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A new approach to the generation of pattern and form in embryology

J. D. Murray and P. K. Maini

1. Introduction

A central issue in developmental biology is the formation of spatial pattern in the early embryo. From the homogeneous mass of dividing cells in the very early stage of development, emerge the vast range of pattern and structure observed in animals. The skeleton, for example, is laid down during chondrogenesis, when chondroblast cells condense into aggregates which lead via cartilage to bone formation. The skin, the major body organ of vertebrates, forms many specialized structures, for example, hair, scales, feathers and glands, as well as antlers and horns. Butterfly wings exhibit spectacular colours and patterns and many animals develop dramatic coat markings.

Although genes play a key role, genetics say nothing about the actual *mechanisms* which produce pattern and structure as an organism develops from the egg to its adult form. The development of structure and form is called morphogenesis and consists of a complex interaction of mechanical, chemical and electrical phenomena. In spite of a vast amount of research the mechanisms involved are not known. It is now a field of intense and genuine interdisciplinary involvement between theoreticians and experimentalists both of which can make major contributions. The aim is to try and elucidate the underlying mechanisms involved in embryology.

Several plausible models, or explanations, have been proposed for the formation of bones, skin organs, butterfly wing patterns and animal coat markings. Some of these are phenomenological interpretations of a set of rules, while others are based on chemical rections coupled with diffusion, for which mathematical models can be formulated. Reaction diffusion or Turing type models form a major class.[9,12–15,25] In these, hypothetical chemicals, called morphogens, react and diffuse and, under certain

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conditions the homogeneous steady state can evolve to a heterogeneous steady state. Cells may be able to interpret the local concentration of morphogen and differentiate accordingly, giving rise to patterned structure — this is the concept of 'positional information' proposed by Wolpert.[28,29] The reaction diffusion model of Thomas,[23] based on empirical chemical laws, can produce patterns observed in animal coat markings.[14] Reaction diffusion theory has had a considerable impact on the field, both in promoting research and in suggesting new experimental investigations. Recently, however, there has been a certain re-appraisal of it since the identification of any morphogens has proved particularly elusive. Also at most stages of development forces must be involved which effect embryological shape change: forces play no role in reaction diffusion models for morphogenesis.

A very different approach has been taken by G. F. Oster and J. D. Murray and their co-workers over the past few years (references will be given at the appropriate places below). They start with known experimental facts about embryonic cells and tissue involved in development and construct models which reflect these facts. Basically they take the view that mechanical morphogenetic movements themselves create the pattern and form. It is this new approach which we shall describe in this paper, in the context of two practical and specific instances of developmental pattern formation of current wide interest. The model analysis will be compared with experiment and will be used to make some specific biological predictions. No mathematics will be given. The phenomenology represented by the model will be described in equivalent word equations.

2. A mechanical model for feather germ formation

Here we shall concentrate on feather germ formation in chicks. This is a widely studied problem of considerable biological interest (for example, Refs. 2, 3, 21, 22). These structures are distributed across the surface of the animal in a characteristic and regular hexagonal fashion. As with other organ systems, this crucial developmental process is not understood. We first give a little biology to set the scene for the modelling.

Vertebrate skin is composed of two layers; an epithelial epidermis overlying a mesenchymal dermis, separated by a fibrous basal lamina (Fig. 1). These layers, or sheets, of epithelial cells can deform and buckle; there is very little movement of individual cells. The dermis is much thicker than the epidermis. It consists of connective tissue, collagen fibres, cells, blood, lymph vessels and sensory nerves. Sweat glands, hair follicles and feather rudiments project down from the epidermis into the dermis. Unlike epidermal cells, the dermal cells are loosely packed and motile. We shall concentrate here mainly on the initiation and subsequent appearance of feather rudiments in the dorsal pteryla — the feather forming region on the chick back.

