

Generation of spatially periodic patterns by a mechanical instability: a mechanical alternative to the Turing model

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SUMMARY

We have studied the generation of spatial patterns created by mechanical (rather than chemical) instabilities. When dissociated fibroblasts are suspended in a gel of reprecipitated collagen, and the contraction of the gel as a whole is physically restrained by attachment of its margin to a glass fibre meshwork, then the effect of the fibroblasts' traction is to break up the cell-matrix mixture into a series of clumps or aggregations of cells and compressed matrix. These aggregations are interconnected by linear tracts of collagen fibres aligned under the tensile stress exerted by fibroblast traction. The patterns generated by this mechanical instability vary depending upon cell population density and other factors. Over a certain range of cell concentrations, this mechanical instability yields geometric patterns which resemble but are usually much less regular than the patterns which develop normally in the dermis of developing bird skin. We propose that an equivalent mechanical instability, occurring during the embryonic development of this skin, could be the cause not only of the clumping of dermal fibroblasts to form the feather papillae, but also of the alignment of collagen fibres into the characteristic polygonal network of fibre bundles – which interconnect these papillae and which presage the subsequent pattern of the dermal muscles serving to control feather movements.

More generally, we suggest that this type of mechanical instability can serve the morphogenetic functions for which Turing's chemical instability and other reaction-diffusion systems have been proposed. Mechanical instabilities can create physical structures directly, in one step, in contrast to the two or more steps which would be required if positional information first had to be specified by chemical gradients and then only secondarily implemented in physical form. In addition, physical forces can act more quickly and at much longer range than can diffusing chemicals and can generate a greater range of possible geometries than is possible using gradients of scalar properties. In cases (such as chondrogenesis) where cell differentiation is influenced by the local population density of cells and extracellular matrix, the physical patterns of force and distortion within this extracellular matrix should even be able to accomplish the spatial control of differentiation, usually attributed to diffusible 'morphogens'.

INTRODUCTION

The development of a spatial pattern by an initially homogeneous tissue requires some sort of autocatalytic instability; one capable of magnifying the

smallest perturbations into spontaneously forming, stable heterogeneities. Turing (1952) showed mathematically how certain combinations of chemical reaction kinetics and diffusion rates should result in chemical instabilities, capable at least in principle of producing the desired kind of spontaneous morphogenesis – even when starting from a state of total homogeneity. Subsequently, some actual combinations of chemicals have been found which spontaneously generate patterns in approximately the desired way, and the general principles underlying all such ‘reaction-diffusion systems’ have become the subject of a large literature (Meinhardt, 1982).

The majority of patterns of interest to embryologists, however, are structural rather than chemical. We want to find out how the parts of our anatomy come to have the shapes and arrangements they do, rather than how chemicals can develop patterns of alternating high and low concentrations. The general assumption has been that chemical patterns must develop first, and that the actual mechanical formation of structures is to be understood as a secondary response of cells to previously developed chemical gradients. These gradients would then provide the cells with ‘positional information’ (Wolpert, 1971).

Rather in contrast to this viewpoint, we have previously encountered several cases in which complex yet regular spatial patterns are created directly by mechanical forces (traction) exerted by cells on extracellular matrices (Harris, Stopak & Wild, 1981; Stopak & Harris, 1982). In those systems, the spatial patterns generated depended upon pre-existing heterogeneities in the organ culture systems – heterogeneities such as local concentrations of contractile cells, rigid fixed points, and so on. Now we report a system in which patterns develop spontaneously from an initially homogeneous distribution of cells and collagen. The mechanical instability which is responsible appears to be in some respects analogous to the chemical instabilities postulated by Turing (1952); except that the ‘reactants’ are not diffusing chemical morphogens. Instead, the ‘reactants’ are actively crawling cells which use the collagen as substrata. Likewise, the interactions between these ‘reactants’ are by mechanical stress and strain, rather than by chemical catalysis. The possibility of such a pattern-generating system was suggested to us by the phenomenon called crazing, which occurs in polystyrene and many other plastics when excessive tensile stress causes them to form what seem to be cracks – but which are really zones of fibre re-alignment (Fig. 1).

The formation of feather papillae in embryonic bird skin is one of the best studied of all cases of pattern formation in vertebrates. The papillae form as clusters of dermal fibroblasts just beneath the epidermis, which thickens and then bulges outward over them, eventually to differentiate into the feather itself (Sengel, 1976). Although these epidermal thickenings become visible before the mesenchymal condensations can be distinguished, grafting experiments indicate that specification of the pattern resides in properties of the mesenchyme. The

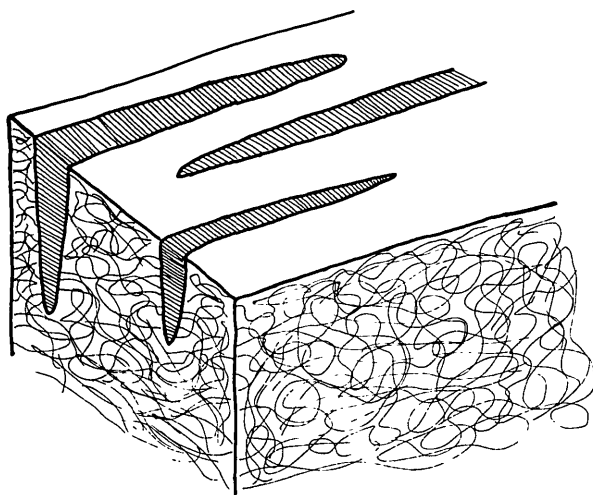


Fig. 1. A simple and easily observed analog of the type of physical instability described here: crazing in thermoplastics. When subjected to sufficiently strong tensile stress, many plastics composed of polymer chains (polystyrene, for example) yield locally by the straightening of these strands to form regions of 'craze matter' (Spurr & Neigisch, 1962). These regions, because of their altered refractive index, appear to be cracks. We have found that collagen gels are subject to a similar periodic yielding when stressed by the traction of dispersed fibroblasts.

problem, then, is to understand the mechanism which creates these papillae and their geometric spacing.

