Diffusion in Embryogenesis

by

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A simple order-of-magnitude calculation suggests that diffusion may be the underlying mechanism in establishing morphogenetic gradients in embryonic development.

It has been a great surprise and of considerable importance to find that most embryonic fields seem to involve distances of less than 100 cells, and often less than 50.

Professor Lewis Wolpert

I am to show that this fear is unfounded, and, on the contrary, that the known facts, sparse as they are, fit rather well to a dynamical equilibrium, and for constant level. At the other end the extreme cell acts as a sink: that is, it destroys the molecule, holding the concentration at a constant level. The obvious model for setting up a simple gradient is illustrated in Fig. 1. At one end of a line of cells one postulates a source—a cell which produces the chemical (which I shall call a morphogen) and maintains it at a constant level. At the other end the extreme cell acts as a sink: that is, it destroys the molecule, holding the concentration at that point to a fixed low level. The morphogen can diffuse from one cell to another along the line of cells. After a time the system approaches a dynamical equilibrium, and it is easy to show that if the effective diffusion constant is everywhere the same, the concentration gradient will be linear.

Of course, real embryological structures will have three dimensions, but if for convenience we restrict ourselves to sheets of cells such as, for example, the insect epidermis or the developing amphibian retinal the problem becomes two-dimensional. The source can be considered to be a line of cells (the line being perpendicular to the paper in Fig. 1) and similarly for the sink, thus reducing the problem to one dimension.

It is not difficult to calculate how long it would take to set up such a system, supposing that both the source and the sink are turned on at time zero. Diffusion is a random walk, and the dimensions of the diffusion constant are DT-1 (where L is length and T is time). This should be contrasted with a mechanism having a velocity (with dimensions LT-1) as proposed, for example, by Goodwin and Cohen. Because in diffusion the length enters as the square, pure diffusion processes are very rapid over rather short distances (say, the size of a cell) and very slow over long distances (say, the size of an organ).

The concentration approaches its final value asymptotically, so one must have some criterion for deciding whether the gradient at any time is sufficiently close to a straight line. I have arbitrarily taken the gradient to be effectively established when it is everywhere within 0.1C of the final value, and chosen AC as 1 per cent of C0 (C0 is the maximum value at the origin). It would make little difference to the argument if AC were considered to have half this value.

The gradient might be set up in various ways. The result in each case can be expressed as

\[ t = \frac{A}{D} \left( \frac{nL}{l} \right)^{2/3} \]

where \( t \) = time in seconds to set up the gradient; \( n \) = number of cells between source and sink; \( l \) = length of each cell, in cm; and \( D \) = diffusion constant, in cm² s⁻¹. \( A \) is a numerical constant, the exact value of which will depend on the way the gradient is developed.

Mathematically the simplest way is to start with zero concentration of the morphogen everywhere at time zero, and thereafter to maintain the source at concentration \( C_s \), and the sink at concentration zero. This gives a value of \( A \) of 0.42. Biochemically more realistic models give values only a little larger than this, so a good general value for \( A \) would be 0.5. It was pointed out to me by Dr Aaron Klug that, if the initial concentration were uniformly \( C_0/2 \), the time required is reduced to a little less than one-quarter, and \( A \) will have a value of about 0.09. More realistic models of this general sort give values of \( A \) of, say, 0.15. The calculations of \( A \) were carried out by Mrs Mary Munro.

In what follows, I shall assume that \( A \) is 0.5 (the simple mechanism), but it should be remembered that the organism might be able to reduce this to about a third of this value.

The diffusion constant in water for all but the smallest molecules—provided they are roughly spherical—is inversely proportional to their mean radius, to a near approximation. Thus, increasing the molecular weight by a factor of 1,000, from, say, a small organic molecule such as ATP (mol. wt. 507) to a very large protein like polynucleotide polymerase (mol. wt. about 0.6 x 10⁹) reduces the diffusion constant only by a factor of 10. Now it is reasonable to expect that the morphogen will diffuse rather rapidly, and should be able to pass fairly efficiently from cell to cell. It is also likely to be a rather specific molecule. For these reasons I doubt if morphogens
will turn out to be large proteins or common ions like $K^+$ or $Na^+$. An obvious choice would be an organic molecule of about the size of, say, cyclic AMP or a steroid. That is, with a molecular weight in the range 300 to 500. The diffusion constant* in water (at $20^\circ C$) for such a molecule is about 4 or $5 \times 10^{-4}$ cm$^2$ s$^{-1}$. (The diffusion of salts like NaCl or KCl is about three or four times as fast as this.)