The first feather rudiments become visible about 6 days after egg fertilization. Each

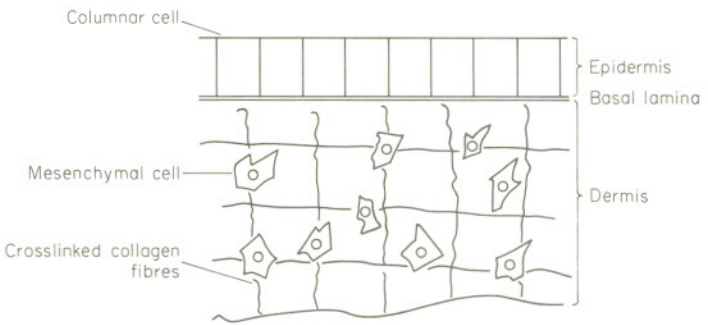


Fig. 1. Diagrammatic representation of chick epithelium at day 6. The epithelium is composed of two layers; an epidermis consisting of columnar cells, and a mesenchymal dermis, connected by anchor filaments. The dermis consists of mesenchymal cells in a jelly-like matrix, the extracellular matrix (ECM), composed of crosslinked collagen fibres and glycosaminoglycans (GAGs).

feather germ, or primordium, eventually consists of a thickening of the epidermis, called a placode, and a condensation of dermal cells, called a papilla. The placode is seen by an elongation of the basal cells perpendicular to the skin, while the dermal condensations are largely the result of cell migration, with localized proliferation playing a secondary role. Whether or not the placodes form prior to the dermal cell papillae, as has been suggested,[5,27] is a controversial issue. The papillae are very small and are difficult to assay. There is considerable experimental work going on to determine if, indeed, the dermal aggregates form first. The dermis exerts a very great degree of control on the epidermis — as shown by epidermal–dermal recombination experiments[20] — but both components are essential for skin organ development.

The mechanical model for dermal cell aggregation is based on the observations of cell behaviour *in vitro* by Harris *et al.*[10] They placed mesenchymal cells on a silicon rubber substratum and found that the cells migrated and exerted large tractions via long finger-like protrusions (lamellopodia) which deformed the substratum, tearing it in some cases. This suggests that cells *in vivo*, moving on the extracellular matrix (ECM) in the dermis (Fig. 1) may deform the ECM. It is also known that, *in vitro*, cell traction increases abruptly at a certain critical age (Harris, pers. comm.).

The model incorporates the traction properties of the cell and its interaction with the elastic properties of the ECM. It suggests the following scenario for mesenchymal cell aggregation and consequent pattern formation in the chick back. Cells are distributed uniformly throughout the domain, migrating and exerting traction forces which deform the matrix. Due to the random fluctuations everpresent in a biological system, the cell density will not be exactly uniform, there will be minor spatial variations. As the cells mature, their traction increases abruptly. Suppose that the random fluctuations are such that in some position there are more cells than in the surrounding neighbourhood. If the combined traction of this small clump of cells is

large enough, it may start recruiting neighbouring cells. As the clump increases in size, the combined traction increases and more cells are dragged towards it. This 'autocatalytic' effect leads to the establishment of an 'organizing centre' attracting more and more cells. Clearly there must be some limiting factors present, otherwise only one cell aggregate would form. There are, in fact, two such limiting processes. The first is the elasticity of the collagen fibres in the ECM. Far enough away from the organizing centre, the traction exerted by the aggregate is balanced by the elastic restoring forces in the stretched ECM, and a separate organizing centre may form. Secondly, as cells come into close contact with one another their metabolic rate decreases rapidly and their traction decreases. This phenomenon is called contact inhibition. Thus the combined traction exerted by the aggregate does not grow unboundedly, rather, it decreases, allowing other aggregates to form nearby. What the mathematical model does is to quantify the cell agregation size and the spacing in terms of the experimentally measureable parameters.

The dermal aggregation pattern exerts a strain on the epidermis leading to the formation of placodes and hence to the formation of feather germ primordia.

To set up a mathematical model to create such a scenario, we simply write down a set of equations which reflect the physical processes taking place. The actual mathematical formulation is rather technical and we refer the interested reader to the paper by Oster *et al.*[17] for full mathematical details. Here we shall present the salient features using word equations.

The model describes the variation of cell and ECM density with time and space, and the mechanical force balance between cells and ECM. Consider a small volume element of cell–matrix material. We need three equations to describe the situation in the element:

Cell conservation

[rate of change of cell density with time] = [flux of cells into element] − [flux of cells out of the element] + [cell proliferation]

The flux through the volume element is composed of:

Random dispersal. Cells move in random fashion throughout the matrix, their motion depending on their immediate surroundings. In the case of mesenchymal cells, however, there is a non-local or long-range diffusion term as well — the cells can detect non-local cell densities via their long lamellopodia, thus random motion depends on non-local cell density as well as the immediate neighbouring density.

