J. Cell Sci. Suppl. 8, 121–140 (1987)
Printed in Great Britain © The Company of Biologists Limited 1987

CELL MOTILITY AND THE PROBLEM OF ANATOMICAL HOMEOSTASIS*

ALBERT K. HARRIS

Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280, USA

SUMMARY

The locomotion of tissue cells need not be invasive or disrupt normal tissue geometry, as occurs in cancer. The normal relationship between anatomical structure and cell locomotion is exactly the reverse, with motility serving to create and maintain the structures of the body. This relationship is most extreme in sponges, where time-lapse films show that the cells move about continually in patterns that restructure these animals' simple anatomy. The cells choose their position according to their differentiated cell type, which is the opposite of what is usually assumed to occur in development and has important implications for the functional significance of histotypic cell sorting.

A particular type of cellular force that seems to be important in morphogenesis is the traction that all motile tissue cells exert. This traction can be studied by culturing cells on very thin sheets of silicone rubber, so that the locations and variations in the cellular forces are made visible by the wrinkles they produce in the rubber substratum. One finding has been that the traction forces exerted by untransformed fibroblasts are very much stronger than is needed for their own locomotion, but are well adapted for the function of rearranging and aligning collagen fibres to form structures like ligaments, tendons and muscles. These forces are found to be greatly weakened by neoplastic transformation, however, suggesting that malignant invasiveness results from some sort of deflection of cell traction forces from their proper morphogenetic functions, so as to produce uncontrolled invasion.

To explain how motile cells create and maintain structures, as well as how their locomotion sometimes becomes perverted into the form of cancerous invasiveness, what seems to be needed is an extension of the concept of homeostasis to apply to the control of geometric relations between cells. This task may not be easy; one obstacle is the widespread belief that asymptotic stability implies the minimization of free energy. Instead, I suggest that stability results from balance of opposing forces within tissues, and that genes control which anatomical shapes will exist by determining the rules by which the relative strengths of these forces vary as functions of shape: to control shape, one must control the way forces vary with shape.

INTRODUCTION: CELL MOTILITY IN RELATION TO ANATOMICAL STRUCTURE

The motility of the body's component cells can be either destructive or constructive. Cell motility is destructive when it takes the form of malignant invasiveness and disrupts the proper geometric arrangements of cells into tissues and organs. But this same motility is constructive when it creates these anatomical structures during embryonic development, and also when it serves to maintain their geometry during subsequent life, despite damage and turnover. We should very much like to understand both the constructive and the destructive effects of cell motility, and it is to be expected that the study of the one should aid our

* This was the Cancer Research Campaign Lecture.

understanding of the other. This will be especially true if, as seems likely, malignancy results from normal cellular maintenance and repair mechanisms that have somehow escaped their normal controls.

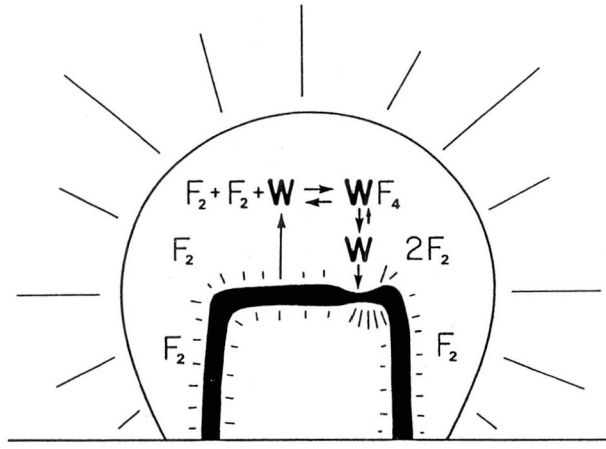
Among the obstacles to understanding these problems is the tendency to think of morphogenesis as having ceased at the end of embryonic development, or at least by the time growth is completed. In actual fact, many parts of the body continue throughout life to replace, rearrange and repair their component cells and molecules; their morphogenesis is continual, since it is by means of this continual renewal and rearrangement (rather than simply by inertia) that the structures are maintained. To mention one example, it has been found that the collagen molecules that make up the periodontal ligament (which holds the teeth in the mouth) turn over constantly throughout life with a half-life of only about one day (Sodek, 1977). There are many other examples, including the continuous replacement by lateral migration of the epithelial cells that line the intestine, reorganization of capillary networks, and a continual breakdown and re-formation of the bones of the skeleton itself. Probably, if we could make time-lapse films of the cell movement within our body, as we can do with sponges, we would be shocked by the amount of cell movement and wonder how it is possible for the geometry of our body to be maintained in spite of it.

The major theme of this article is that it is not *in spite of* the movements and turnover that anatomical structure is maintained; it is actually *by means of* these movements that anatomy maintains itself. Cell locomotion itself should not be considered as equivalent to invasiveness, or as inherently disruptive to tissue architecture. Controlled locomotion is a necessary part of the maintenance of our anatomy. We therefore need to think of anatomical shapes as being less like those of an inert statue, and more like the shapes formed by water shooting up in a fountain. The external shape of a statue is, of course, very much more similar to that of the human body; but this shape has been imposed by external forces, rather than arising from the properties of the material itself. In contrast, the shapes taken by the water of a fountain reflect properties of the water itself, and it is not in spite of the water's continuous movement that these shapes are maintained, but precisely by means of this movement.

When a biological property is maintained relatively unchanged, despite disturbances, it is said to be in homeostasis. Physiologists have developed the concept of homeostasis to a high degree of sophistication over the past century, with Bernard and Cannon having been the major contributors (see Langley, 1973). It has even become part of our collective 'common sense' as biologists that, when one encounters a constancy of some property like body temperature or blood pH, one should seek explanations in terms of negative feedback and countervailing influences, the relative strengths of which vary as functions of whatever physiological variable is being kept constant. Unfortunately, however, when the variable being kept constant happens to be a geometric or mechanical one (the shape of an organ, perhaps, or the relative positions of some cells) it has not been usual to think of this as being a case of homeostasis, or to ask what the counter-balanced forces or the feed-back cycles that function to maintain tissue geometry might be.

There are some exceptions to this general lack of attention to anatomical homeostasis: the Chalone hypothesis, for example, seeks to explain the constancy of size in the liver, skin and other organs in terms of negative feedback by hormone-like substances (Bullough, 1975; see also Lord *et al.* 1978); there is also the theory that bone mass, shape and internal structure may be controlled by electrical fields (whether piezoelectric or electro-osmotic), which the bones themselves generate when strained (Bassett, 1971; Currey, 1984). Thoma's (1893) comparable work on the control of arterial pathways and diameters by physical tensions and blood flow-rates, however, is seldom even cited.