An important clue to the nature of this morphogenetic mechanism is the coordinated development in the skin of aligned bundles of collagen fibres. These interconnect adjacent pairs of dermal papillae, so as to form a network of interlocking polygons (Stuart & Moscona, 1967; Wessells & Evans, 1968). Although these polygons consist of a mixture of triangles and squares, this is the pattern which has somehow come to be called 'hexagonal'. These aligned bundles of collagen presage the formation of an intricate network of ligaments and muscles which serve to crosslink the feather bases in the mature bird and control their movements in flight (Lucas & Stettenheim, 1972).

The causal relationships between the formation of these collagen fibre bundles and of the papillae which they interconnect have been the subject of two sorts of hypothesis: either that the fibre bundles determine the papillae because cells migrating along them aggregate where they collide (Stuart, Garber & Moscona, 1972; see also Weiss & McMurray, 1959, for a similar explanation for the

formation of teleost fish scales), or alternatively that the fibre bundles might be secondary consequences of the papillae, perhaps created by their concentrated traction (Harris *et al.* 1981). However, what we have now found is that fibroblast traction is capable, in principle, of both generating the cellular aggregations and of simultaneously aligning the collagen between them – both as simultaneous consequences of a simple mechanical instability.

MATERIALS AND METHODS

Cell culture

Dispersed populations of primary fibroblasts were prepared by dissociation of minced skin from stage-33 to -36 chicken embryos (Hamburger & Hamilton, 1951). The skin was first washed in calcium-/magnesium-free saline, then incubated for 20 min in 0.1 % trypsin, after which the cells were gently aspirated in culture medium. After dissociation, fragments containing epithelial cells remained and were allowed to settle by gravity and removed. The remaining cells consisted of nearly 100 % fibroblasts. These cells were washed twice in medium, collected by centrifugation, and dispersed onto the surface of a collagen gel. To determine cell population densities on the collagen, an eyepiece micrometer was used to count cells 6–12 h after plating. Cultures were prepared having initial cell densities ranging from 1×10^4 to 1×10^5 cells per cm^2 . In addition, equivalent cultures were also prepared by dissociation of skeletal muscle from embryos of the same age; however, these cultures contained a mixture of myoblasts and fibroblasts.

Culture medium in all cases consisted of Dulbecco's modified Eagle's medium with 25 mM-HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid), 10 % foetal calf serum and 50 $\mu\text{g}/\text{ml}$ -gentamycin (all from GIBCO, Grand Island, NY).

Preparation of collagen gels

Gels of reprecipitated collagen were prepared using the method of Ehrman & Gey (1956) as modified by Elsdale & Bard (1973). Rat tails were frozen and then thawed in 70 % ethyl alcohol, and the tendons were then pulled out mechanically. After separation from other tissues, these tendons were cut into short fragments and placed in 0.5 M-acetic acid at 4 °C, with all subsequent steps up to gelation also being carried out at this temperature. Tendon fragments from two tails were dissolved for 48 h in 100 to 200 ml of acetic acid (depending upon the amount of tissue obtained), after which time the collagen expanded to fill the total volume. This solution mixture was then dialysed for 24 h against two changes of 4 l each of Dulbecco-Vogt's modified Eagles medium (DMEM) diluted to 1/10th normal strength and at a pH of 4.0. The remaining insoluble collagen and other materials were removed by centrifugation at 25 000 r.p.m. in

a 35Ti rotor on a Beckman L-4 ultracentrifuge. The viscous supernatant was used to make the collagen gels. The concentration of the collagen solutions was determined by the method of Lowry, Rosenbrough, Farr & Randall (1951). To prepare collagen gels, ingredients were mixed in the following proportions: five parts collagen solution, two parts $5\times$ DMEM (with HEPES and gentamycin), one part 0.15 M-NaOH, and one part 1/10 DMEM. Gelation of these mixtures occurred within 10 min at 37°C. The final concentration of collagen in these gels was from 1.3 to 1.7 mg/ml. Suspensions of cells dispersed in culture medium were allowed to settle out onto the upper surfaces of these collagen gels.

Mechanical restraint of collagen contraction

Collagen gels were prepared and cells cultured in commercial 35 mm diameter, 10 mm deep polystyrene Petri dishes. First, 2 ml of collagen solution was poured into a dish, allowed to cover the bottom and was then gelled by warming to 37°C.

In those experiments in which free contraction of the collagen by cell traction was to be permitted, the relatively non-adhesive, bacteriological Petri dishes were used (instead of tissue culture dishes) to minimize the attachment or other unintended mechanical interactions between the culture container and either the collagen gels or the cells. In these cases, the newly formed gel was also gently pulled loose from any attachments to the dish using a probe.

Two methods were used to study the effect of mechanically restraining the contraction of the collagen gels. One was simply to use the relatively adhesive 'tissue culture' dishes whose surfaces have been mildly oxidized by the manufacturer in a low air pressure radio frequency discharge (Falconization). A more effective mechanical restraint was achieved using Whatman GF/C glass microfibre filter paper. 10–15 mm holes were punched in 24 mm filters using an ordinary conductor's punch, the punched filter was placed in a Petri dish and the collagen solution was poured into this punched hole so that the collagen gel formed would extend several mm into the glass fibre matrix and be firmly gripped by it.

Fluorescent labelling of collagen

Collagen was labelled by covalent conjugation to fluorescein isothiocyanate (FITC) following procedures designed for the preparation of fluorescent antibodies (Nairn, 1969). The pH of the collagen solution was raised to 9.5 by dialysis against a 0.25 M-carbonate buffer. The collagen was then diluted 1: 1 with this buffer (from an original concentration of 3.2 mg/ml), while FITC was added progressively to a final concentration of 0.08 mg per mg of collagen. After mixing on a clinical rotator for 24 h at 4°C, the collagen was separated from unbound FITC by gel filtration on a Sephadex G-25 column. Several eluting buffers were tried; 0.05 M-phosphate plus 0.25 M NaCl gave good results. After recovery from the column, the FITC-collagen was first dialysed against dilute acetic acid, and

then further dialysed twice against 1/10× DMEM. Collagen gels were then prepared from this labelled collagen in the usual way.

