability to undergo contact inhibition; are the mechanisms similar in all cases; and, where they are similar, what degree of specificity between different cell types is there in the reaction.

Homologous Interactions

Fibroblasts. On a plane surface, populations of fibroblasts of many different kinds, when not too dense, are well known to have a strong tendency to be arranged at any moment in a monolayer. The weight of evidence seems to be that this monolayering, which has in some cases been quantitatively estimated (48, 17), is caused by contact inhibition, as it is in the

similar monolayering of the intensively studied chick heart or neonatal mouse muscle fibroblasts. It should be noted that Sanford et al. (49), have remarked that contact inhibition does not occur amongst embryo hamster fibroblasts observed in time-lapse films.

Sarcoma cells. Perhaps the most extensive application of the concept of contact inhibition has been made by tumour virologists. It is extremely common for a colony of cells, probably of fibroblast origin, that has been transformed by a tumorigenic virus, to be multilayered. The cells, if they are of elongated form, are often criss-crossed over each other. They therefore contrast clearly with the more monolayered and mutually aligned cells of origin (50). A similar disturbance has been described for presumed fibroblasts transformed in vitro by irradiation (51) or chemical carcinogens (52–54). There is therefore a presumption here of a diminution of contact inhibition. The picture is not however uniform. Quite a number of virally transformed tumorigenic fibroblasts show well-marked monolayering (48, 55, 56). Cultured strains that have become spontaneously tumorigenic in vitro may also show a tendency to multilayering (57) but here too there are tumorigenic strains whose social relations do not seem to be seriously disturbed (58).

Sarcomas normally carried in vivo by transplantation may show an unusual degree of piling up in culture (59). Our quantitative estimations of monolayering in populations of mouse S180 cells on a plane substrate show however that while there is more overlap than is usual amongst normal fibroblasts, there is a good deal less than is to be expected were they randomly distributed. Filming demonstrates that collisions are frequent, so some form of contact inhibition seems to be present. Mouse MCIM sarcoma cells also show considerably fewer overlaps than if they were moving randomly, and they are often mutually well aligned, as are a number of cultured fibrosarcomas (e.g. 60). A series of mouse polyoma tumours induced in vivo showed when explanted all variations from monolayering to multilayering (61). It is interesting that the first description of contraction on contact, suggesting contact inhibition, was apparently that of Canti (62), and the cells concerned were from the Jensen rat sarcoma.

The indications are therefore that homologous contact inhibition tends to be diminished in sarcomas, as compared with normal fibroblasts, though to a very variable extent. Some degree of homologous contact inhibition commonly remains. It has been pointed out that if there is any selection for invasiveness in a tumour, it might be expected to prevent total loss of homologous contact inhibition (63). Whether the contact inhibition between transformed fibroblasts that remains is of the same nature as the homologous contact inhibition between normal fibroblasts is unclear. Behaviour in culture has in some cases (e.g. 3) suggested that the strong mutual adhesion associated with the contact inhibition of normal fibroblasts is missing amongst sarcoma cells.

Epithelia. As earlier mentioned, it is often remarked that a migrating epithelial sheet of a healing wound in vivo is halted by collision with a homologous sheet (references in (6)). The situation is not favorable for detailed resolution of the cell behaviour involved. In vitro the same cessation of movement occurs (6) and correspondingly cell overlapping after collision of epithelial cells is relatively slight. In vitro it can be seen that the active ruffling of the lamellae at the cell edge is suppressed by contact (64, 65, 6). The same suppression of ruffling was also observed in vivo, amongst the epithelial cells of the enveloping layer of Fundulus embryos (66). Conversely, the spontaneous appearance or artificial production of an intercellular gap in an epithelial sheet leads to the appearance of ruffling lamellae on the free edges of the bordering cells (67, 64–66). It seems evident that these epithelial contacts quickly involve strong adhesions (11). Contraction of the contacting cells has been fully described by Bereiter-Hahn (67) when cells of Xenopus epidermis meet each other. We have also observed signs of contraction, reminiscent of those shown by fibroblasts, in our films of various epithelia. From all these observations it can be concluded that contact inhibition occurs between homologous epithelial cells of a number of different kinds, and that this inhibition is very similar to that found between homologous fibroblasts, involving stopping of locomotion in the direction of the contact, adhesion, paralysis of ruffling, and contraction. The strong lateral adhesion between epithelial

cells seems to be their main behavioural difference from fibroblasts (6, 64).