Likewise, in modern technology, it is much more usual for homeostatic servo-mechanisms to be used to control quantitative variables, rather than geometric or structural ones. Lest this imbalance suggests some inherent limitation to the capacities of negative feed-back (rather than limitations of human imagination), and as an example of the general sort of mechanisms we need to look for, let me interject the interesting example of the 'quartz–halogen' lamp (Dettingmeijer *et al.* 1975) (see Fig. 1). These are incandescent light-bulbs whose tungsten filaments are heated to such high temperatures that the housings would melt were they made of glass rather than quartz; the hotter filaments produce a brighter light, but at the cost of considerable evaporation of tungsten atoms from the filament. Halogen gasses are included in the bulb in order to react with the free tungsten atoms, and because the



Quasi-homeostatic regeneration
of tungsten filaments
in quartz–halogen light bulbs,
as people wish it would work

Fig. 1. Diagram of the principle of operation of a quartz–halogen lamp as an illustration of geometric homeostasis achieved by means of a continuous expenditure of energy and a recycling of material. The intense heating of the filament by an electric current causes evaporation of tungsten atoms from the filament, but these react with halogen gas to form a tungsten halide, which is then itself broken down by heat to redeposit tungsten onto the filament. Ideally, this redeposition is concentrated on the thinnest parts of the filament, where it is hottest.

resulting tungsten halides are themselves broken down by high temperatures, tungsten metal is continuously redeposited back onto the filament. The most truly homeostatic feature is the tendency for this deposition to be concentrated wherever the filament is hottest (since the highest temperatures will be produced where the filament is thinnest, this effect can tend to even out filament diameter). Unfortunately, this last effect is seldom achieved.

The development of the concept of contact inhibition by Abercrombie & Heaysman (1953, 1954) can (and, I think, should) be regarded as having been the crucial first step towards understanding how the motility of our component cells can be homeostatically controlled in such a way as to permit renewal and repair of tissues, whilst blocking invasiveness. But now we need to go still further towards understanding how cell behaviour and interaction can bring into existence and maintain those complex spatial arrangements of cells and matrix that constitute anatomy.

SPONGES: AN EXTREME CASE OF PERPETUAL MORPHOGENESIS

Both fresh-water and marine sponges, at least those of the majority morphological type called 'leuconoid', can be cultured in thin spaces between microscope coverslips, slides or Petri dishes (Ankel & Eigenbrodt, 1950). The sponges obligingly adapt their anatomy to these 'sandwich cultures' (Fig. 2), so that one can observe the movements of their component cells, much as one observes the behaviour of ants living in colonies between parallel sheets of plate glass. By this and similar methods, the behaviour of cells within living sponges has been studied extensively by Weissenfels (1981), Harrison (1972) and more recently by Calhoun Bond (1986) in my own laboratory.

Time-lapse films of sponges growing between coverslips and slides show that their component cells are continually moving about (Fig. 3). The most active are the several classes of mesenchymal cells in the sponge interior; these carry the spicules (skeletal elements) from place to place, here installing and joining them into skeletal supports, there moving other spicules to new locations. The special type of epithelial cell peculiar to sponges (called 'pinacocytes') also shows active movement and locomotion; sheets of these cells form the sponge's surface layer, including what one might call the floor, by which the sponge adheres to the substratum. As Bond (1986) has shown, these floor pinacocytes actually exert a substantial traction force on the external substratum, and the net result of this traction is that the entire sponge crawls laterally; in aquaria, for example, one finds sponges climbing the glass walls at speeds of the order of 1 mm per day.

Individual pinacocytes move around within their sheets; not infrequently some of these cells detach themselves from the edge of the sponge and crawl around for a few minutes on their own before rejoining it. Water is sucked into the sponge through many small pores in the pinacocyte layer; these pores run through and between the pinacocytes themselves, so that the diameters and number of these incurrent pores are a constant function of the cells' motile activity and the constriction of these pores.

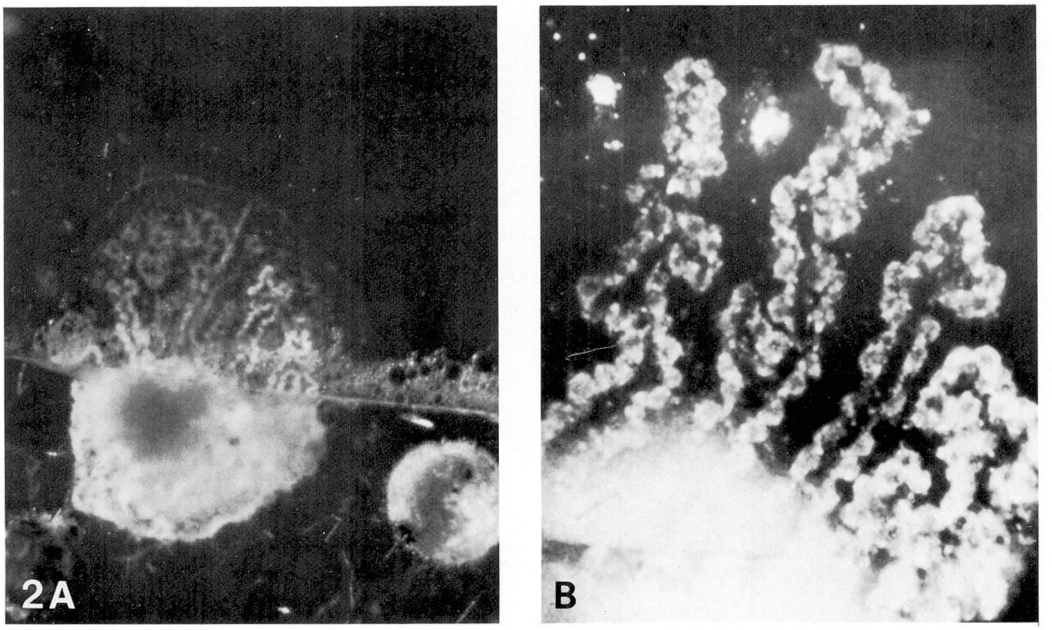


Fig. 2. Photographs of living fresh-water sponges (Ephydatia) living in 'sandwich cultures' between coverslips. A. Low magnification; sponge extending under coverslip at top. Thicker region of sponge at bottom, flanked by two gemmules. B. Higher-magnification view of choanocyte chambers and excurrent canals from the same species. This is a single frame from a time-lapse film taken by fluorescence microscopy; the choanocytes appear white because of the fluorescent plastic beads that they have filtered out of the water.

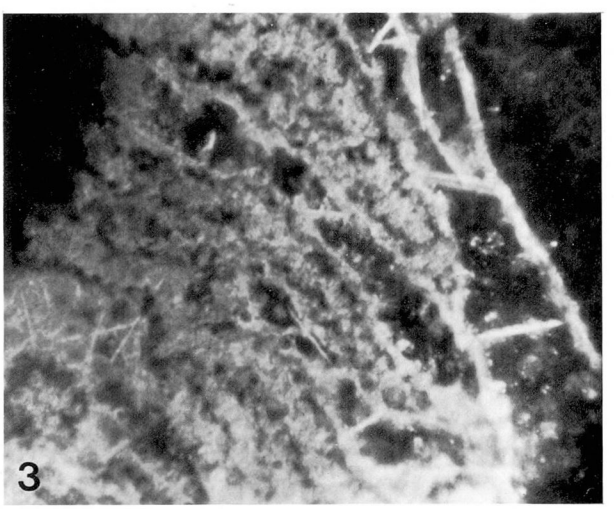


Fig. 3. Single frame from a time-lapse film showing movements and anatomical rearrangements inside a living *Ephydatia* sponge.

Other pinacocytes (whether these are interchangeable with those forming the outer layer seems not to be known) arrange themselves into a branching network of excurrent canals, which conduct the water away from clusters of the flagellated cells called choanocytes.