Microscopy and photographic techniques

Time-lapse films were made by Zernike phase-contrast microscopy of the cells moving on the collagen gels using a one-minute interval between exposures. Bolex 16 mm cameras were used, sometimes with a Wild intervalometer and a Zeiss IM35 inverted microscope and Zeiss 2.5×, 10×, or 16× Neofluar objectives, and in other cases using a Sage intervalometer and an Olympus CK inverted microscope with 10× objective. Kodak 16 mm plus-X reversal film was used and processed commercially. In addition to filming, cultures were photographed periodically with Kodak Plus-X 35 mm film. For fluorescence microscopy, the Zeiss IM35 microscope was configured for epi-illumination with an HBO 50 mercury arc and BG12 excitation filter and standard barrier filter. Kodak Tri-X film was exposed for periods of 2–8 s and developed in diafine and 'pushed' to an effective ASA of 1600.

OBSERVATIONS

Formation of periodically spaced condensations by cell traction

As fibroblasts spread on the surface of the collagen gels and extend themselves into the interstices of the collagen fibre network, traction forces exerted by the cells pull centripetally against the collagen fibrils. A progressive distortion of the collagen gel matrix is observed by time-lapse photography, and an overall contraction of the entire gel becomes apparent macroscopically within 24 h. The net morphological effect of fibroblast traction on a collagen gel depends upon what mechanical forces restrain the overall shrinkage of the gel itself. When there is no such mechanical restraint, the whole gel becomes compressed into a smaller and smaller area and volume, as has previously been described (Bell, Ivarsson & Merrill, 1979; Harris *et al.* 1981). Within days, the cumulative cell traction will reduce a collagen gel to 0.1 % or less of its initial volume. As has also been described, the effect of restraining this shrinkage of a collagen gel at a series of separate individual fixed points, physically analogous to a series of nails driven through the gel, is to bring about a strong alignment of both collagen fibres and fibroblasts into taut strands stretched around and between the fixed points (Bell *et al.* 1979; Stopak & Harris, 1982).

However, when the contraction of the collagen gel is firmly restrained by mechanical attachment continuously all around its periphery (either to a glass fibre 'doughnut', as described in the methods section, or more simply by adhesion to a wettable polystyrene culture dish) fibroblast traction produces instead a complex internal rearrangement of both cells and collagen. When the overall contraction of the collagen matrix is mechanically prevented by rigid and

continuous restraint at the periphery, the cumulative effect of the fibroblasts' traction is to break up the initially homogeneous collagen gel into a series of compacted clumps. These clumps of cells and collagen are interconnected by strands of stretched collagen fibres (see Fig. 2). At points distributed from 0.1 to 0.8 mm apart through the matrix, the actively motile fibroblasts coalesce and aggregate gradually together. In time-lapse films, taken at high magnification, these aggregating cells can be seen to pull on the collagen fibres and to draw both collagen and other fibroblasts together into the developing aggregations. By this process of fibroblast traction pulling on the collagen, the developing aggregations progressively enlarge themselves, and collagen becomes further compacted into and onto the accumulating masses of fibroblasts. These cells' traction draws still more collagen, and the additional fibroblasts attached to that collagen, into the developing aggregations. This traction has the additional effect of aligning collagen fibres into linear tracts running directly between adjacent concentrations of cells (Fig. 3).

The initiation of this process of cluster formation was difficult to record but seemed to require that the distribution of cells within the preparation be uneven in one or another respect. When the cells' population density was made perfectly

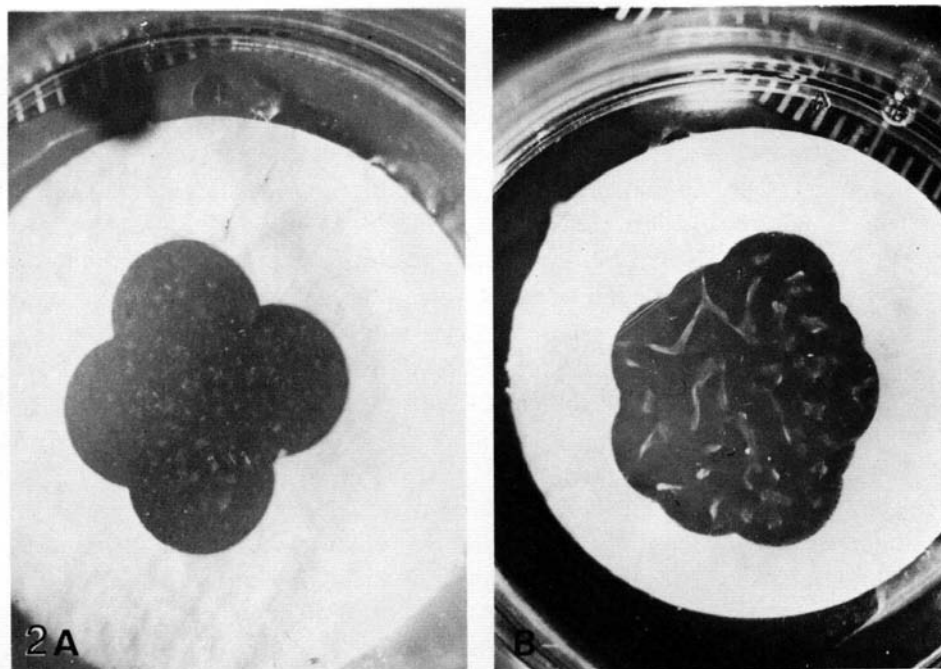


Fig. 2. Very low magnification views of 'doughnut' preparations, with reprecipitated collagen gels supported mechanically around their peripheries by attachment to glass-fibre filters. A millimeter scale is visible beside each culture. A. Punctate pattern of clumping which develops when fibroblasts are plated onto gels at an initial density of 4×10^4 cells/cm². B. Larger and more elongate clumps form when fibroblasts are plated at a higher population density, 7×10^4 cells/cm².