Haptotaxis. Cells move by attaching their lamellopodia to certain specialized adhesive sites in the ECM. As the cells deform the matrix, a gradient in adhesive site density appears (Fig. 2) and cells have a tendency to move up such a gradient. The movement of cells up a gradient in adhesive site density is called haptotaxis.

Convection. Cells are passively dragged along on the ECM by the contraction forces exerted by their neighbours on the ECM.

These factors appear to be the important ones for cell flux. Experimentally, convection appears to be the key effect.

Fig. 2. Cell tractions deform the ECM leading to variable density in adhesive sites. (a) Undeformed ECM. (b) Establishment of a gradient in adhesive site density due to cell tractions deforming the matrix. The tendency of cells to move up such a gradient is called haptotaxis.

Cells proliferate in sigmoidal fashion (Fig. 3), and we take the simplest parameterization of this as the proliferation term.

Matrix (ECM) conservation.

[rate of change of matrix density with time] = [flux into a volume element] − [flux out] + [matrix secretion]

The matrix moves only through convection, that is, it is dragged along by cell traction. Although, initially, cells secrete matrix, at the time of feather germ primordia formation matrix secretion is negligible and so we can ignore it.

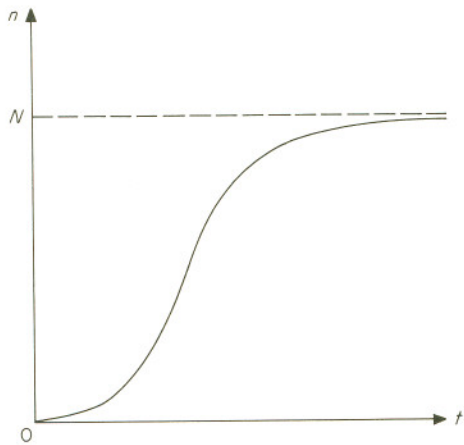


Fig. 3. The qualitative sigmoidal character of cell proliferation typically observed in cell cultures.

Force balance equation. In the cell–matrix composite, we may neglect inertial terms in comparison to the viscous and elastic terms (we are dealing with low Reynolds number[16,19]) that is, motion instantly ceases when the applied forces are switched off. Thus, the net force on a volume element is zero. Experimental observations indicate that displacements and strains are small, so we may model the cell–matrix composite as a linear, viscoelastic material; that is, it is capable of generating viscous and elastic forces. These forces oppose the traction exerted by the cells on the matrix. Fig. 4 illustrates the form this traction per cell must take. Note that as cell density increases, the traction exerted by each cell decreases. This simply reflects cell–cell contact inhibition which we mentioned above. The cell–matrix composite is tethered by anchor filaments to a sub-dermal layer. This we model as a simple elastic body force.

The force balance equation simply sums these various contributions and sets the total equal to zero.

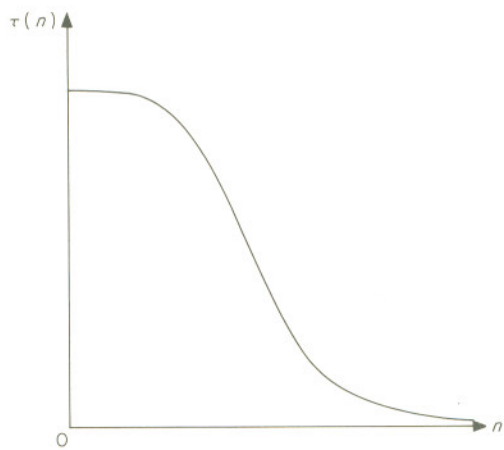


Fig. 4. Typical cell traction per length per cell as a function of cell density. For small cell densities, the traction is almost constant. However, at large cell densities, the traction decreases due to cell–cell contact inhibition.

The above is a verbal description of the model. All the mathematics does is to express these various components in a concise form in terms of the parameters. The actual mathematical equations are a set of nonlinear partial differential equations. These were simulated, in one dimension, on the Los Alamos CRAY 1 and some of the results are shown in Fig. 5. Note that if we take the case of four cell aggregations as initial conditions, the cell density evolves to a solution of four aggregations which are out of phase with the initial aggregations. This is of crucial importance for the model from a biological viewpoint, which we now consider.