The choanocytes are the third major cell type and are the ones that do the actual pumping of water. It is these cells that filter food from the water; one might even paraphrase the cliché about the egg and the chicken by saying that the rest of the sponge is just the choanocytes' way of channelling water past themselves most efficiently (as well as affording themselves some degree of protection). Groups of choanocytes spontaneously arrange themselves into rosettes (or hollow balls), with their flagella all pointing inwards; the beating of these flagella pulls water through narrow spaces between adjacent choanocytes and produces a pressure that pushes the water out of the sponge through the tubular excurrent ducts made of pinacocytes (mentioned above), to which these hollow clusters of choanocytes spontaneously connect themselves.

From my observations of food particles and fluorescent plastic beads being filtered out of water by sponges in sandwich cultures, it appears that the water reaches the choanocytes by flowing directly through the space (called the 'mesohyl') where the mesenchymal cells, spicules and collagen are located. Text illustrations of leuconoid sponges always show 'incurrent ducts' (see Hyman, 1940), but these seem to be imaginary. The true situation is apparently analogous to the way air reaches the fire in an ordinary wood stove, i.e. by coming through the surrounding room. The water is then sucked between adjacent choanocytes, which take up the fluorescent beads as the water goes by. The beating of the chanocyte flagella then pushes the water out of the excurrent canals, while it simultaneously creates a suction in the mesohyl space, so that water is drawn into this space through pores in the surrounding sheet of pinacocytes.

Time-lapse films of sponges cultured in these 'sandwich' chambers show very extensive and continuous rearrangements of cells, spicules, choanocyte chambers, excurrent canals, and the 'spongin' collagen matrix. Pronounced waves of contractility sweep repeatedly through all the tissues and the shapes of the chambers and canals change radically from hour to hour. When initially shown such films, people usually assume that the subject matter must be the fabled reaggregation of dissociated sponge cells, as was originally observed by H. V. Wilson (1908), late of my department. They are surprised to be told that these are not reaggregating or otherwise experimentally treated sponges; this is just the way their component cells behave all the time. These ordinary day-to-day movements of cells within sponges are at least as rapid, active and extensive as one sees in time-lapse films of reaggregation.

In effect, sponges are always in a perpetual state of spontaneous anatomical reorganization. Thus, Wilson's remarkable reaggregation phenomenon should apparently be regarded as the imposition of a somewhat more extreme version of events that are normally going on all the time inside sponges. As Wilson pointed out, sponges living under poor conditions frequently form one or another sort of

'reduction body'. In the case of fresh-water sponges, these are special well-organized spherical structures called 'gemmules'. The reorganization of a functioning sponge from the cells of a gemmule or other reduction bodies is a normal equivalent of the reaggregation phenomenon. Nor are sponges unique in this respect: fresh-water ectoproct bryozoans (such as *Pectinatella*) also reorganize their anatomies from cells stored in special reduction bodies called 'statoblasts'.

WHAT IS THE FUNCTIONAL SIGNIFICANCE OF HISTOTYPIC CELL SORTING?

The constant renewal of sponge anatomy by cell motility may have important implications for the whole phenomenon of histotypic cell sorting, including that of vertebrate cells. Let us look at the problem very abstractly. The central task of metazoan development is to put the correct differentiated cell types into the proper geometric locations relative to one another. Logically, there are two extreme alternative ways to get the right cell types in the right places. The first alternative is *to have position control differentiation*, so that the specialized cells simply develop where they ought to be, and then stay there. The second alternative is exactly the reverse: *to have differentiation control position*, by which I mean the inclusion among the differentiated characteristics of whatever behaviour or properties will make the cells move spontaneously to their correct relative positions.

The first of these two extreme alternatives (position determining differentiation) is the common-sense one; it is also the one posited by the conceptual system called 'positional information'. Many cases are definitely known in which position controls determination and subsequent cytodifferentiation.

The second of these alternatives (differentiation determining position) may well be counterintuitive and seem impractical to the point of absurdity. But this absurdity seems not to deter the sponge cells, because it is apparent from the time-lapse films of their perpetual reorganizations that it is the second of these alternatives that the sponges are actually using to create and maintain their humble anatomies. Their cells choose position on the basis of their differentiated state, rather than the other way around, and they do so constantly.

What I am led to suggest is that the morphogenesis of all metazoans should be regarded as employing some mixture of these two stratagems for getting the right cell types into the right geometric locations: either having position control differentiation, or having differentiation control position. In the case of sponges, a rough estimate might be that they are using 90 % in the second stratagem and only 10 % in the first; in the case of our own anatomies, the estimated percentages might be approximately the reverse. Histotypic cell sorting, then, would represent the cells' capacity for this second stratagem being forced to operate in isolation from their capacity for the first. Any behaviour or property (and not just selective adhesiveness), which has the effect of moving cells to certain relative geometric positions (according to their differentiated cell type), should likewise cause cell sorting. But

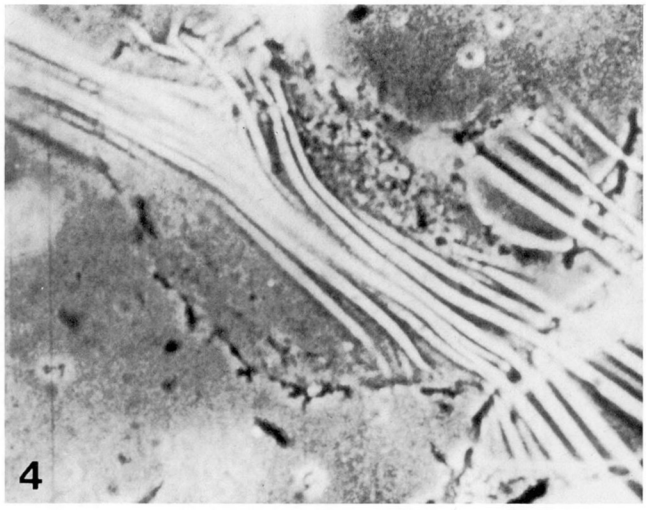


Fig. 4. A single frame from a time-lapse film showing a chick heart fibroblast crawling on the surface of a thin sheet of silicone rubber. The traction forces are made visible by the wrinkles they form in the rubber substratum. Notice how these wrinkles begin behind the leading margin of the cell where the ruffling activity is concentrated. The cell is crawling toward the upper right.

what kind of cellular behaviour and what forces would have the ability to rearrange cells, spicules and matrix into their proper geometric patterns?

CELL TRACTION AS A MORPHOGENETIC FORCE

The silicone rubber substratum technique was originally invented as a means of studying the mechanism of locomotion in tissue cells. In this technique, liquid silicone fluid is cross-linked by brief exposure to a flame, so as to form a layer of silicone rubber only about 1 μm thick. This layer coats the fluid's surface. Tissue culture cells are then plated out directly onto the surface of this rubber layer; the cells adhere to the rubber, spread on it and crawl about, just as they would on a glass or polystyrene surface. The rubber layer, however, is sufficiently weak and elastic to be visibly distorted by the propulsive forces exerted by the individual cells. These cellular forces produce complex patterns of wrinkles in the rubber substrata; the wrinkles are easily observed by phase-contrast microscopy, and provide information about where, and in what directions, the cellular forces are exerted (Fig. 4).