One further problem relevant to the homologous contact inhibition of epithelial cells needs mention, and this concerns how epithelia move (12). Is a sheet of epithelium dragged across a substratum solely by the activity of its peripheral cells (e.g. 69)? Is there continual momentary dissociation of the cells, allowing individual cells to move to fill the gap (e.g. 68)? Or does a premanently continuous sheet move by the activity of cells behind the periphery as well as at the periphery? The first two views, both of which may be correct in different circumstances, are compatible with the mechanism of contact inhibition, involving a signal to contraction, favored here. The third is not. Something of the same problem arises with sheets of fibroblasts (3), though in this case there is usually little doubt about the existence of temporary gaps between the cells. The existence of this problem led to the suggestion that the really essential point of contact inhibition might be that one cell could not move over the surface of another. A cell could therefore move in the direction of an existing contact with a second cell, provided this second cell kept moving away. Obviously if this is the situation it speaks strongly in favour of one of the hypotheses (numbers 3 and 4 above) depending on failure to adhere adequately to the dorsal surface of a cell. It has further been suggested that one cell may actually guide the active movement of another cell adhering to it, leading it along, which would account for the concerted movement of epithelial and fibroblast sheets (3). Shaffer has coined the term "contact following" for a similar phenomenon in slime mould amoebae. This general problem of locomotion despite contact may ultimately prove to be an important count against the signal theory (hypothesis number 6) favoured in this review. As yet however there is no clear evidence that epithelial or fibroblast sheets move in a way incompatible with contact inhibition of this kind.

Epithelial tumors. The mutual contact behaviour of the cells of epithelial tumours has been rather little studied. Most of the relations described that differ from those in normal epithelia concern the apparent strength of mutual cohesion, the relation of which to contact inhibition is uncertain. Microdissection studies for instance have shown that the cells of a carcinoma can be more easily separated from each other than can those of normal epithelia (70–72), which correlates with the known defects of carcinoma cells in the apparatus of junctional structures that link up a normal epithelium (73, 74). Correspondingly, intercellular communication is impaired (33). In culture, the diminished mutual adhesion may be reflected in a tendency for the cells to move more independently of each other than those of normal epithelia (65, 75); sometimes they separate completely (76) though less frequently than do the cells of sarcomas (77). There is however great variability between different carcinomas in the compactness of the population (78), which probably depends not simply on mutual adhesiveness, but on intensity of adhesion relative to locomotor activity (79).

Bearing more directly on the contact inhibition between cells of an epithelioma are the frequent descriptions of their growth substantially as a monolayer, like the cells of normal epithelia. There is however a tendency towards a less regular and more crowded arrangement of the cells, which Santesson (80) in particular described in a series of mouse mammary tumours of varying grades of anaplasia. Easty and Mercer (81) found from electron microscopy of replicas of cultured cells that those of a kidney tumour overlapped while corresponding normal cells did not. In a study of a mouse epithelioma with time-lapse filming, Trevan and Roberts (79) found that a retraction occurred when two cells collided, which is reminiscent of that occurring in fibroblasts. With the epithelioma cells, however, only one of the two in collision usually retracts and the authors interpret the phenomenon as an undercutting so that one cell loses its grip on the substratum in the region of contact. Wilbanks and Richart (65) have found in human cervical carcinoma in situ cells in culture, that membrane activity at a cell margin is diminished when contact is made with another cell, but there is more cytoplasmic overlapping than with normal epithelia, and the cells usually quickly separate again after contact.