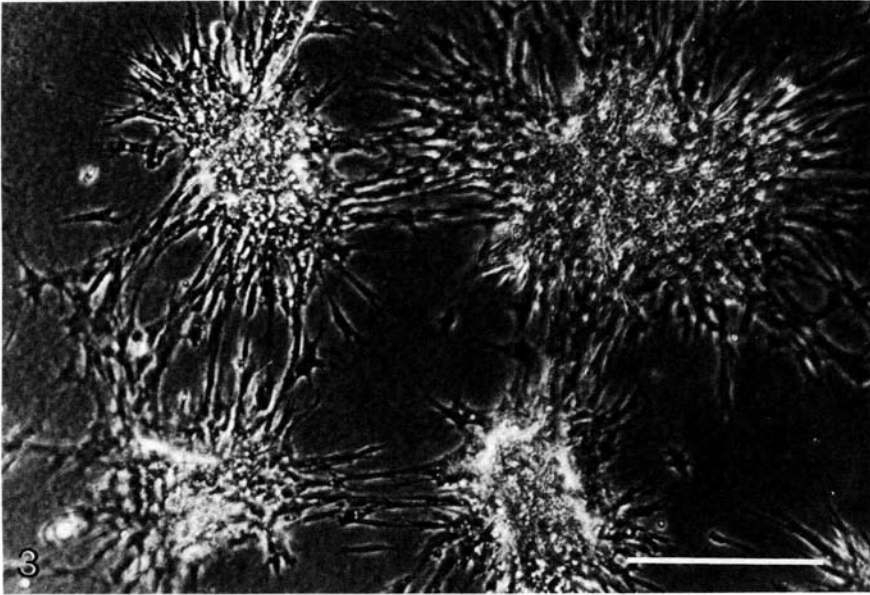


Fig. 3. High magnification view of the completed pattern, showing similarity to the normal anatomical pattern of papillae and interconnecting bundles of aligned collagen and fibroblasts. The scale bar is $200\text{ }\mu\text{m}$ long.

homogeneous, then the condensation process failed. Several different kinds of heterogeneities were found to be effective in triggering the instability; both locally increased and locally decreased cell population densities had this effect. We originally introduced local discontinuities in the population density by cutting a small hole in the gel before the cells were plated onto it and then after plating filling this hole with cell-free collagen. We subsequently found that areas where fewer than the average number of cells had settled were equally effective as initiators, as were gradients of population density created by plating the cells from uneven depths of medium. Physically torn edges in the collagen gel itself were also effective initiators of this progressive spontaneous subdivision of the cell–collagen mixture into aggregations.

Centres of higher cell density began to form on the second day in culture, and continued to increase in density over the course of the following 2 to 3 days; however, this time course showed considerable variability between different sets of culture preparations (Fig. 4). We do not yet understand the factors which cause this unpredictable variability, except insofar as such behaviour is presumably to be expected of spontaneous instabilities. Time-lapse films taken during the condensation of the cells into aggregations show that, although the cells continue their active to-and-fro locomotion throughout this period, the progressive concentration of cells occurs primarily because these cells are pulled passively into the aggregations (along with the collagen to which they are

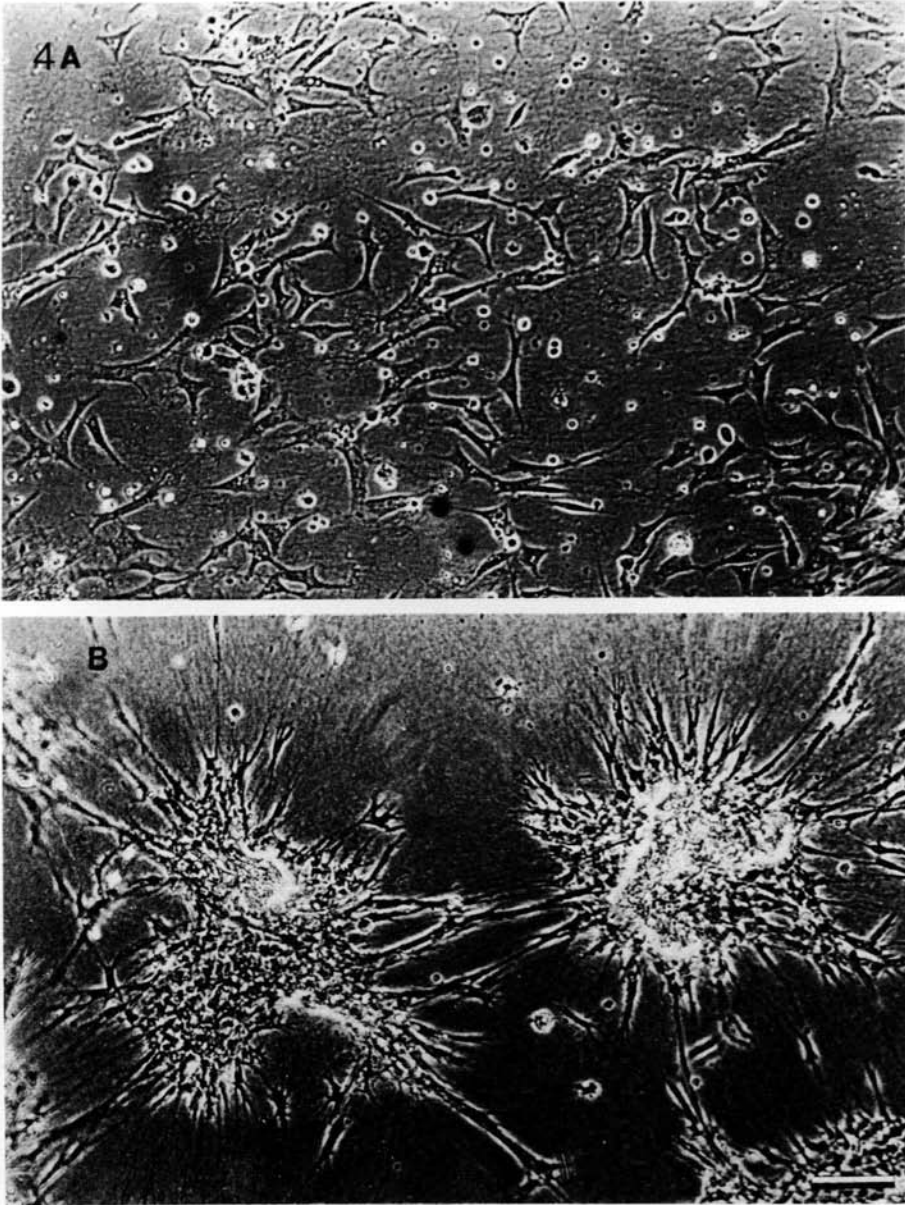


Fig. 4. Time sequence of pattern development. A. 24 h after plating, fibroblasts are still evenly distributed. B. After 6 days, the formation of periodic condensations is complete. The scale bar equals $100\text{ }\mu\text{m}$.

attached). The net displacement is thus apparently less the result of the aggregating cells' own locomotion than it is the result of traction exerted on the collagen meshwork by the cells already concentrated into these aggregations. The cells appear to be pulled passively into the aggregations. Cells peripheral to these

growing aggregations were often drawn inward as a group, almost as if carried on a conveyor belt.