This method does not lend itself to quantification of the forces exerted, partly because of the difficulty of making two sheets of rubber with exactly the same elasticity, but even more because of the mathematically complex relationship between tensile stress and wrinkle size and number. The method can, however, yield unambiguous information about which cell types exert stronger forces than others, or whether a given experimental treatment causes a strengthening or a weakening of

these forces. Changes in the forces exerted are most clearly seen when the wrinkling patterns are recorded by time-lapse cinemicrography.

The cells whose motility has been most intensively studied using this technique are those generalized mesenchymal cells called fibroblasts. These cells exert strong centripetal forces on the silicone rubber substrata; bipolar fibroblasts, for example, will pull rubber in from both directions toward their centre. The result is that the rubber sheet becomes thrown into 'compression wrinkles' directly beneath the cell body, but stretched into 'tension wrinkles' that radiate outward beyond the cell margins (Fig. 5). The latter type of wrinkle, especially, can often extend beyond the cell for many hundreds of μm, and sometimes a mm or more.

These pulling forces, which fibroblasts and other tissue cells exert, have been given the name 'traction'. The locations and directions in which a given cell exerts its traction are found to be correlated with the ruffling and blebbing activities along the cell margins, as well as with the direction in which the different parts of the cell margins spread outwards over the substratum. Traction is concentrated in those regions directly behind (centripetal to) the particular parts of the cell margin where the plasma membrane is undergoing its most active ruffling movements. The direction in which the traction force is exerted is rearward or centripetal; in other words, the direction of traction tends to approximate the predominant direction of ruffle backfolding.

We should definitely not conclude, however, that the ruffles or other protrusions from the cell surface play a direct role in the exertion of the traction force; in particular, there is no indication of the cell margin reaching out, attaching, and then pulling back on the substratum, intuitively attractive as such a scenario might be.

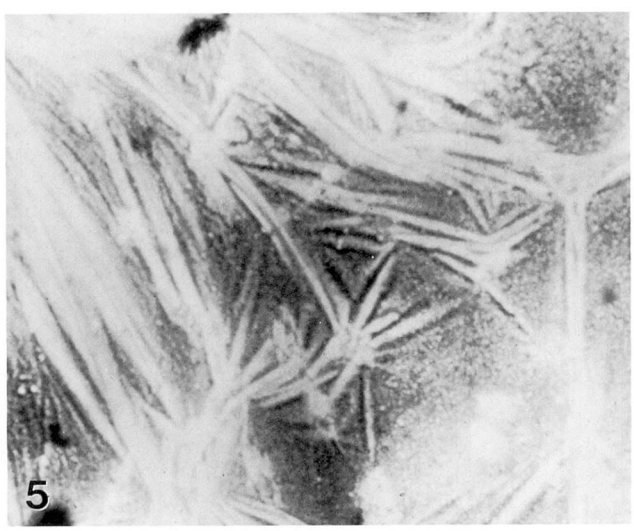


Fig. 5. A single frame from a time-lapse film showing chick heart fibroblasts at low magnification compressing a sheet of silicone rubber. Within an hour of the time this picture was taken, the entire area of rubber visible in this field had been compressed to a small wrinkled mass and pulled out of the field of view at the upper left.

Low-magnification observations might suggest such a cycle; but when filmed at high magnification, the ruffles themselves do not appear to exert any appreciable forces on the substratum, neither to pull it nor to push it, nor usually even to touch it. On the basis of the locations where the wrinkles appear in the rubber substrata, cell traction is exerted 5–10 μm or more behind the ruffling margins, in the area where dark contact areas and points are seen by interference reflection microscopy (fortunately, the optical properties of the silicone rubber are similar enough to those of glass to permit observation of contacts by interference reflection). Moreover, this traction seems to be exerted as a steady shearing force, one that can vary over time but without any apparent element of oscillation or pulsatory quality. It is not unusual for opposite sides of a single cell to exert traction in opposite directions; all sides towards the middle.

Spread fibroblasts remain in a state of tensile stress, stretched taut between their adhesions to the substratum, through which they continue to transmit a pulling force. If one or more of the cell margins happens to break its adhesions to the rubber substratum, and this is not infrequent, the tension is released and the wrinkles in the rubber re-expand and disappear within one or two seconds. In such cases, it is not unusual to see a partially detached fibroblast pulled bodily sideways by the elastic re-expansion of the rubber; the cell is pulled away from the sites of its newly broken adhesions at speeds of 10 μm s^{-1} or more (enough to produce blurred exposures in films). The continued existence of the wrinkles in the rubber substrata depends on the continuing exertion of the contractile forces by the cells; the wrinkles in the rubber are not some kind of 'footprint' left by the motile cells. The only exceptions to this rule are when so much tension has been exerted that the rubber sheet has actually been torn, or when cells have held the rubber continuously in a highly wrinkled state for several days and deposited a layer of collagen fibres on its surface, which can effectively lock some of the wrinkles in place.

I conclude from these and other observations that these traction forces that distort silicone rubber substrata are the same as those that cause cell spreading and locomotion, and that they are also probably equivalent to the forces that move adherent particles centripetally across cell surfaces. They seem also to be the same as the forces responsible for the condensation or compression of fibrin and collagen substrata around cultured cells. It was once generally accepted that this condensation of extracellular matrix around cells was a shrinkage of the matrix itself, stimulated in some way by uptake of water or by other biochemical effects of growing cells; considerable evidence against that type of explanation was accumulated by Stopak & Harris (1982), who showed that those cell lines that exerted the weakest traction on rubber substrata also produced the least compression of collagen gels, regardless of the cells' growth rate or metabolic activity.

Comparisons of the amounts of distortion produced by cells, both on silicone rubber substrata and in gels of re-precipitated rat-tail tendon collagen (Fig. 6), show very large differences in tractional strength between different classes of differentiated cells and also between normal cells and their transformed equivalents (Harris *et al.* 1981). Blood platelets exert the strongest traction, followed by fibroblasts. Epithelial