Feather germ formation on the chicken back takes place in a well-ordered fashion[2,3]

as illustrated in Fig. 6. Initially, a row of aggregates forms along the mid-dorsal line on the chick back. Rows on either side then form sequentially, the aggregates in the adjacent rows being at interdigitating positions to those of the previous row, eventually leading to a hexagonal array of feather germs on the chick back.

The numerical simulations clearly exhibit this type of behaviour (Fig. 5) where the pre-strain set up in the ECM by an initial row of papillae directs aggregates in the neighbouring row to form at interdigitating points. Thus the model exhibits the type of sequential pattern formation observed experimentally.

We shall come back to this model, and its predictions, in Section 4.

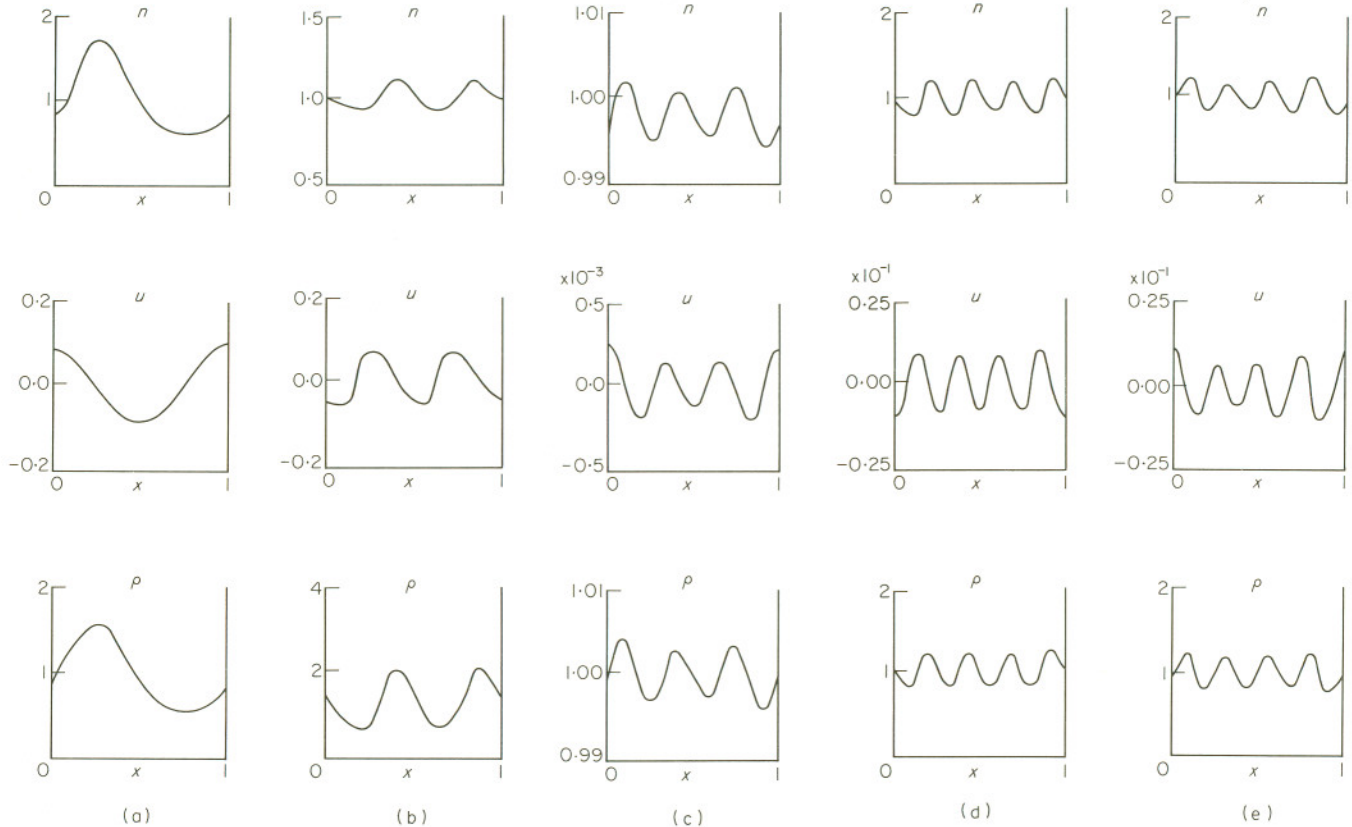
3. A mechano-chemical model for chondrogenesis

