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Positional Information and the Spatial Pattern of Cellular Differentiation†

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The problem of pattern is considered in terms of how genetic information can be translated in a reliable manner to give specific and different spatial patterns of cellular differentiation. Pattern formation thus differs from molecular differentiation which is mainly concerned with the control of synthesis of specific macromolecules within cells rather than the spatial arrangement of the cells. It is suggested that there may be a universal mechanism whereby the translation of genetic information into spatial patterns of differentiation is achieved. The basis of this is a mechanism whereby the cells in a developing system may have their position specified with respect to one or more points in the system. This specification of position is positional information. Cells which have their positional information specified with respect to the same set of points constitute a field. Positional information largely determines with respect to the cells' genome and developmental history the nature of its molecular differentiation. The specification of positional information in general precedes and is independent of molecular differentiation. The concept of positional information implies a co-ordinate system and polarity is defined as the direction in which positional information is specified or measured. Rules for the specification of positional information and polarity are discussed. Pattern regulation, which is the ability of the system to form the pattern even when parts are removed, or added, and to show size invariance as in the French Flag problem, is largely dependent on the ability of the cells to change their positional information and interpret this change. These concepts are applied in some detail to early sea urchin development, hydroid regeneration, pattern formation in the insect epidermis, and the development of the chick limb. It is concluded that these concepts provide a unifying framework within which a wide variety of patterns formed from fields may be discussed, and give new meaning to classical concepts such as induction, dominance and field. The concepts direct attention towards finding mechanisms whereby position and polarity are specified, and the nature of reference points and boundaries. More specifically, it is suggested that the mechanism is required to specify the position of about

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50 cells in a line, relatively reliably, in about 10 hours. The size of embryonic fields is, surprisingly, usually less than 50 cells in any direction.

1. Introduction

The central problem of the development of form and pattern is how genetic information can be translated in a reliable manner to give specific and complex multicellular forms and varying spatial patterns of cellular differentiation. In considering this problem it is important and convenient to distinguish between molecular differentiation, spatial differentiation and morphogenesis, while recognizing their interdependence (see Waddington, 1962). Molecular differentiation places the emphasis on the changes occurring within a cell with time and is mainly concerned with the control of the synthesis of specific macromolecules which are characteristic of the cell type: for example, the processes involved in specifying the synthesis of the muscle proteins in a developing muscle cell or chondroitin sulphate by a cartilage cell. Spatial differentiation is the process by which the individual cells within a population are specified to undergo a particular molecular differentiation, which results in a characteristic spatial pattern. For example, the differences between the forelimb and hindlimb of a tetrapod probably do not lie in the processes of molecular differentiation of, say, the muscle or cartilage cells but rather in the spatial processes which specify which cells will form cartilage or muscle. As is well known the development of spatial patterns of differentiation is capable of considerable regulation when disturbed, this regulation requiring intercellular communication and it is this intercellular communication system that is one of the distinguishing features of spatial differentiation. The spatial pattern of differentiation may be regarded as the process whereby a cell has its spatial position specified—positional information—and that it is this which can determine its molecular differentiation. Finally, with morphogenesis—moulding of form—the emphasis is on the forces bringing about changes in shape. Examples of such forces are pseudopodal contraction and other localized cellular forces and it is the space-time distribution of such forces that leads to change in shape (Gustafson & Wolpert, 1963, 1967).

While considerable attention is at present being given to the control of molecular differentiation, it is important to recognize that few, if any, of the present lines of thought on the control of gene action lead directly to the solution of spatial pattern formation. We have, for example, the very much exploited model of Jacob & Monod (1963) for the control of gene transcription, which even in its various modifications for eukaryotes is at least one level of organization removed from problems involving intercellular communication. Dealing as it does, with intracellular regulatory

phenomena it is not directly relevant to problems where the cellular basis of the phenomena are far from clear. This paper is firmly based on the belief that until the cellular basis of a multicellular phenomenon such as pattern formation is understood, it is not possible to pose the appropriate molecular questions. We have used a similar argument in relation to morphogenesis where we have suggested that an understanding of the cellular forces is a prerequisite for posing appropriate questions at the molecular level, and thus linking gene action with the development of form (Gustafson & Wolpert, 1963, 1967). For example, our analysis of gastrulation in the sea urchin embryo which suggests that it is brought about by the pseudopodal activity of a few cells at the archenteron tip leads on the one hand to meaningful questions about the molecular basis of pseudopodal activity and cell contact, and on the other hand poses a problem in pattern formation by asking how pseudopodal activity becomes specified in those particular cells at a particular time.