Individual cells in these films could also be observed being passively stretched and aligned between these aggregations. Cells attached to the matrix were moved along with it as concentrations of cellular traction pulled the matrix from place to place (convection), and this occurred even when the locomotion of these individual cells happened to be propelling them in a different direction, relative to the matrix itself. Periods of rapid inward convergence toward the aggregations often alternated with periods of relative stability, in which few additional cells were added to these clusters. During these periods of relative quiescence, cells could be seen to migrate to-and-fro, some toward and some away from the centres of aggregation, until another period of matrix retraction toward these centres began.

A stable state was eventually reached (after 2–3 days), in which the tensile strength of the aligned tracts of collagen resists the traction of the fibroblasts. The aligned collagen then remains tensely stretched between adjacent cell aggregations, balanced between them, instead of being drawn into either. The end result is thus a tessellation or interlocking network of polygons. These polygons are primarily rhombi (squares or parallelograms) and triangles (Fig. 3). The dense self-aggregations of fibroblasts and compacted collagen make up the vertices of these polygons, while the tracts of aligned collagen form the polygons' sides. The polygon networks formed in this way by dissociated fibroblasts plated out onto reprecipitated collagen gels, are generally less regular than the networks of feather papillae and interconnecting collagen tracts which are found in developing bird skin. The difference is often very marked. We have not been able to determine why the resulting pattern is so much more regular in some culture preparations (Fig. 3), and in some parts of preparations, compared with others.

Condensations also formed spontaneously when embryonic skeletal muscle (rather than dermis, as in the rest of these experiments) was used as the source of dissociated cells. In this case, myoblasts present in the dissociated muscle tissue fused and differentiated to form myotubes, and these even underwent irregular spasms of contraction. However, these muscle cells did not appear to participate directly in the formation of the condensations of fibroblasts and collagen. The lengths of these myotubes was such that they typically spanned two or more of the fibroblast aggregations. The tensile stress exerted by the fibroblasts in these aggregations caused stretching, elongation and eventually tearing of these myotubes. As the fibroblast aggregations developed, myotubes stretched between adjacent aggregations gradually became narrowed in their middles, before being torn in two. By the end of a week in culture, most myotubes had been disrupted by this tensile stress and had degenerated, apparently as a result of this mechanical fragmentation.

Effects of cell population density on the pattern of condensation

The size and spacing of the aggregations (or condensations) varied as a function of the original population density at which the cells had been plated out (Fig. 5). At densities below 10^4 cells/cm², cells spread and migrated on and through the collagen matrix, visibly distorting the gel in their immediate proximity. But at these low population densities, the cells' collective strength was apparently insufficient to produce the massive, permanent rearrangements of the matrix, which results in the formation of the condensations. Instead, only occasional, small and irregular aggregations were formed. At population densities of 2×10^4 cells/cm² small condensations of ten or fewer cells formed.

When the cell density was further increased, the numbers of cells composing each resulting condensation likewise increased. For example, at a plating density of 5×10^4 cells/cm², the condensations which formed had diameters averaging about 500 μ m. Each of these consisted of many hundreds of cells. At still higher population levels, a different pattern of condensation arose, instead of the punctate tessellation pattern. Between 7×10^4 and 1×10^5 cells per cm², instead of condensing into more or less round aggregations as at lower densities, the cells condensed into elongate columns which coursed irregularly through the matrix, roughly parallel to one another (Fig. 2B). Just as the rounded condensations

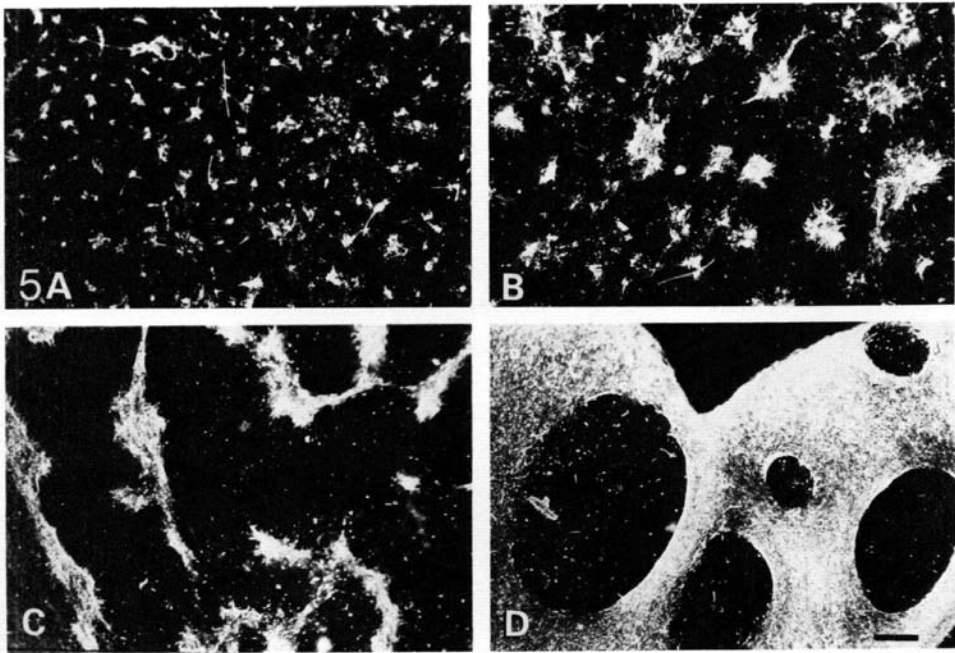


Fig. 5. Effects of differing initial population densities of fibroblasts on the resulting spatial pattern. Scale bar equals 400 μ m. A. 2×10^4 cells/cm². B. 4×10^4 cells/cm². C. 7×10^4 cells/cm². D. 9×10^4 cells/cm².

formed at lower cell concentrations were reminiscent of the dermal papillae of feathers or hair, these elongate aggregations formed at higher population densities had shapes roughly similar to the scales on developing avian hindlimbs. At initial cell concentrations of 1×10^5 or above a continuous monolayer of cells was quickly established which was mechanically stable and did not break up into condensations, but did develop small, irregular foci of locally increased cell density.