Once again the model is based on the mechano-chemical interactions that are known to occur *in vivo*, and in particular, the developing chick limb bud. The model is based on the scenario[18] illustrated in Fig. 7. Referring to the figure, chondroblast cells emerge from the progress zone secreting hyaluronic acid (HA), a highly osmotic glycosaminoglycan, which swells the limb bud and keeps the cells apart (1). At a certain stage, perhaps when the concentration of HA has reached a certain critical level, the cells begin secreting the enzyme hyaluronidase (HAase) (2) which breaks down the HA, causing the interior of the limb bud to deswell (3). Cells can now come into close contact with one another (4) and begin to exert traction forces to set up an organizing centre leading to aggregation. Throughout this process the limb bud is surrounded by a 'sleeve' of HA[8] which is not degraded and stops the limb bud from collapsing. These cell aggregates become cartilage and eventually bone.

Once an aggregate is formed, cells in the centre begin to secrete HA once again, causing the aggregate to swell. If we consider the condensation as a cylinder, then the longitudinal stress is half the circumferential stress (Fig. 8): this is a standard property of a cylinder (it is the explanation of why a sausage splits longitudinally rather than circumferentially). If, as is reasonable, we assume that cells align along stress lines in the ECM (there is some evidence for this — see Ebendal[6]), then cells on the outer region of the aggregate will lie circumferentially. This is what is observed in the perichondrion — the membraneous envelop that surrounds a condensation. This structure is essential to the condensation — remove it and it dissolves.

The full mathematical details of this model may be found in Oster *et al.*,[18] where it is shown that the model exhibits the expected aggregation formation. In particular, the model indicates three key types of bifurcation (Fig. 9).

We can now propose a scenario based on the model, using Fig. 9, for the formation of the cartilage pattern in the chick limb, and indeed for vertebrate chondrogenesis in general. As the limb bud grows, it is reasonable to suppose that the model parameters vary smoothly from the progress zone and so we expect condensations to form sequentially in proximal to distal order. If the constant cell-age contours (which determine the model parameters) are not planes normal to the cylindrical limb axis,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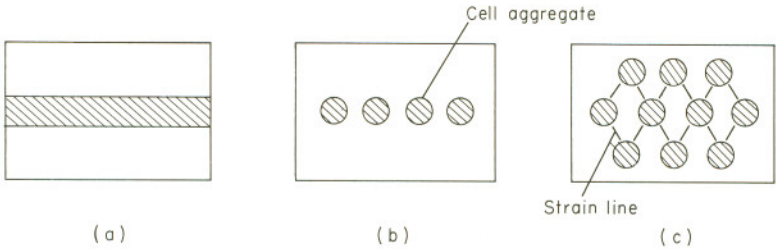

Fig. 6. Illustration of feather germ formation on an idealized section of chick back. An initially uniform density of cells (a) along the mid-dorsal line forms aggregations (b). The aggregation pattern spreads out laterally with primordia forming in adjacent rows at intermediate points to those in the original row, giving rise to a hexagonal pattern (c). The scenario for such pattern formation, based on the mechanical model, is as follows. Initially the cell traction is small and the uniform steady state (a) is stable. As the cells mature, their traction increases and the homogeneous steady state is no longer stable and evolves to an inhomogeneous steady state (b). This initial row of aggregates imposes a strain field causing aggregation along neighbouring rows at intermediate points (c).

then the bifurcation boundaries between one and two aggregations will also not be planes normal to the limb axis. Thus, the size and shape of the condensations will be affected by the curved geometry of this 'critical age' contour. Also, if the limb bud is not perfectly elliptical in cross-section, but rather more like an aerofoil — thicker on one side — then, as the limb grows, the cell tractions will accentuate this, and affect the size and sequence of the bifurcations so that they do not occur symmetrically with respect to the anterior–posterior axis. In general, we expect that at a level where two or more condensations coexist (for example, radius and ulna), when one of the elements bifurcates, the bifurcation of the other element will be delayed distally. This

Fig. 5. Some results from numerical simulations of the model equations for feather germ formation in one dimension (Perelson, Maini, Murray and Oster, pers. comm.). The full nonlinear system is simulated numerically and the pattern predicted by linear analysis was observed. With different sets of parameters we can isolate modes 1–4 ((a)–(d)). Initially the strain displacement, u, and the matrix density, ρ, were set at the uniform steady state of 0 and 1 respectively. The initial conditions for cell density, n, were random spatial perturbations about the homogeneous steady state $n = 1$ (this simulates the fluctuations that occur in any biological system). If we take as initial conditions, random perturbations about $n = 1$ for cell density and u and ρ as in (d), that is, we impose a pre-strain on the domain, the cell density evolves to four aggregates which are 180° out of phase (e) with the cell density that set up the pre-strain (d). This is very important from the biological viewpoint and gives rise to the hexagonal pattern that is observed *in vivo* (Fig. 6). The simulations were carried out using a multi-purpose FORTRAN subroutine on a CRAY at the Los Alamos National Laboratory.