A feature of developmental processes which is not often discussed is the extent to which there are, or will emerge, general or universal principles which are applicable to development in the same way that there appears to be universal rules for genetics, or, of more relevance, for the transcription and translation of the genetic material at the molecular level. It is too often implicit in embryological thinking that each step in development is a unique or special phenomenon with little general significance. One might, for example, view development as a sequential process involving the synthesis of a large number of different proteins, the essential feature of each stage being dependent on the nature of the proteins synthesized (see, for example, Lederberg, 1967). Viewed in this light, the possibility of obtaining a set of general principles enabling one to deal with the translation of genetic information into cellular patterns and forms would seem almost hopeless, since it would be dependent on the specific properties of a large number of different, and perhaps quite unrelated proteins. I would like to suggest that such a view is quite misleading and that there is good reason for believing that there are a set of general and universal principles involved in the translation of genetic information into pattern and form. While some would argue that such a view is gratuitous, it can find some support in consideration of the evolutionary process and our present knowledge of developmental mechanisms. From an evolutionary point of view development is the process whereby the phenotype is specified by the genotype. Selection acts on the phenotype but it is the genotype which is evolving. Considering the universality of the genetic code and of genetic processes, it seems hard to believe that some sort of equally general principles are not involved in the 'translation' of genotype into phenotype. In viewing, for example, the

evolutionary divergence of the vertebrate limb, or the co-ordinate transformation in vertebrate skulls (D'Arcy Thompson, 1941), it is again hard to resist the impression that general principles are at work. In spite of our ignorance of the developmental mechanisms, there is nothing to suggest that general principles will not emerge. On the contrary the very concepts of field and gradient in pattern formation suggest basic underlying principles. In the area of morphogenesis cell motility and cell contact are increasingly emerging as the basis elements in a wide variety of systems, and one can begin to conceive of quite general principles (Steinberg, 1964; Gustafson & Wolpert, 1967).

Pattern formation is a rather neglected area of developmental biology and with a very few notable exceptions, such as in studies on insects, it could be argued that almost no progress has been made since the 1920's when the concepts of mosaic and regulative development, field, dominance, gradients and induction were elaborated. [This earlier work is effectively summarized by Huxley & de Beer (1934), Child (1941), Spemann (1938), Weiss (1939) and Dalcq (1938).] One reason for this was the almost obsessive involvement with the process of induction and inducing substances which almost totally obscured the problem of pattern formation, by emphasizing the importance of the inducing substances (cf. Rose, 1957*a*) rather than the behaviour of the responding tissue. In this paper an attempt is being made to put forward a conceptual framework within which the development and regulation of a variety of spatial patterns may be discussed. By specifying certain rules for cellular behaviour in pattern formation it is hoped that the main problem will be identified and stated in such a form that both new experimental and theoretical approaches may be initiated. Not least my aim is to suggest that a set of 'simple' universal principles may in fact be operative in translating genetic information into pattern.

(A) STATEMENT OF THE PROBLEM

The key to the problem of pattern formation lies in the correct posing of the problem so that an answer can be obtained in terms of cellular behaviour. In very general terms it is the problem of assigning specific states to an ensemble of identical cells, whose initial states are relatively similar, such that the resulting ensemble of states forms a well-defined spatial pattern. It is probably convenient to distinguish from the outset between the 'mosaic' type of development in which the specification occurs during the growth of the ensemble from a single cell and in which communication within the system is rather local, and the 'regulative' in which specification occurs in an ensemble of cells and global interactions are highly relevant. It is almost entirely with the latter that this paper is concerned, though it is recognized

that a sharp distinction does not necessarily exist (Weiss, 1939). The effective distinguishing feature between mosaic and regulative development is that when a portion of the system is removed, then the mosaic system will largely lack those regions which the removed portion would normally form, whereas in regulative systems a normal pattern would still be formed. I have formalized the problem of the regulative development of axial patterns, whose pattern is size invariant, in terms of the French Flag problem (Wolpert, 1968). This problem is concerned with the necessary properties and communications between units arranged in a line, each with three possibilities for molecular differentiation—blue, white and red—such that system always forms a French Flag irrespective of the number of units or which parts are removed; that is the left-hand third is always blue, the middle third is always white and the right-hand third always red. This abstraction of the problem corresponds quite well with experimental observations on the early development of sea urchin embryos, and regeneration of hydroids as well as a large variety of other systems. For example, the proportions of the mesenchyme, endoderm and ectoderm of the sea urchin embryo remain constant over about an eightfold size range; a fragment of hydra, one-hundredth its volume can give rise to an almost complete animal. In more general terms, such systems obey what I have called Spiegelman's rule which may be stated as follows: the amount of material in a developing or regenerating system that is capable of developing into a particular region or part of a pattern is larger than normally does so (see Spiegelman, 1945). This rule emphasizes the problem of assigning the appropriate states to the cells with reference to the system as a whole.