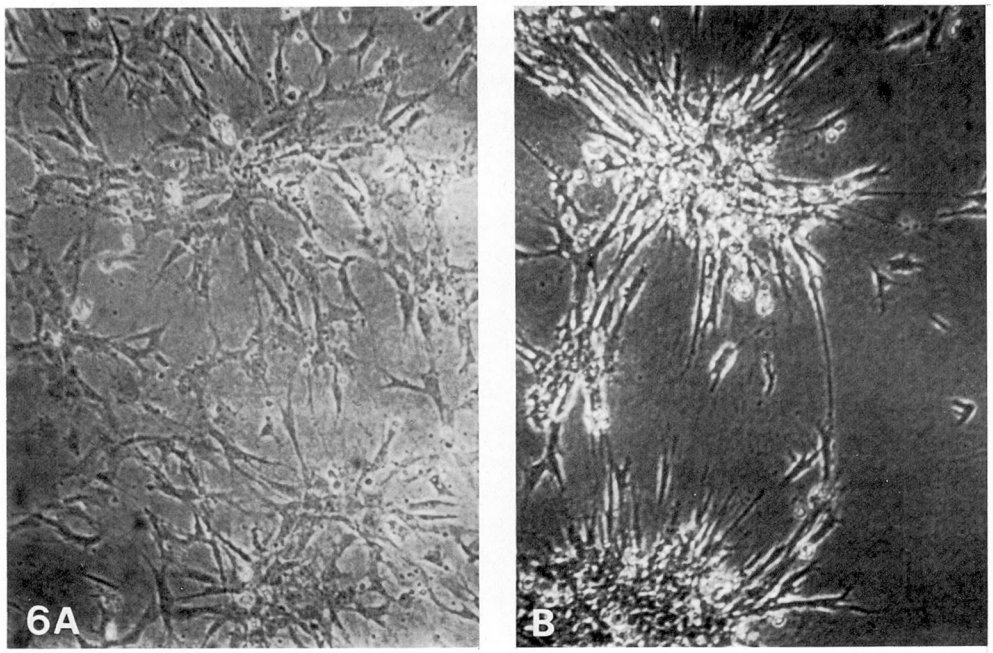


Fig. 6. A,B. The cumulative effect of fibroblast traction in mechanically reorganizing a gel of reprecipitated rat-tail tendon collagen. Individual frames from a continuous time-lapse film; 36 h separates the two time points. Note how the population density of cells becomes progressively more uneven, due to the cells' traction pulling more cells and matrix into tight concentration.

cells are considerably weaker, macrophage traction is barely detectable, and no visible distortion of the rubber is caused by either polymorphonuclear leucocytes or nerve growth cones.

Transformed fibroblasts exert traction on the rubber substrata in the same pattern as do normal fibroblasts, but this traction is very much weaker; the wrinkles produced are smaller and fewer. The systematic comparison by Steinberg *et al.* (1980) of the population densities required to produce a certain degree of collagen gel distortion confirmed that transformed cells are characteristically weaker than normal cells. Using reverse transformation of CHO cells by dibutyryl cyclic AMP, Leader *et al.* (1983) showed that these cells exert much weaker traction in the transformed state, compared with the reverse transformed state. The transformed cells' adhesions to the substrata were also found to be larger in total area, but weaker in total strength. Most recently, Danowski & Harris (1986) found that phorbol ester tumour promotors cause an immediate weakening of fibroblast traction, combined with more gradual changes in cell morphology and adhesion to the substratum. In this case, cells temporarily in a transformed state show decreased adhesion to hydrophilic surfaces but an increased ability to attach to hydrophobic surfaces.

When fibroblasts are explanted from embryos into tissue culture, however, their tractional strength undergoes an increase in strength. This occurs between 3 and 4

days after initial explantation and is accompanied by a conversion of their substratum adhesions to the tight, focal adhesion type. We believe that this increase in the force of the cells' traction corresponds to the increased development of actomyosin stress fibres by such cells and is a response of normal fibroblasts to trauma; as such, it would be equivalent to conversion of the cells to 'myofibroblasts', as described by Gabbiani *et al.* (1973).

Our studies of fibroblast traction were undertaken on the assumption that the only biological function of these forces was to propel the cells exerting the forces from one place to another. The observations described above raised several paradoxes, however: one paradox was that the more motile cells, such as macrophages, leucocytes and transformed fibroblasts, were the ones that exerted the weakest traction forces; another was that fibroblast traction was so strong as to produce large-scale rearrangements in collagen matrices. These rearrangements can easily extend for centimetres. So not only does fibroblast traction seem to be wastefully strong, it seems to pose some danger of disrupting the normal mechanisms that arrange collagen and other matrix materials into their correct geometric patterns within the body.

Stopak and I proposed a simple solution for both these puzzles (Harris *et al.* 1981; Stopak & Harris, 1982), which was that fibroblast traction is itself the principal mechanism for arranging collagen fibres into their correct anatomical patterns. In other words, the traction exerted by macrophages and leucocytes actually is intended to move those cells from place to place; but the much stronger traction that fibroblasts exert has a quite different function; fibroblast traction is intended primarily to distort and rearrange the collagen in their vicinity. Whatever displacement of the fibroblasts might result from their traction would be a secondary effect. When placed on an abnormal, rigid substratum such as glass, fibroblasts will still exert traction, but now the only visible effect of that traction will be a rather inefficient locomotion.

Stopak & Harris (1982) were also able to produce evidence that fibroblast traction can produce several important kinds of morphogenesis; these include the alignment of collagen into ligaments and tendons, as well as the compaction of collagen into sheets much like organ capsules, perichondria and certain dermal structures. Subsequently, Stopak showed that rat tendon collagen, covalently labelled with fluorescein and injected into developing chick limb buds, is subjected to forces that compress and rearrange this collagen so that it becomes part of whichever of the chick's anatomical structures form at the site of the injection (Stopak *et al.* 1985). Although there was no way of observing whether it was cell traction that generated the forces responsible for this *in vivo* remodelling of collagen, the results were those to be expected of traction. A series of mathematical and computer simulations have been started to determine what net geometric consequences are to be expected from traction and other cellular forces, as well as whether these correspond to actual anatomical structure (Oster *et al.* 1983). These continue to be encouraging and it has been possible to show that fibroblast traction can generate periodic spacing of cells and matrix (Harris *et al.* 1984).

ASYMPTOTIC STABILITY AND THE ENERGY MINIMIZATION FALLACY

Although it is well known that our body temperature normally gravitates to about 37 °C, no one concludes that this temperature therefore must represent some kind of state of minimum free energy; nor do people draw such conclusions from other cases of physiological homeostasis. However, when it comes to tissue geometry and to the tendency of cells to sort out into different patterns, this minimization-of-free-energy interpretation has many militant advocates. Their misinterpretation of thermodynamics represents, therefore, a formidable obstacle to any effort to understand the generation and maintenance of tissue geometry in terms of homeostatic mechanisms. Imagine what the effect on the progress of physiology would have been if people had believed that the constancy of body temperature or blood pressure required (or even implied) that the stable states had to minimize free energy.

Of course, there really are many cases in which systems gravitate to certain configurations that do minimize either potential energy or one of the forms of free energy; the latter constitute much of the subject matter in thermodynamics courses, which appears to be the source of this fallacy. When the only stable systems people are told about are those that minimize free energy, it is only natural for them to draw conclusions. The pair of physical analogies illustrated in Fig. 7 are intended to clarify this issue.