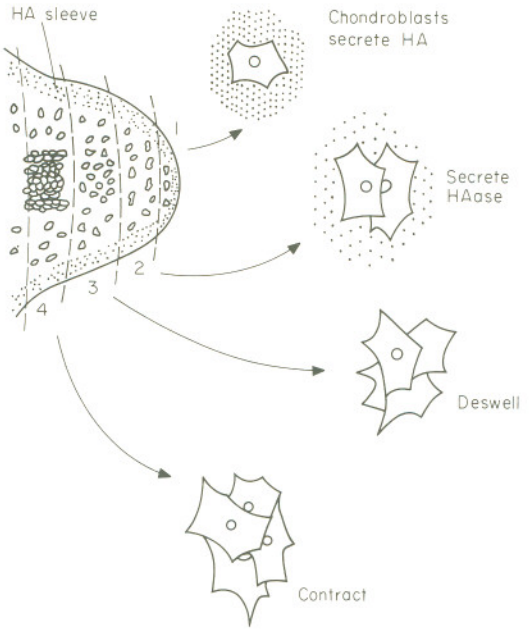

Fig. 7. Scenario for chondrogenesis described by the mechano-chemical model. As cells emerge from the progress zone (1) a series of changes in the physico-chemical makeup of the cell–matrix milieu (2 and 3) leads to cell aggregation (4). See text for details.

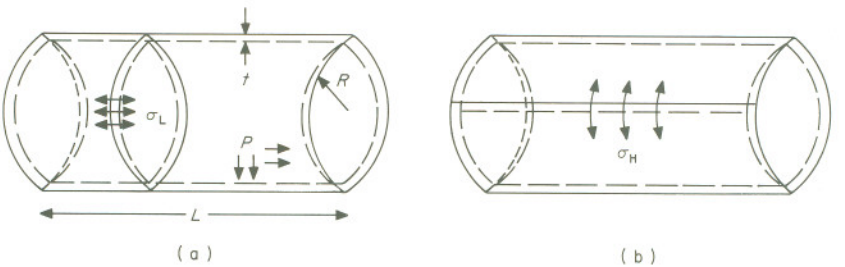

Fig. 8 Stresses in a thin walled cylinder, thickness t and length L, under internal pressure, p, The longitudinal stress, σ_L, is given by σ_L = axial force/area = $pR/2t$ and splits the cylinder circumferentially (a). The hoop stress, σ_H, is given by σ_H = hoop force/area = pR/t and causes a longtitudinal split (b).[26] Clearly the hoop stress is twice the longitudinal stress — this is why a boiled sausage splits longitudinally rather than circumferentially.