The inside of a cell is very far from being made of water, and one must estimate the effective diffusion constant within a cell. This amounts to estimating the effective viscosity. The cytoplasm being a concentrated mixture of molecules having a large variety of sizes, the relative viscosity will be considerably higher than water at the same temperature. For a small molecule, which can, as it were, slip between many of the other molecules, the effective viscosity is unlikely to be as big as the bulk viscosity of the cytoplasm (wherever that may be). It is difficult to make any precise estimate of the effective diffusion constant, which in any case may worry considerably between different types of cell. Allowing a factor of increase of viscosity of $\times 6$ (corresponding to a sucrose solution 40 per cent by weight), which seems not unreasonable, would make the effective diffusion constant about $0.8 \times 10^{-4}$ cm$^2$ s$^{-1}$.

The diffusion constant in water (at 20°C) for such a molecule is about 4 or $5 \times 10^{-4}$ cm$^2$ s$^{-1}$. This is easy to show that the effective diffusion constant, $D'$, for our problem is given by $1/D' = 1/4D + 1/P$, where $l$ is the length of each cell in the direction of diffusion. Thus, if $D$ is $0.8 \times 10^{-4}$ cm$^2$ s$^{-1}$ and $l = 10 \mu m$ (say) we see that if the "resistance" to flow of the morphogen because of permeability between cells was equal to that due to diffusion within a cell, $P$ would have to have the value $8 \times 10^{-4}$ cm/s. This is a high value, but probably not impossible high. If we arbitrarily take $P$ as about half this and $D$ as before, we obtain $D' = 0.27 \times 10^{-4}$ cm$^2$ s$^{-1}$.

We now need an experimental estimate of the time needed to set up a gradient. This is not easy to obtain. Most embryologists would feel that a day is too long. A minute seems far too short. A few hours would seem about right. An expert has suggested that between 5 to 10 h is not unreasonable for many of the well-studied cases (ref. 1, page 41). I shall assume a figure of 10$^4$ s (approximately 3 h), because some time must be allowed for the changes which take place after the gradient is set up. Combining our formulae we obtain

\[
t = A/nh \left( \frac{1}{D} + \frac{1}{P} \right)
\]

and substituting the chosen values: $t = 10^4$ s, $A = 0.5$, $l = 10 \mu m$, $D = 0.8 \times 10^{-4}$ cm$^2$ s$^{-1}$, $P = 4 \times 10^{-4}$ cm$^2$ s$^{-1}$ we obtain $n = 70$ cells. If $l$ were 30 $\mu m$, $n$ would come to a little more than thirty cells. If we take the approximate nature of the calculations, the agreement with the figures given in the quotation at the start of the article is striking. In broad outline what the calculation shows is that, for the times considered, distances or the order of a millimetre (or less) are possible, but distances of a centimetre are too great. Of course, for organisms which develop very rapidly the distances would have to be smaller than a millimetre.

We can take it, then, that assuming the effective viscosity of cytoplasm has not been grossly underestimated, and provided there is a special mechanism to increase the rate of permeability of the morphogen between cells, there are many cases in embryology where the times and distances involved are quite compatible with a mechanism based on diffusion. It is important, however, to make two reservations. There may be special cases, involving setting up gradients quickly over large distances (of the order of several centimetres) which may require other mechanisms, such as the signalling devices suggested by Goodwin and Cohen. Cases of "mushroom growth" (as, for example, the growth of mushroom) are unlikely to be due to diffusion alone. Secondly, in one's enthusiasm for diffusion, it is important to realize that the many other problems remain to be tackled. Even when the gradient has been set up the cell has to recognize it. Because at least in the insect integument the "gradient" appears to impose a polarity on those epidermal cells which become scales and bristles the cells must have some additional mechanism (involving microtubules?) to do this.

In the case of the amphibian eye the retinal ganglion cells must not only recognize the presumed gradient (so that they know where they are in the retina); each cell must also convey this information to the far end of its growing axon, so that it can make the connexion at the appropriate place on the optic tectum. Moreover, there are likely to be subsidiary mechanisms to guide the growing bundle of nerves along the right path to the tectum.