In the first of the two examples, a motor boat propels itself upstream against a current, with this current becoming progressively stronger upstream; the boat thus

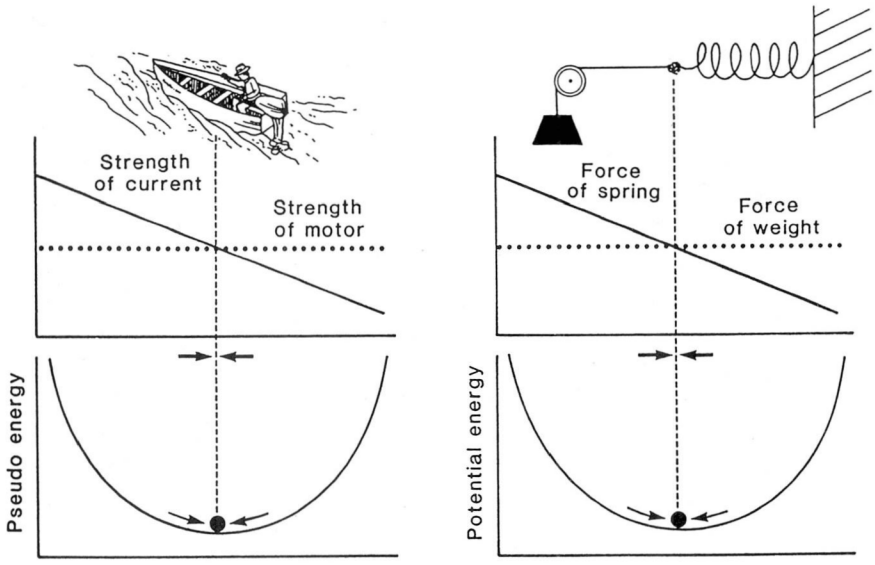


Fig. 7. Diagrammatic illustration of the relationship between force balance, energy minimization and the achievement of an asymptotically stable configuration. On the left side, a boat is propelled by a constant force up a river whose current becomes progressively stronger the farther upstream the boat progresses. On the right, a weight falls until the elastic pull of a spring exactly balances its weight. In both cases, a state of asymptotic stability is reached, but only the situation on the right minimizes energy.

gravitates to a stable, stationary position at which the strength of its motor is exactly counterbalanced by that of the current.

In the second example, the gravitational pull on a weight stretches a spring until the elastic pull of the spring exactly counterbalances the gravitational pull. This configuration is then stable and will spontaneously restore itself if disturbed.

The point of these two examples is that both gravitate to a certain configuration, but neither potential energy nor free energy is minimized in the first example, while in the second potential energy really is minimized. The difference is that the forces exerted by gravity and the spring happen to be conservative forces and do not expend energy in the static situation; in contrast, the forces exerted by the river current and the boat's motor would not be conservative forces, so it would not even make a great deal of sense to speak of the potential energy. The property of gravitating to some particular configuration depends on the balance between opposing forces, the crucial aspect being the rules by which the opposing forces vary as functions of position. The question of energy minimization, on the other hand, depends on whether the opposing forces happen to be conservative or not. These are completely different questions. Stability does not imply the minimization of energy, neither does it imply that the forces responsible have to be conservative.

In both our imaginary examples, the state of force-balance is what is called 'asymptotically stable' Hirsch & Smale (1974), in that the system will gravitate to it from either direction. Both also possess what Rene Thom (1975) meant by 'structural stability', which sounds as if it had something specifically to do with the involvement of physical structures, but actually refers to the fact that the qualitative behaviour of the system is relatively insensitive to qualitative changes in the variables (like the absolute strength of the boat's motor, or that of the current). As long as these qualitative changes are not too large, the qualitative result is the same. Notice that the laws by which the forces vary have been chosen to be the same in both these examples; even though the physical cause of the forces could hardly be more different, the same laws govern how the forces vary as functions of position. Which configurations will be stable does not depend on the physical causes of the forces, it depends on the rules by which the forces vary as functions of configuration. The same rules generate the same geometries.

THE CAUSATION OF SHAPE BY PHYSICAL FORCES

The relationship between physical forces and the shapes they create is not simple or free of logical pitfalls. As part of what must be the greatest single biological treatment of this topic, D'Arcy Thompson (1942) catalogued many examples in which cells or organisms take on shapes resembling those of soap bubbles and liquid droplets. Although Thompson was rather non-committal in his interpretations, the conclusion often drawn from such similarities is that the same physical force (i.e. surface tension) must be shaping cells and organisms. I suggest, however, that we should conclude only that the forces that shape the cells happen to obey rules rather similar to those obeyed by surface tension. Forces that obey the same rules will create

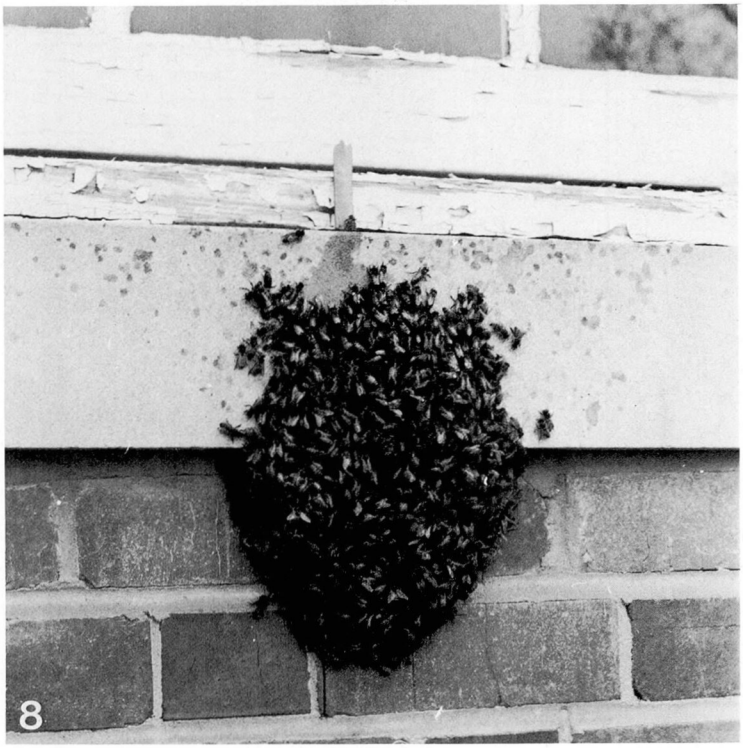


Fig. 8. A mass of swarming bees, which collectively behave much like a liquid droplet with a 'surface tension'.

and maintain the same shapes; their physical causation need not have anything in common.

Fig. 8 shows a mass of swarming bees suspended from my laboratory window; notice how much this mass resembles the shape to be expected of a liquid droplet. Because the individual bees tend to crawl from the surface of the mass into its interior, the net result is as if the surface were contractile and tended to minimize its area. When sugar/water was sprayed on the mass, the bees became more willing to remain at the surface; so the effect was equivalent to a surfactant.

A comparable example is the tendency of aggregates of dissociated embryonic cells to round up, as if they collectively possessed a surface tension. One hypothesis is that the cells tend to maximize their areas of contact with one another, with the minimization of the area of the exposed surface being a secondary consequence. A rather different mechanism, which would have exactly the same net effect, would be for the cell cortices to contract actively (as by actomyosin filaments), but for this cortical contractility to be inhibited over those parts of the cells' surfaces that lie directly adjacent to another cell; this would mean that the strongest cortical contractility would be in those areas directly exposed to the medium. In that case, the minimization of exposed area would be the primary cause, while the maximization of

contact area would be the secondary effect. Note that if the sum of exposed surface area and contacted surface area is a constant, then minimization of the first will equal the maximization of the latter.