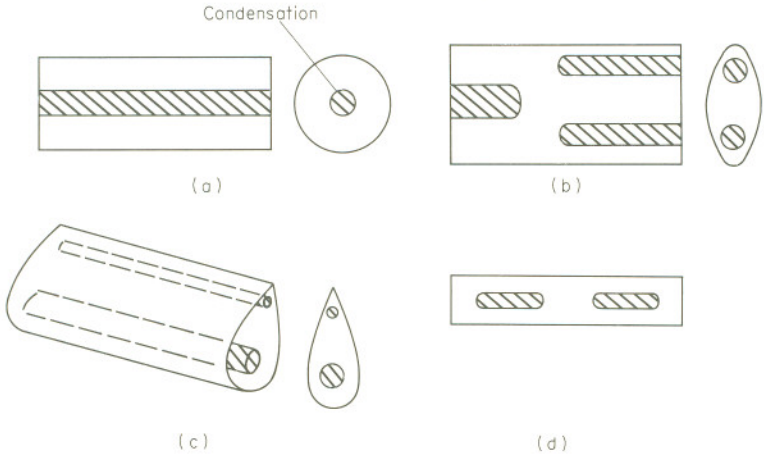

Fig. 9. The three types of bifurcation generated by the model. (a) An axial bifurcation (type A) initiates an axial condensation (for example a humerus or femur) in a cylindrical domain from an initially homogeneous cell distribution. (b) A transverse bifurcation (type T) splits an axial condensation producing a doubling of the original condensation. (c) An aerofoil-shaped domain will produce unequal axial condensations (radius/ulna, tibia/fibula). (d) In a long, thin cylinder, the axial condensations will be broken up into segments by axial bifurcations perpendicular to the long axis of the cylinder. The size and proportions of the segments depend on the tissue geometry and parameter values. These bifurcations are termed longitudinal (type L) bifurcations.

is because a condensation has a 'domain of influence' wherein it recruits cells into itself. This creates a focus of compressive stress and will tend to inhibit nearby condensations until they grow past the stress focus and can commence their own bifurcation centre. Fig. 10 illustrates the scenario.

4. Experiments to distinguish between models

As previously mentioned, several models based on different mechanisms can give rise to the observed biological pattern. The key question is how to distinguish between them so as to determine which may be the relevant mechanism *in vivo*. These different models, or explanations, for how pattern arises suggest different experiments which may lead to a greater understanding of the biological processes involved. The final arbiter of a model's correctness is not so much in what patterns it generates (although a first necessary condition for any such model is that it must be able to produce biologically observed patterns), but in how consistent it appears in the light of subsequent experiments and observations.

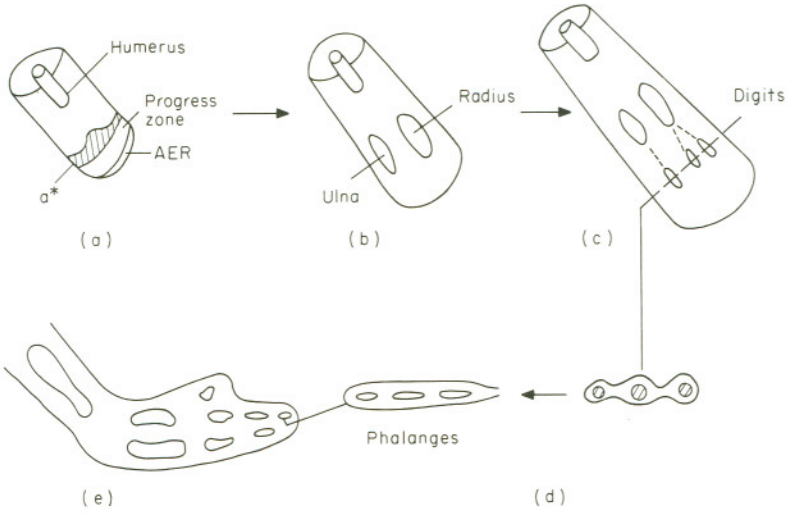

Fig. 10. A hypothetical scenario showing a possible pattern of bifurcations according to the mechano-chemical model. Condensations will form when the cells have aged through the 'critical age' contour. (a) A type A bifurcation leads to the formation of a humerus as the critical age contour, a^*, progresses distally down the limb. (b) When a critical cross-sectional ellipticity is reached, a type T bifurcation occurs leading to two asymmetrical axial condensations (radius and ulna). (c) The asymmetry induced in the limb geometry by the cell tractions leads to a type T bifurcation only on the radius. The domain of influence of these condensations prevents the ulna condensation from bifurcating. (d) By now the limb bud is flattened and type L bifurcations can occur leading to formation of the phalanges. (e) Asymmetrical bifurcations lead to the formation of the digits in the developing chick limb.

The existence of morphogens, on which the reaction diffusion theory for morphogenesis is based, is a controversial issue — no conclusive evidence for their existence has been presented, although retinoic acid or calcium may be possible candidates (see for example, Tickle,[24] B. Goodwin, pers. comm. 1983). This makes reaction diffusion models very difficult to test at this stage. For example, let us look at the predictions made by the models on spacing in feather germs. A mathematical analysis of reaction diffusion models shows that the spacing or wavelength w, predicted when pattern starts to form, is a simple algebraic relation involving the chemical reaction rate constants and the diffusion coefficients. Clearly this can be varied by changing the temperature which affects rates of reaction and diffusion coefficients. However, since temperature affects every parameter term in the expression and, as the morphogens have not yet been isolated, it is impossible to say what will happen to w.