It is clearly impossible to interpret these data unequivocally in terms of contact inhibition. But it appears that the contact relations of neoplastic epithelial cells are often disturbed; and though mutual adhesion is most obviously

affected, the descriptions are consistent in a number of cases with a diminished, but still persisting mutual contact inhibition.

Wandering cells. Oldfield (82) found that chick polymorphonuclear white cells are strongly monolayered, and that their colonies have a pronounced tendency to take up a circular form. While no measurements have been made on macrophages, common observation indicates that they too are monolayered (83, 84); and when in epithelioid form they are monolayered in mutual contact. There is, it seems clear, some form of mutual inhibition of movement at work; but because these cell types are susceptible to chemotactic control of movement, including in the case of mass populations of polymorphs a mutual chemotactic repulsion (82), there must be some doubt as to whether contact is required to elicit the cell to cell inhibition.

Heterologous Interactions

Between different fibroblasts. That heterologous contact inhibition occurs when fibroblasts from embryonic chick heart collide with fibroblasts from neonatal mouse muscle was shown in detail by Abercrombie, Heaysman and Karthauser (16) by means of an analysis of confronted explants. The restricted overlapping in mixed dissociated populations of these two cell types also indicated the operation of contact inhibition, and mixtures of embryonic chick heart with adult guinea pig subcutaneous fibroblasts behaved in a similar way (17). An attempt was made to find what specificities there may be in these interactions between unlike cells, but it cannot be considered to have been very successful because of the difficulty of rigorous interpretation of overlap frequencies in terms of contact inhibition. Judging by the overlap index, contact inhibition is more effective in the heterologous collisions of mouse with chick than in the homologous collisions between chick cells or between mouse cells (the two homologous indices did not differ significantly). The heterologous index for overlaps between chick and guinea pig cells had a value between the homologous index for chick cells and the homologous index for guinea pig cells, these two homologous indices being widely different. It is at least clear that overlap indices, and hence perhaps contact inhibition, do not depend on some single property of the cells, say general adhesiveness, which takes different values in different cells. Something more complicated, that is to say some specificity, is involved.

Heterologous epithelial contacts. Because of the marked functional differences between epithelia, the study of heterologous contact inhibition between them should be particularly valuable for investigation of the specificity of the reaction. The relations between two sheets of different epithelia after their collision can indicate whether contact inhibition has occurred, and there is evidence both in vivo and in vitro that a "seamless" junction, with the different cells in close contact but not overlapping, can occur between diverse epithelia (for references see 6). Chiakulas (85) has however obtained some evidence of specificity in vivo and there is also evidence from observations in culture that heterologous inhibition may not be as effective as homologous inhibition (6). Weiss (11) found that homologous epithelial cells tended to retain their mutual contact along a broad front, while contacts between heterologous cells usually soon break up. This may however reflect differences in mutual adhesion rather than in contact inhibition.

Epithelial cells and fibroblasts. Knowledge of interactions between these two kinds of cell relevant to contact inhibition has been reviewed by Abercrombie and Middleton (6). There are clear indications of some sort of mutual obstruction when collision occurs in culture, though to some extent fibroblasts can slip under the advancing edge of an epithelial sheet, moving between it and the substratum.

Sarcoma cells and fibroblasts. The first examination made of cells in this heterologous combination concerned the transplatable mouse sarcomas S180 or S37 with mouse or chick fibroblasts (16). The radial lengths and populations of outgrowths from confronted explants, measured before and after the outgrowths had collided showed no sign of contact inhibition. The fibroblasts had somewhat diminished movement towards the sarcoma explant, but this was not necessarily a contact effect, since it was appreciable before any collision had occurred. Overlapping of the two cell types after collision was no different from random. There was perhaps some reason to doubt whether the

sarcoma populations were uniform in this behaviour; but it can be said that at least a substantial proportion showed no contact inhibition against fibroblasts. It is not so clear from these data that fibroblasts show no contact inhibition by the sarcoma cells; the point is obscured by the diminution of fibroblast movement in the direction of the sarcoma cells. Observation of living preparations, direct and by time-lapse filming (3), showed however no reaction of either fibroblast or sarcoma cell when they collided. The sarcoma cells moved across any part of the *dorsal* surface of the fibroblasts, with no sign of close adhesion. The overlaps were not therefore produced in the same way as those between normal fibroblasts, which are due to one cell moving ventrally to part of another.