The preceding examples are meant to illustrate that it is the rules obeyed by forces that determine what shapes these forces will create and render stable. Surface tension contracts the surfaces of liquids with a force that is isotropic and (usually) homogeneous; any other forces that contract a flexible surface (or resist its expansion) isotropically and homogeneously will generate this same range of shapes. The force need not tend to minimize energy or have any other specific property. Physical similarities and analogies to inorganic shape can be helpful when they show us some of the shapes that can be rendered stable by forces obeying certain combinations of rules; but these same analogies can also be very misleading. We must avoid the conclusion that similar shapes imply causation by the same forces; but it is still more important to realize that the range of geometric shapes that can be generated and rendered asymptotically stable (even by quite simple combinations of force-rules) is not limited just to that paltry range of shapes that liquid surface tension can create. Thus, in the attempt to explain morphogenesis in terms of physical forces, we need not feel ourselves limited to those few organisms whose contours happen to mimic soap bubbles. Other force-rules can create other shapes, and the range of variation that can be achieved has only started to be explored by advanced mathematics and computer simulation.

A good example of the dependence of shape on the rules obeyed by forces is the familiar (but odd) behaviour of elongate rubber balloons when these are partly inflated. Such balloons develop a fat end and a thin end, with such different tensions in the two parts of their surfaces as to make it seem almost impossible that the air pressure is the same in both places (as it is). Not only this effect, but also the greater pressure required to initiate the inflation than to continue it, turn out to result from a subtle non-linearity in the elasticity of rubber (see Chater & Hutchinson, 1984). If stress in rubber happened instead to be exactly (linearly) proportional to strain, then neither effect would occur. So imagine what would happen if you could make a balloon out of a material whose rules of contractility could be changed at will; when the elasticity was non-linear (as in actual rubber) the balloon would adopt one set of shapes; but if the elasticity were then made linear, the forces would cease to be balanced and the balloon would jump to some new shape. The same rules produce the same shapes; different rules (can) produce different shapes; a large enough change in the rules obeyed by forces will produce 'spontaneous' jumps to new shapes.

Of course, we want to explain anatomical shapes in terms of the actual forces (such as traction) that create and maintain them; but to achieve this goal, we must not be distracted from also asking what it is about these forces that decides which particular shapes they will create and maintain. My suggested answer is that the shape forces produce is determined by the rules by which the strengths of the forces vary as functions of the existing geometry. Consider how the homeostasis of body temperature depends on having the rates of heat production and heat dissipation vary as functions of the existing temperature to the body. Likewise, in the earlier

examples of the boat on the river and the weight hanging from the spring, the property of gravitating to one configuration in preference to others, depended on having the relative strengths of the opposing pairs of forces vary as functions of geometry. The same was true of the quartz–halogen lamp and we ought to expect similar principles to apply to cellular forces.

If we extrapolate to the problem of how to cause some anatomical structure to have this same kind of stability, the requirements seem to be these; first, you need physical forces that have sufficient strength to change the geometry of the system, as well as other forces that can resist or counterbalance these; second, you need the relative strengths of the forces to vary as functions of the existing geometry. The forces that can change geometry must themselves vary in strength as functions of the same geometrical properties that they change. Only if the forces vary as functions of geometry can they be stably counterbalanced when the right geometry exists, but not otherwise (Fig. 9).

It is essential to realize, however, that variations in the relative strengths of forces can occur for several different kinds of reasons. The change can be because of what amounts to leverage, as when the net force exerted perpendicular to a stretched sheet varies locally in proportion to the curvature. Or, alternatively, you can have actual inhibitions or stimulations of the force generators themselves. For example, suppose that crowding cells together weakens their traction; this will tend to even out their distribution. Conversely, the increased strength of fibroblast traction that occurs in response to trauma (as well as explantation) seems well designed as a homeostatic mechanism (Fig. 10) for constricting sites of injury, either for closing surface

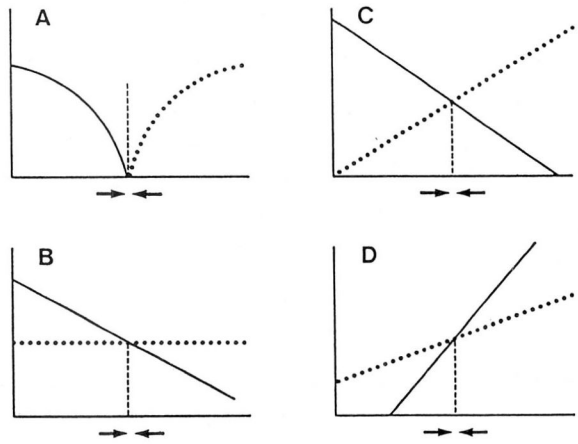


Fig. 9. Stability results from a balance of opposing forces. At which point the stability will occur depends on the rules by which the forces vary, in this case as functions of position. If the continuous lines represent the strengths of forces acting towards the right, while the dotted lines represent the strengths of forces acting towards the left, then for each of these four combinations of force-rules, asymptotic stability will occur at the points towards which the arrows point. Notice how different the rules can be and still produce the same result.

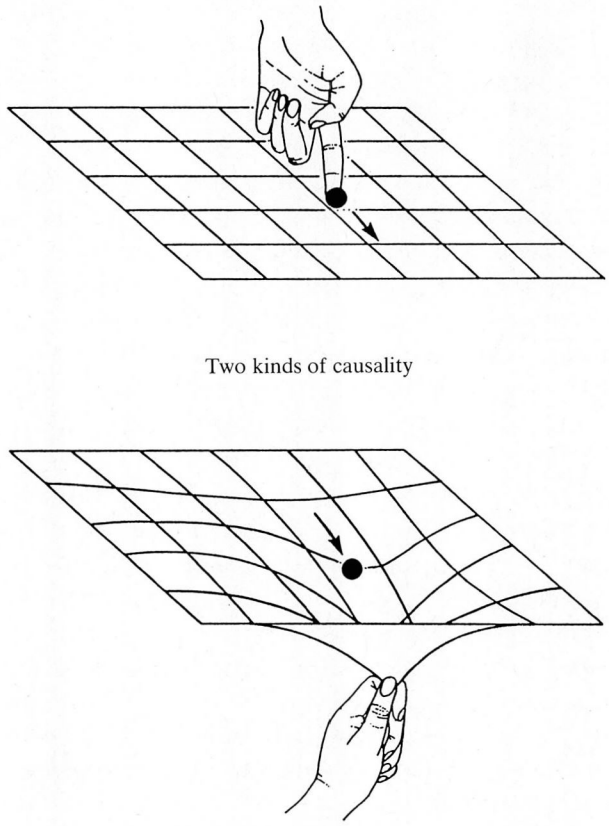


Fig. 10. Two fundamentally different ways of causing a certain combination of variables to come into existence: either (top) by imposing some outside force on the system, or (bottom) by changing the rules of interaction among the component parts, so that the desired combination of variables will arise spontaneously. The bottom sketch is meant to represent the kind of geometric homeostasis discussed in this paper.

wounds or for bringing torn tissues back together in the case of sprains or broken bones.

We should also realize that the absolute strengths of opposing forces will not need to fall to zero in order to achieve a stable balance. For example, in the case of the original observations of contact inhibition, Abercrombie & Heaysman (1953) reported that cell motility was reduced, but by no means completely stopped, in cells having many contacts; such cells reduced their movement only by about half. Others have sometimes concluded that contact inhibition could not be enough to control cell invasiveness. But, depending on what other controlling or countervailing effects are acting, an inhibition by half may be all that is needed. The forces acting on a soap film do not become zero when the stable shape is achieved; they merely become equal and opposite.