Observations on changes in matrix density using fluorescein-labelled collagen

As has been mentioned, the formation of the periodic aggregations of cells within the collagen matrix is accompanied by a corresponding redistribution and realignment of the collagen fibres. The packing density of collagen became markedly greater at the sites of cell aggregation and became depleted around them, except in the bundles of aligned fibres which formed connecting these aggregations. This much was apparent from phase-contrast microscopy, but to determine whether the original collagen of the gel was being drawn into these centres, as seemed to be the case (instead of perhaps being secreted there and degraded in surrounding areas) it was necessary to follow the redistribution of the fluorescent label covalently bound to the collagen.

The covalent conjugation of FITC to the isolated monomeric collagen fortunately did not inhibit its precipitation as a gel. Gelation of the FITC-collagen occurred within 10 min by the usual methods and the appearance of the resulting matrix under phase contrast optics was normal. These gels, made of fluorescently labelled collagen, supported normal cell growth and locomotion; no apparent differences in either the morphology or the behavior of cells were detected in the labelled, as compared with the unlabelled gels.

Before the addition of cells, and for about 24 h thereafter, the fluorescence of these labelled gels remained diffuse when observed with the fluorescence microscope, with an even intensity throughout. The fluorescence was distributed as a delicate meshwork having a finely textured and interwoven appearance, but without any overall direction orientation. This corresponds to the appearance of the gels by phase-contrast microscopy. Once the centres of aggregating cells began to form, locally bright regions of fluorescence also appeared, and these regions of concentrated fluorescence exactly matched the locations of the developing cell aggregates (Fig. 6). This progressive concentration of the fluorescent label indicates that the increased concentration of collagen fibres, within and around the cellular aggregations, must be due primarily to physical displacement of collagen from the original gel into the aggregations (as opposed to local secretion of new collagen, for example, although this may well occur, in addition).

As the fluorescently labelled collagen became concentrated into these aggregations, the level of fluorescence in the regions between the aggregations was correspondingly depleted. However detectable amounts of collagen (fluorescent label) did remain along linear regions running directly between the aggregations.

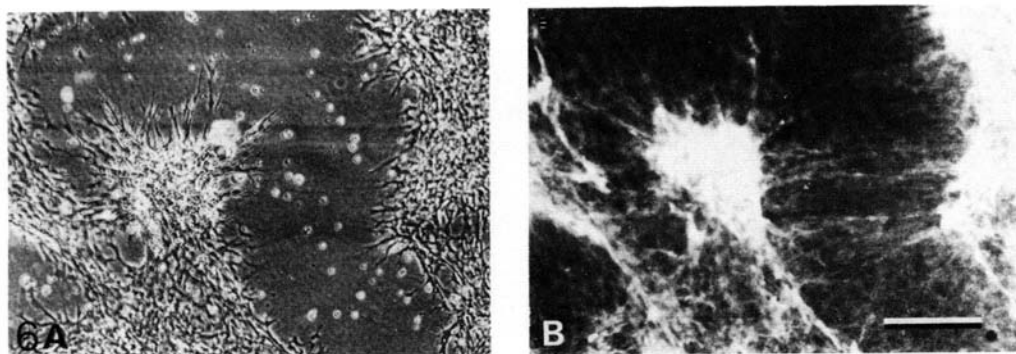


Fig. 6. Accumulation of fluorescently labelled collagen into papilla-like clumps. The scale bar equals 200 μm . A. Phase-contrast view. B. Fluorescence excitation view.

DISCUSSION

Convection

The displacement of cells by being pulled along bodily with the extracellular matrix is a type of morphogenetic cell movement which seems not previously to have been reported or hypothesized in the embryological literature. We propose to call this type of cell movement 'convection', as was suggested to us by George Oster, based on the analogy with the carrying of heat or substances from place by the flow of a liquid or gas. Not only is the etymology of the word convection quite appropriate (Latin for 'carry along with') but the mathematical expressions needed to treat this class of cell displacements turn out to be the same as those used for the convection of heat by fluids (see Murray, Oster & Harris, 1983). We would also propose that whenever cells within embryos are found to bunch together into compact masses (as for example in the initial condensation of skeletal rudiments), the possibility should be considered that these cells may also have been pulled along with extracellular matrices by a conveyor-belt-like effect of cell traction – rather than, for example, having become especially adhesive to one another. Note the similarity of convection to the phenomena aptly termed 'passive locomotion' by Trinkaus (1982), in which cells are pulled along by the locomotion of others to which they are attached.

The generation of spatial patterns by convection.

Our observations on the (admittedly experimental) system of fibroblasts dispersed on collagen gels suggest explanations for several aspects of normal dermal morphogenesis in birds. For one thing, these observations show that neither papillae nor the aligned tracts of collagen between them need be the cause of the other's formation, and that neither class of structure requires a chemical prepattern. Instead, our results suggest that both events (the aggregation of fibroblasts into papillae, as well as the alignment of collagen fibres into linear bundles interconnecting these aggregations) may, at least in principle, be

explained as parallel consequences of the same mechanical instability. This instability occurs when the initially homogeneous and isotropic dermal matrix yields plastically to fibroblast traction. The ability to generate patterns depends upon the tendency of both cells and matrix to be displaced toward sites of locally greater cell concentration (and thus of locally greater cell contractility). This movement of cells up local gradients of cell population density not only has the needed autocatalytic effect of magnifying small or even random differences in cell density (much as do the chemical reactions of Turing's model, though of course for different reasons), it also entails a capacity for lateral propagation of alternately concentrated and depleted cell population densities (Fig. 7). Propagation of the instability would occur because the aggregation of cells at one site depletes the surrounding area below the prevailing population level of the area beyond the depleted area. This depletion would thereby create additional gradients of population density, up which cells will be pulled to form secondary aggregations of cells at some distance from the first. These would then deplete the areas around themselves, and so on. The rectilinearity of the avian dermal pattern can be interpreted as a consequence of positive feedback between cell orientation by contact guidance along matrix fibres and matrix orientation by the traction exerted by the oriented cells, with new condensations tending to form where the resulting fields of tension intersect (cf. Stuart *et al.* 1972).