Further work (63) with other mouse sarcomas, primary and transplanted, has, mostly in a qualitative way, indicated diminished contact inhibition. With sarcoma MCIM the evidence includes living observations of the sarcoma cells invading a fibroblast monolayer, and counts of numbers of overlaps. The latter do not show a random distribution of sarcoma cells on the fibroblasts, but they do show considerably more overlapping than between homologous fibroblasts. In experiments with these and other sarcoma cells, however, the inhibitory effect on fibroblasts of some diffusing agent from the sarcoma cells, that showed up in a mild way with S180, is often pronounced (see also 86, 87), and makes analysis difficult.

Barski and Belehradek (88) observed the penetration of populations of normal fibroblasts by some cells ("sentinel cells") of a population of mouse sarcoma cells, especially where the fibroblast outgrowth is not highly coherent. Obstruction by contact with coherent sheets, some identified as endothelium, was observed; this is discussed below. Stoker (89) found that hamster cells transformed by polyoma virus, when in contact with normal fibroblasts became aligned and obstructed and therefore presumably showed contact inhibition of movement; while they apparently exerted no such inhibition on each other. He therefore made the valuable suggestion that emission of and response to a contact-promoted signal could become defective independently: his polyoma cells seemed to receive but not emit signals in the normal way. We now have some evidence that cells of a sarcoma (mouse MCIM) may emit but not receive properly, since they inhibit fibroblasts but are not inhibited by them.

In view of the variability of malignancy in vivo, the likelihood of differences between sarcomas in contact inhibitory behaviour is high. The simple view that sarcoma cells lack all contact inhibition is most unlikely to be right. The appropriate hypothesis at the moment seems to be that sarcoma cells show a contact inhibition to normal fibroblasts that is reduced to varying degrees. The relation of the behaviour in culture to invasiveness in vivo is fully discussed in L. Weiss' book (90) (and see 8).

Epithelial tumor cells and fibroblasts. Published work indicates that in culture the outgrowth from a carcinoma is better able to penetrate amongst a population of fibroblasts than is a normal epithelial outgrowth (80, 91), and there are several other reports, though without normal comparisons, of malignant epithelial cells moving through a fibroblast population (92–94). Wilbanks and Richart (65) observed in time lapse films that carcinoma in situ cells did not adhere to fibroblasts, which moved round but not across the carcinoma cells or became detached from the substratum by collision. As with the invasion of normal fibroblasts by sarcoma cells, the situation is complicated by the readiness with which carcinoma cells, after the invasion, seem to kill the normal cells (91, 92, 86).

Epithelial tumour cells and normal epithelium. The only information seems to be from Wilbanks and Richart (65) who observed that cells from cultured carcinoma in situ pushed normal epithelium aside as it grew, or detached it from the substratum.

Wandering cells and fibroblasts. Oldfield (82) confronted explants of chick buffy coat with explants of chick embryo heart. Collision of the outgrowths checked neither; outgrowth lengths, numbers of cells and population densities were unaffected by the presence of the other cells. Overlaps of both polymorphs and macrophages on fibroblasts were approximately random. It may be provisionally concluded that no contact inhibition is manifested between these cell types; though the homologous interaction of each type separately produced a strong inhibition.

Wandering cell or sarcoma cell versus endothelium. It has been mentioned that Barski and Belehradek (88) found sarcoma cells were often unable to invade continuous sheets of fibroblast-like cells, and they identified some of these sheets as probably endothelium. We have found the same, not only with a sarcoma (S180), but also with macrophages. While they freely move over the ordinary fibroblast-like cells from chick heart, they are completely unable to move over sheets which we presume (though the identification is purely morphological) to be endothelium or mesothelium. It seems possible that this complete inhibition may result from the nonadhesiveness of the endothelial (or mesothelial) cell surface: these cells are after all normally highly nonadhesive in vivo. If the interpretation is valid, this would be an instance of contact inhibition of the kind listed above as hypothesis number 3.