Conversely, we also need to bear in mind that no new forces need to come into existence in order to shift a state of force balance; an existing force may instead

become (relatively) stronger, or its opposing force could become weaker. The crucial thing is the rule by which the opposing forces vary; if you change this rule, the forces will shift the geometry to a new balance; and if you change the rule too much, the old state can cease to be stable at all, so that the components jump (spontaneously) to some new and perhaps very different configuration. By saying 'spontaneously', I mean that forces unrelated to those actually causing the change will become able to initiate it. Unless the experimental biologist consciously prepares his mind for such effects, he will have little chance of escaping the worst misinterpretations.

These considerations should also be relevant to the problem of invasive locomotion by neoplastic cells. To the extent that structural cells of our body are continually using their motility and traction for purposes of renewal and repair, it is not the motility of malignant cells that we should view as abnormal. What is wrong is that their propulsive forces somehow fail to obey the proper rules.

CONCLUSIONS

The constant renewal and repair of body structures, in sponges and also in higher animals, seems to require the equivalent of homeostatic mechanisms for arranging cells, collagen and other materials into their correct geometric arrangements. By using rubber and collagen substrata, it has been shown that fibroblasts exert shearing forces (traction) of sufficient strength to achieve large-scale geometric rearrangements of cells and matrix. It is a mistake to think that the tendency of a system to gravitate homeostatically to some particular configuration should require or imply energy minimization; the actual requirement is balance between opposing forces, the relative strengths of which need to vary as functions of the geometric properties to be controlled.

I am indebted to my collaborators David Stopak, Patricia Greenwell, Calhoun Bond, Mark Leader, Barbara Danowski, John Dmytryk and Sally Gewalt. I also thank Susan Whitfield for preparation of illustrations and Elizabeth Harris for help with the manuscript.

REFERENCES

ABERCROMBIE, M. A. & HEAYSMAN, J. E. M. (1953). Observations on the social behavior of cells in tissue culture. I. Speed of movement of chick heart fibroblasts in relation to their mutual contacts. *Expl Cell Res.* **5**, 111–131.

ABERCROMBIE, M. A. & HEAYSMAN, J. E. M. (1954). Observations on the social behavior of cells in tissue culture. II. 'Monolayering' of fibroblasts. *Expl Cell Res.* **6**, 293–306.

ANKEL, W. E. & EIGENBRODT, H. (1950). Uber die Wuchsform von Spongilla in sehr flachen Raumen. *Zool. Anz.* **145**, 195–204.

BASSETT, C. A. L. (1971). Biophysiological principles affecting bone structure. In *The Biochemistry and Physiology of Bone* (ed. G. H. Bourne), vol. 3, pp. 1–76. New York: Academic Press.

BOND, C. (1986). Locomotion of entire sponges is a result of amoeboid locomotion of their component cells. *Am. Zool.* **26**, 14A.

BULLOUGH, W. S. (1975). Mitotic control in adult mammalian tissues. *Biol. Rev.* **50**, 99–127.

CHATER, E. & HUTCHINSON, J. W. (1984). Mechanical analogs of coexistent phases. In *Phase Transformations and Material Instabilities in Solids* (ed. M. E. Gurtin), pp. 21–36. Orlando: Academic Press.

CURREY, J. (1984). *The Mechanical Adaptations of Bones*. Princeton: Princeton University Press.

DANOWSKI, B. A. & HARRIS, A. K. (1986). A time lapse study of phorbol ester effects on the contractile and adhesive properties of fibroblasts in tissue culture. *J. Cell Biol.* **103**, 428a.

DETTINGMEIJER, DITTMER, G., KLOPFER, A. & SCHRODER (1975). Regenerative chemical changes in tungsten-halogen lamps. *Phillips tech. Rev.* **35**, 302–306.

GABBIANI, G., MAJNO, G. & RYAN, G. B. (1973). The fibroblast as a contractile cell: the myofibroblast. In *Biology of Fibroblasts* (ed. E. Kulonen & S. Pikkarainen), pp. 139–154. New York: Academic Press.

HARRIS, A. K., STOPAK, D. & WARNER, P. (1984). Generation of spatially periodic patterns by a mechanical instability: a mechanical alternative to the Turing model. *J. Embryol. exp. Morph.* **80**, 1–20.

HARRIS, A. K., STOPAK, D. & WILD, P. (1981). Fibroblast traction as a mechanism for collagen morphogenesis. *Nature, Lond.* **290**, 249–251.

HARRISON, F. W. (1972). The nature and role of the basal pinacoderm of *Corvomeyenia carolinensis* Harrison (Porifera: Spongillidae). A histochemical and developmental study. *Hydrobiology* **39**(4), 495–508.

HIRSCH, M. W. & SMALE, S. (1974). *Differential Equation, Dynamical Systems and Linear Algebra*. New York: Academic Press.

HYMAN, L. H. (1940). *The Invertebrates*, vol. I. *Protozoa through Ctenophora*. New York: McGraw-Hill.

LEADER, M., STOPAK, D. & HARRIS, A. K. (1983). Increased contractile strength and tightened adhesions result from reverse transformation of CHO cells by dibutyryl cyclic adenosine monophosphate. *J. Cell Sci.* **64**, 1–11.

LANGLEY, L. L. (1973). *Homeostasis: Origins of the Concept*. Stroudsburg, Penn.: Dowden, Hutchinson & Ross.

LORD, B. I., POTTEN, C. S. & COLE, R. J. (1978). *Stem Cells and Tissue Homeostasis*. Cambridge University Press.

OSTER, G. F., MURRAY, J. D. & HARRIS, A. K. (1983). Mechanical aspects of mesenchymal morphogenesis. *J. Embryol. exp. Morph.* **78**, 83–125.

SODEK, J. (1977). A comparison of the rates of synthesis and turnover of collagen and non-collagen proteins in adult rat periodontal tissues and skin using a microassay. *Archs Oral Biol.* **22**, 655–665.

STEINBERG, B. M., SMITH, K., COLLOZO, M. & POLLACK, R. (1980). Establishment and transformation diminish the ability of fibroblasts to contract a native collagen gel. *J. Cell Biol.* **87**, 304–308.

STOPAK, D. & HARRIS, A. K. (1982). Connective tissue morphogenesis by fibroblast traction. I. Tissue culture observations. *Devl Biol.* **90**, 383–398.

STOPAK, D., WESSELLS, N. K. & HARRIS, A. K. (1985). Morphogenetic rearrangement of injected collagen in developing chicken limb buds. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2804–2808.

THOM, R. (1975). *Structural Stability and Morphogenesis: An Outline of a General Theory of Models*. Reading, Mass.: W. A. Benjamin.

THOMA, R. (1893). *Untersuchungen uber die Histogenese und Histomechanik des Gefasssystems*. Stuttgart: Verlag Ferdinand Enke.

THOMPSON, D'A. W. (1942). *On Growth and Form*. Cambridge University Press.

WILSON, H. V. (1908). On some phenomena of coalescence and regeneration in sponges. *J. exp. Zool.* **5**, 245–258.

WEISSENFELS, N. (1981). Bau und Funktion des susswasserschwamms Ephydatia fluviatilis L. (Porifera). VIII. Die Entstehung und Entwicklung der Kragengeisselkammern und ihre Verbindung mit dem ausfuhrenden Kanalsystem. *Zoomorphologie* **98**, 35–45.