Both in spacing and size, the aggregations formed in our cultures were less regular than actual feather papillae, often very much so (see Figs 2, 5 & 6), although even these least regular cases compare favourably with patterns created by computer simulation of Turing's mechanism (Turing, 1952; Bard & Lauder, 1974). While we have not been able to determine the causes of this irregularity, or of its variation from case to case, the explanation may lie in the ability of these pattern-generating mechanisms (both Turing's and the one proposed here) to create their peaks either by autocatalysis of random perturbations or secondarily, by propagation from previously formed peaks nearby. In the former case, the pattern generated is apt to partake of the randomness which triggered it (Bard & Lauder, 1974), but in the latter case the spacing is determined by the ranges and strengths of the interactions involved and can therefore be very regular. Thus if the papillae in the skin are mostly secondary effects (of propagation), while in our cultures they are mostly primary, the contrast in regularity would result. In simple computer simulations of our model, this was the case. These considerations suggest the possibility that embryos solve the problem of achieving regularity of results, despite using stochastic mechanisms, simply by developing sensitivity to the instability progressively across a tissue field – so that most of the peaks will be secondary to pre-existing ones. Although we were unable to devise such a gradation of sensitivity in our cultures, we would suggest that if dermis could be caused to mature simultaneously over a broad area, and the papillae thus to form simultaneously, then their spacing would be less regular. This may be the reason for the lesser regularity of hair (cf. Davidson, 1983).

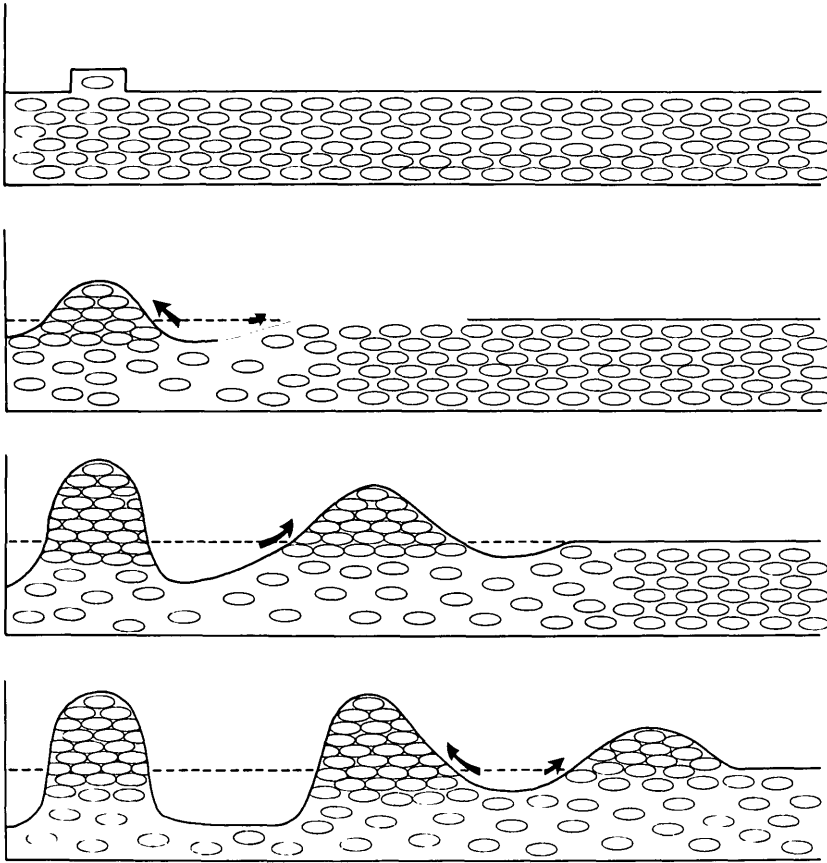


Fig. 7. Schematic diagram of the type of mechanical instability responsible for re-arranging collagen matrices into the patterns described here. When cells move up gradients of their own concentration (as well as pulling collagen fibres up these gradients), then even a small local deviation from the average population density will initiate an accumulation of cells. The accumulation of cells into a growing peak will deplete the surrounding area, thereby creating secondary gradients on the opposite side of the depleted areas. Since cells also move up these secondary gradients, the instability propagates itself. Thus the resulting pattern of alternating high and low population areas can develop either simultaneously over the field, if the system is excited by widespread initiating irregularities, or sequentially when initiated in one area from which the instability then propagates.

Several different factors may contribute to the antidiffusive tendency of cells to move up gradients of cell and matrix density. One is haptotaxis – the migration of cells up gradients of adhesiveness (Carter, 1967; Harris, 1973). As the cells are adhesive to sites on the collagen molecules, a gradient of collagen concentration should therefore be a gradient of adhesiveness for these cells (Murray *et al.* 1983). Among other factors which should contribute to the autocatalytic

aggregation effect is the simple physical tearing of the collagen matrix (which is indeed the most apparent feature in our time-lapse films of the phenomenon), non-linear proportionality between stress and strain in collagen, the contact guidance effects mentioned above, as well as any cooperative effects or mutual stimulation of contractility by cells crowded together. The situation within the stressed matrix is rather like a many sided tug-of-war in which pulling an opponent (cell) close enough to oneself adds it to your team (aggregation). The autocatalytic (or antidiffusive) consequence of such recruitment is obvious.

As to the possible operation of this pattern-generating mechanism in the development of bird skin, our observations on the effects of varying cell population density suggest that the formation of the bare areas ('apteria', see Lucas & Stettenheim, 1972) may be the result of insufficient traction (relative to the elastic resistance of the matrix) to pull fibroblasts together into aggregations; much as when too few fibroblasts are plated onto a collagen gel. This interpretation is supported by the observation of Wessells (1965) that the dermis is indeed less dense in presumptive apterial regions, as well by the observations that experimental implants which cause feathers to form in normally apterial regions first cause the appearance of a dense mesenchyme (Sengel & Kieny, 1967; Sengel, Dhouailly & Kieny, 1959). Also supporting this interpretation is the finding of Mauger (1972) that the induction of bare regions where feathers usually form is accompanied by the formation of a sparse dermis.