It is clear that signs of contact inhibition in the general sense are widespread amongst different kinds of vertebrate cell in culture. But at present there can be legitimate doubts as to whether this very general behaviour always has the same basic mechanism. One mechanism seems to be common to fibroblast-like cells and epithelia. This is associated with a characteristic adhesion between the colliding cell and contraction of the leading lamella that makes the adhesion: it is the contact paralysis of Wolpert and his colleagues (28, 29). The existence of quite a different mechanism, in which the nonadhesiveness of a major part of the exposed surface of a cell makes it unavailable as a substratum for movement of another cell, is suggested, though in no way validated yet, by the interaction of presumed endothelium with any other cell type. It is conceivable that this second mechanism is also at least potentially present in epithelia, and indeed amongst fibroblasts too, though usually obscured by the occurrence of the contractile type. Other mechanisms may yet emerge.

Total failure of contact inhibition has only so far been found in a few heterologous interactions: in wandering cells with fibroblasts, and in some sarcoma cells with fibroblasts. Where different basic mechanisms are at work such specificity of the reaction is to be expected. It will be of considerable interest to determine whether there are also specificities of the reaction amongst epithelial and fibroblast-like cells, which seem to share the same basic mechanism. Heterologous contact inhibition certainly occurs within each of these groups of cells, and possibly between them. The small amount of data suggests that some degree of specificity in the reaction may nonetheless exist; but this important aspect is not yet satisfactorily explored.

Relation to Inhibition of Replication

As remarked in the Introduction, some years after the term contact inhibition was introduced discussion of the subject sank into a state of profound confusion, because a number of authors gratuitously added inhibition of mitosis to, or substituted it for, the inhibition of movement with which the concept was actually concerned. Subsequently, evidence suggesting that there may really be such a phenomenon as contact inhibition of replication began to appear, and the subject of growth control by contact, or at least by proximity, has become of major importance in its own right. Aside from the historical accident which coupled the two kinds of contact inhibition, there seems little to connect them at present. Castor (95) has suggested, at least for one interesting cell strain, that extensive mutual contact inhibition of movement at high density, by restricting cell spreading and hence surface area, alters uptake from the medium and hence replication (see also 96, 97). The main suggestive evidence that a connexion exists between the two contact inhibitions is the finding that transformed fibroblasts, of many but not all kinds, differ from normal fibroblasts (in certain culture conditions) both in their ability to grow at high density and in their assumption of a spatial pattern that suggests a low homologous contact inhibition of movement. If transformation thus disturbs both inhibitions, they may well have a common basis. The possibility has appealed to many that the common basis lies in cell surface structure, which is changed by transformation. Exploration of this idea has hardly begun.

Conclusions

Contact inhibition of movement is here defined simply as the stopping of the continued locomotion of a cell in the direction which has produced a collision with another cell; so that one cell does not use another as a substratum.

Amongst fibroblasts and epithelial cells this inhibition seems to be brought about by a mechanism which it is suggested consists essentially of a spasm of contraction in the region of the contact, set off by some signal from the cell contacted. Many other kinds of cells show the general phenomenon of contact inhibition; but there is no certainty that they have the same contractile mechanism.

The survey of the literature which this review has entailed suggests that it might be useful to end with four somewhat negative points: (1) Contact inhibition as originally defined is not concerned with mitosis. It may of course become so. (2) Contact inhibition of movement is difficult to analyse reliably without quantitative estimations and deliberate experiments. Anecdotes are not enough. (3) Malignant cells are not properly described as being devoid of contact inhibition. It is suggested that they are defective as compared with their cells of origin. (4) From the point of view of invasion in vivo, the homologous contact inhibition of tumour cells by tumour cells is of little direct interest. It is the heterologous inhibition of tumour cells by normal cells that is relevant.

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