In two brilliant articles published about ten years ago, Lock* showed that the pattern of wrinkles obtained on

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the adult cuticle of Rhodnius after operations on the
earlier larval stages (usually the last larval stage) can only
be explained by a "gradient" of some sort, running from
one intersegmental membrane to the next, and repeating
in successive segments. He showed convincingly that
neither mechanical expansion alone, nor polarity alone,
could explain the results. Dr Locke kindly sent us the
original photographs of some of his material. Mrs Mary
Munro and I, together with Dr Peter Lawrence, have
attempted to fit these patterns to a pure diffusion model.
Although, following Locke's arguments, the observed
wrinkles have roughly the expected pattern, they differ in
detail from the computed pattern. Moreover, various
estimates of the diffusion constant disagree drastically.
We are therefore currently exploring a model in which
each cell in the epidermis attempts to maintain the con-
centration of the morphogen within itself to a previously
present level, determined soon after the gradient is first set
up. This model, which has only one disposable parameter,
is a much better fit with Locke's data. More elaborate
hypotheses, within the basic diffusion framework, are also
under consideration. It is important, therefore, not to
approach these problems with too naive a model.1

Finally, one should emphasize that gradients are unlikely
to command general acceptance until their biochemical
basis is discovered experimentally, and that this may not
prove an easy task. Mathematically minded biologists
could well object that any theory which has the same
mathematical formalism would necessarily fit the observed
patterns, and that the agreement between the calculated
and observed distances (on the diffusion theory sketched
above) may only be coincidental. In spite of these possible
objections it is my belief that mechanisms based on
diffusion are not only plausible but rather probable.
Nature usually has such difficulty evolving elaborate
biochemical mechanisms (for example, those used in
protein synthesis) that the underlying processes are often
rather simple. If this approach serves to make the idea
of diffusion gradients respectable to embryologists it will
have served its purpose.

I thank my wife for drawing the figure, my colleagues,
especially Dr Peter Lawrence and Mrs Mary Munro, for
many helpful discussions, and Professors Lewis Wolpert
and W. D. Stein for sending me information.

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Information and the Spatial Pattern of Cellular Differentiation", should
be consulted both for a modern statement of the problem and also for
references to earlier work.

2 The basic idea of this article was presented at a lecture given to students
at the Fourth NATO Advanced Study Institute of Molecular Biology
in July 1968 at Special.

3 Child, G. M., Patterns and Problems of Development (Chicago University
Press, Chicago, 1941).

4 For a review see Lawrence, P. A., Adv. Insect Physiol. (in the press). See

5 For a review see Gaze, R. H., Growth and Differentiation, Ann. Rev.
Physiol., 28, 50 (1967).


7 The mechanism for forming a source and a sink for ions also presents
special problems, whereas for organic molecules rather simple enzymatic
processes could do the trick.

8 The effect of temperature on the viscosity of water does not seriously
affect the calculations. Taking the viscosity of water (in arbitrary units)
as 1.0 at 20°C, its value at 5°C is about 1.4 and at 38°C is close
2.9.

9 See, for example, a very ingenious fluorescent method used by Victor W.
organic molecule. He obtained a factor of about 3 for Euglena. The
figure for yeast was about twice this.


11 This assumes that the permeability is fairly evenly distributed over the
cell membrane. If it were concentrated in a small patch the effective
diffusion would be slower.

12 For example, P for glucose in ascites cells at 37°C in about 4.5 10^-6 cm/s
(Kolb, K. B., and LaFevre, P. G., J. Gen. Physiol., 41, 1967; 1967),
or in human red cells at 37°C about 1 x 10^-7 cm/s (Millar, D. M.,
Biophys. J., 6, 497, 1963). Admittedly these are among the higher
values of P known so far. See Stein, W. D., The Movement of Molecules
should be remembered that in going from one cell to the next the
morphogen may have to cross two cell membranes.

13 An upper limit can be calculated assuming that the morphogen is an ion,
that it diffuses (at 37°C) in the cytoplasm as freely as in water, that the
permeability between cells is so high that it does not slow down the
process at all, and that the most efficient method is used to set up the
gradient. The distance then comes to 1-5 cm, but I feel that this com-
Bination of assumptions is quite unrealistic.

14 Fehlin, R., Naturwissenschaften, 85, 372 (1968); Lawrence, P. A., J. Exp.


16 The present model could be elaborated by assuming two different mor-
phogens, one having a gradient sloping from left to right, and the other
with a gradient from right to left: on this model the position of a cell
would be characterized by the ratio of its concentration of the two
morphogens. This particular elaboration, however, would not signifi-
cantly alter any of the arguments given in this article.