Likewise, the replacement of small, punctate aggregations by larger, elongated ones when cells are plated out at higher population densities, would seem to suggest an explanation for the replacement of feathers by the similarly broad scales (scuta and scutella) of avian feet. However, according to most workers, these scales lack well-organized papillae. Otherwise, we would suggest that the replacement of scales by feathers (ptilopody) in certain mutants, or in embryos treated with retinoic acid (Dhouailly, Hardy & Sengel, 1980), might prove explicable in terms of an inhibition of cell traction. In a similar way, the dependence of this type of instability on the maintenance of tensile stress may help to explain the results of Novel (1973), in which early feather germs dispersed when skin fragments were explanted. In addition, our observation of the ability of free edges to initiate this instability suggests an explanation for another of Novel's observations: that explants subsequently develop new papillae in rows parallel to their long edges.

Mechanical pattern generation and non-diffusible morphogens

The implications of this mode of pattern generation are not confined to the skin, or to birds. For one thing, certain morphological similarities suggest that a very similar self-propagating mechanical instability may be responsible for generating the pattern of scales in bony fish, even though these scales are essentially dermal bones, and as such not strictly homologous to the epidermal scales of birds. Weiss (1959) has reported the observation by McMurray in developing

teleost dermis of a pattern of collagen alignment nearly identical to that subsequently found in bird skin, but with the formation of bony scales (rather than papillae) at the fibre intersections. If one were to generalize the mechanism still further to include the mechanical interactions among the epidermal cells themselves, it might be able to explain the remarkable splitting of the feather germ itself to form its barb ridges.

Mechanical instabilities of the same basic kind may also help to explain certain features of the spatial control of cartilage differentiation. Thorogood & Hinchliffe (1975) have described the transient compaction of the presumptive chondrocytes which immediately precedes their differentiation as cartilage. This compaction could be causal, with the mechanical compression of cells and matrix locally stimulating the cells' differentiation, since it is known that chondroblast differentiation is promoted by cell density (Holtzer, Abbot, Lash & Holtzer, 1960) and by the concentration of extracellular matrix materials (Lash & Vasan, 1978). Thus the morphogen or pre-pattern for cartilage formation could be compressive strain, an explanation which is further developed for the case of the limb skeleton in a separate paper (Oster, Murray & Harris, 1984). This effect of compression may correspond to the second regulatory step in cartilage differentiation postulated by Solursh *et al.* (1982). The possibility is also tempting that the segmentation of the somites represents the operation of such a mechanism along a one-dimensional cell column. However, we note that because the segmental muscles and cartilages (vertebral arcualia) to which the somites give rise are spaced in alternation with each other (with the cartilage forming at the boundaries between somites and the muscles connecting these cartilages), therefore, if somite segmentation is mechanically analogous to the aggregation of dermal fibroblasts into papillae, then it would have to be the ends of the somites (where the cartilages form) which are mechanically equivalent to the dermal papillae. The somites themselves would have to be equivalent to the aligned bundles of matrix between these papillae.

Mechanical versus chemical pattern generation

For the generation of anatomical patterns, mechanical instabilities, such as those described here, differ in at least five important respects from chemical instabilities, like the Turing model. Several of these differences may prove useful as criteria for distinguishing between the operations of the two classes of mechanism. The first and most obvious difference is that mechanical instabilities yield actual physical structures, not just chemical 'blueprints' capable of guiding the subsequent formation of these structures by separate and unspecified forces. If mechanical instabilities have as much (or more) ability to create regular geometric patterns as chemical instabilities, why would evolution retain the latter? Physical forces are always necessary for morphogenesis anyway.

The second difference is the longer distance over which mechanical forces can be effective. Crick (1970) has estimated the maximum distance over which diffusion

gradients could be expected to stabilize within a reasonable period of time inside an embryo. His estimate was only about 1 mm, whereas the mechanically created patterns we have been encountering in organ culture have dimensions in the centimetre range (Stopak & Harris, 1982), as do many actual embryonic patterns – such as those of the dermis. The third difference is closely related and is the greater speed (in comparison with chemical diffusion) with which mechanical forces can be propagated within developing tissues – and perhaps more important, the greater speed at which these forces can reach equilibrium (see Oster *et al.* 1984). A fourth difference is that mechanical systems are much less vulnerable than diffusion gradients to disruption by flows of liquids through the developing field, as, for example, when the circulatory system begins to function. Conversely, pattern-generating systems which depend upon stress and strain should be more susceptible to mechanical interference, and this difference might be made to provide an experimental criterion for their identification.

The fifth and last of these differences is that mechanical instabilities depend upon tensor properties (like stress, strain and curvature), which can differ in value in different directions at each point in space. This is in contrast to chemical concentration, which as a scalar property has no inherent directionality, but only a quantity at each point. While it is true that fields of scalar properties can determine direction by their gradient (the gradient of a scalar field being a vector field), as in chemotaxis or in the determination of bristle alignment in the insect epidermis, the range of possible direction fields which can be determined in this way turns out to be quite circumscribed. In particular, there are many vector fields which are not the gradients of any possible scalar field. This is true of all vector fields whose curl is anywhere not equal to zero (Davis, 1961). These vector fields are called non-conservative, or rotational, and the familiar spiral whorl of hair alignment on the back of the human head (not to be confused with the curl of the hair itself!) is a good example of an actual anatomical pattern whose vector field has a non-zero curl. Thus, it is inherently incapable of being determined by a gradient of a morphogen concentration or of any other scalar property. Conversely, if certain anatomical patterns were found persistently to possess zero curl, then this could be taken as evidence of their determination by the gradient of some scalar property, such as chemical concentration.

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Our efforts to explain pattern generation in terms of mechanical interactions between cells have been inspired by the example of our friend and colleague, H. Eugene Lehman, whose work on the generation of pigmentation bands in salamanders (*J. of Experimental Zoology*, vol. 135, pp. 355–86, 1957) paralleled Turing's theory, in that it showed how certain combinations of mutual attraction and repulsion between cells could achieve much the same morphogenetic results as equivalent combinations of chemical autocatalysis and inhibition.

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