It is surprising how little work on theoretical aspects of pattern development there has been, and none of the available models provided a means of solving the problem. In fact with the exception of Rose (1952, 1957a) and Dalcq (1938) almost no models are available which may be tested on the French Flag problem. The classical field-gradient systems first formulated by Child (1941) and elaborated by Huxley & de Beer (1934) are not very helpful. In terms of these concepts there would be a gradient in some property along the system, to which the non-committal term activity-gradient could be assigned, implying some sort of metabolic gradient (Huxley & de Beer, 1934) or the less informative term morphogenetic potential. The apical or distal region would always form at the high point of the system and its formation would be autonomous. The apical region exerts an influence on other regions and is known as the dominant region. "In terms of the field concept, the apical region establishes a field of a certain extent, which it dominates so as to control the morphogenetic processes of the other regions of the field. The control is exerted in such a way that the various morpho-

genetic processes occur in harmonious relation with each other: this is because it exerts its control through the establishment of a field" (Huxley & de Beer, 1934). These concepts have been extended in detail, and while being useful in that they are applicable to a large variety of systems, they also illustrate the essential weakness of gradient-field concepts. These are (i) they fail to provide a mechanism whereby the apical or dominant region should be established at the high point of the gradient, (ii) they give no indication as to the form of the gradient or mechanism whereby the gradient is maintained or regulated, (iii) they do not even consider how the size of the apical region is determined, (iv) nor do they consider how the apical region controls the order or size of the adjacent parts; this is sometimes assigned to inducer activity by the dominant region. As Spemann (1938) has pointed out, the gradient theory of Child failed to provide a mechanism whereby quantitative differences were translated into pattern.

The single attempt to develop a more specific model and one which attempts to get over the limitations just mentioned is that of Rose (1952), but we have found this model unsatisfactory (Webster, 1964). He suggested that there is a gradient in rate of differentiation and a hierarchy of self-limiting reactions. The most favoured reaction would occur at the high point in the gradient and after a certain time would, by self-inhibition, resulting from production of diffusible inhibitor substances, restrict both its spatial extent and prevent the same reaction occurring elsewhere in the system. When the most favoured reaction was completed, the next in the hierarchy would be permitted, and this would occur in the adjacent region. An essential feature of his model is that of specific inhibition, that is the spread of inhibitory information from one region to another in the form of region specific substances, this flow of inhibitory information being polarized (Rose, 1957*a*,*b*). For recent experimental justification of his model, see Rose (1967). In our hands this type of model can provide the basis for pattern formation but it has severe limitations particularly in the difficulties it meets in accounting for size invariance and proportionate regulation. This difficulty results from the assumption that the size of any region will depend on the rate of differentiation and for how long this occurs. Both of these will depend on the gradient, the concentration of inhibitors, and rate of diffusion of inhibitors. The dependence on the specific temporal sequence makes a reliable regulating model almost impossible, since one is not dealing with a system involving dynamic equilibrium. The extension of the model to two-dimensions seems particularly difficult. Another feature of this type of model is that it regards different parts of the model as distinct regions and requires an interaction between the regions as such. For example, in applying his ideas to regeneration of *Tubularia*, Rose (1957*a*,*b*, 1967) regards the regions

in a distal proximal direction as comprising hypostome, distal tentacles, gonophores, proximal tentacles and stolon. It is assumed by him that hypostome must form before distal tentacles and so on. It is however highly questionable as to whether the division of the hydranth into a set of distinct regions, as is essential for the theory of specific inhibition, is in fact legitimate. There is no reason to believe that from a developmental point of view they do in fact constitute different and separate regions. An alternative view is to regard them as being the morphological expression of the spatial variation of but a few cellular activities such as cell adhesion. This would be more in line with our studies on sea urchin morphogenesis where a restricted number of cellular activities are responsible for morphogenesis. We have, for example, reason to believe that the cellular activities responsible for gastrulation are very similar to those responsible for coelome formation and the development of the primary pore canal (Gustafson & Wolpert, 1963, 1967).