On the other hand, in the mechanical model which involves cell densities, we also

obtain an algebraic expression for the spacing in terms of the cell densities, elasticity of the matrix and so on (all in principle measureable). Now we can change several of the real parameters. If we simply reduce the number of cells (for example, by radiation) the model predicts that the spacing will vary in a predictable way. One version of the model predicts that spacing will increase.[17] Davidson (pers. comm. 1984), using radiation techniques, decreased the total number of cells on the chick back and found that the spacing increased. Davidson[2,3] also found that the 'wave' of aggregates that spread out from the dorsal midline was not stopped by incision of the skin. The mechanical model is in agreement with this — it predicts that the 'wave' will simply start up again and progress as before.

We remarked earlier that the temporal sequence in which dermal papillae and epidermal placodes formed was a controversial issue. In the mechanical model we assumed that the dermal aggregates formed first and then exerted a strain on the epidermis. The reverse situation would be modelled by epidermal placodes forming first and exerting a force on the dermis, leading to dermal papillae. This would involve including another term in the force balance equation — this would simply enhance the pattern forming capabilities of the dermal mechanical model.

Edelman[7] has isolated cell adhesion molecules (CAMs) which seem to be involved in skin organ formation. A mechano-chemical model based on the linking of epidermal and dermal processes via these cell adhesion molecules has been proposed by Cheng *et al.*,[1] who have shown that the mechanism can give rise to structure.

In the problem of chondrogenesis, if, as we have asserted, the osmotic swelling of the extracellular matrix is a crucial factor in creating cell aggregations, then experiments which modulate this process should have profound morphogenetic effects. In this context, it may be possible to modify the extracellular ionic strength and thus the osmotic pressure, without disrupting normal cell functions. Antibodies to hyaluronidase are also a possible mode of intervention.

Dhouailly[4,5] found that mesenchymal morphogenesis in feather and limb development was greatly altered by treatment with retinoic acid, a substance present in vitamin A. Recent experiments by Kochhar *et al.*[11] suggest that this disruption may be due to the effect of retinoic acid on hyaluronidase production. They found that mouse limb bud mesenchymal cells, treated with retinoic acid, synthesized HA to a much greater degree than untreated cells. With the mechano-chemical model, it is easy to see how disrupting the hyaluronate/hyaluronidase system can alter morphogenetic patterns.

The role of theory in morphogenesis is to point out the physical possibilities and to suggest experiments to distinguish between them. As we have seen, the mechano-chemical models lend themselves to experimental scrutiny more readily than reaction diffusion models. It is likely that both types of models are involved in development, but until more is known about the morphogens involved it seems that mechanical models can indicate experimental activity to elucidate the underlying mechanisms involved in morphogenesis in a more productive way.

5. Potential future applications

The encouraging results from the above applications (and others not described here) of the mechano-chemical approach to pattern formation suggests that it might be useful and informative to investigate other areas where cell traction may play a key role. Here we mention only a few.

The actual process of wound healing is a complex sequence of events which is not understood. In the case of burns for example, epidermal cells at the wound site appear to adopt mesenchymal cell characteristics and are capable of exerting large traction forces, which pull the wound edges together. These large traction forces can cause puckering of the skin and can lead to severe scarring and disfigurement. This process can be modelled using the mechano-chemical approach, with a view to trying to minimize the traction-caused puckering, either by artificial or other means, suggested by the model. Mathematically this would be a formidable free boundary problem. However, the potential practical rewards justify a detailed study.

One of the problems associated with artificial hip joints is that of rejection: the cement for fixing them in place inside the femur frequently does not form a good bond with the living tissue. The concepts used in setting up the mechano-chemical models discussed here suggest that a method of obtaining a better bond might be to use an adhesive which is sufficiently porous to allow migration of cells into it. Experimental work motivated by this mechano-chemical approach is currently in progress (Y. K. Liu, pers. comm. 1983).

In the eyeball, mesenchymal cells may migrate from the back of the eye into the vitreous humour in front of the retina. There they exert traction forces which can detach the retina from the back of the eye, leading to irreversible blindness. It is not known why cell migration occurs but an understanding of normal mesenchymal cell behaviour is the first necessary step to understanding why they behave abnormally.

Acknowledgment

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