Our own analysis of the French Flag problem (Apter, 1966; Webster, 1965; Wolpert, 1968; Mary Williams, personal communication) has suggested that there are, in principle, only two types of solution to the problem (Wolpert, 1968). The one as illustrated by Webster's (1966*b*) model and Wolpert's (1968) balancing model, makes use of a balancing principle; the amount of a substance being made in one region being altered until it 'balances' with the amount being made or destroyed in another region. In such models the position of a cell within the system is indeterminate. By contrast, the other types of model make use of a positional principle. That is they employ a mechanism whereby the cell's position within the system, with respect to the two ends, is uniquely specified and this information is used to determine the nature of its differentiation. It is with this concept of positional information that much of the remainder of this paper is concerned. It is perhaps also worth pointing out that all solutions to the French Flag problem appear to require three basic elements: (i) a mechanism for specifying polarity; (ii) a mechanism for the differential response of the cells, such as thresholds; and (iii) at least one spontaneous self-limiting reaction (Wolpert, 1968).

(B) POSITIONAL INFORMATION

The main points about the concept of positional information which will be expanded on, are:

(1) There are mechanisms whereby cells in a developing system may have their position specified with respect to one or more points in the system. When cells have their positional information specified with respect to the same set of points, this constitutes a field.

(2) Positional information largely determines, with respect to the cell genome and developmental history, the nature of the molecular differentiation that the cell will undergo. The general process whereby positional information leads to a particular cellular activity or molecular differentiation will be termed the interpretation of the positional information. The specification of positional information in general precedes and is independent of molecular differentiation.

(3) Polarity may be defined in relation to the points with respect to which a cell's position is being specified: it is the direction in which positional information is specified or measured.

(4) Positional information may be universal, that is the same mechanisms that specify positional information may be operative in different fields within the same organism as well as in quite different organisms from different genera or even phyla.

(5) The classical cases of pattern regulation whether in development or in regeneration, that is the ability of the system to form the pattern when parts are removed or added, and to show size invariance, as illustrated by the French Flag problem, are largely dependent on the ability of the cells to change their positional information in an appropriate manner and to be able to interpret this change.

The concept of positional information will be shown to provide a unifying conceptual framework for a variety of systems including regeneration of hydroids, sea urchin development, and pattern formation in the insect epidermis. It also may provide some insight into problems of size and growth control. Probably the most important aspect is that it focuses attention on aspects of development which have received far too little attention, particularly where the reference points are and how positional information is specified. It is hoped that it poses questions concerning pattern formation in a new form, for unless the correct questions are asked there is little hope of obtaining useful answers.

Positional information as here defined has features in common with the double gradient theory of Dalcq (1938) and the concept of prepattern as proposed by Stern (1956) and extended by Kroeger (1959, 1960). Dalcq's detailed concepts have been little used and will thus not be discussed further here. The prepattern concept is discussed below. It should be emphasized that the idea that a cell's position is important in development is not a new one but has been explicit and implicit in the writing of various authors at various times (see, for example, Weiss, 1962). However, the implications have not been developed nor has the idea of specification of position formed the basis of either theories or experiments in pattern formation.

(C) POLARITY AND THE SPECIFICATION OF POSITIONAL INFORMATION

A cell's positional information is specified with respect to one or more reference points. The identification of such reference points is of great importance and by no means always obvious; two relatively clear-cut examples are in hydra where one reference point is almost certainly at the hypostome and in the early development of the sea urchin embryo the animal and vegetal poles are very likely reference points. The specification of positional information with respect to such points can be essentially of two main types. One is a quantitative variation in some factor such as concentration of a substance such that it increases or decreases in some well-defined monotonic way with distance from the reference point. It is thus possible to talk about a positional information/distance curve, several examples of which are shown in Fig. 1, and the positional information of

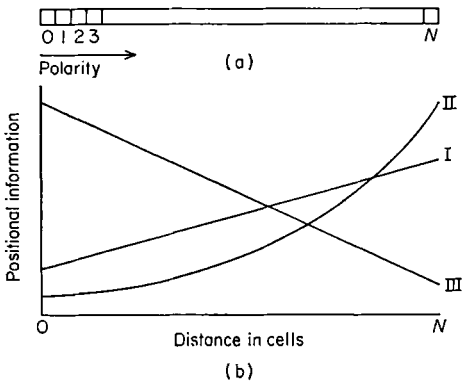

FIG. 1. In (a) a line of cells, N cells long, is shown and the arrow shows the polarity of the system. In (b) three examples of positional information/distance curves are illustrated.

Curve I is a case in which there is a linear increase in positional information with distance from the end, and this could represent the increasing concentration of a substance or the phase angle difference between two periodic events spreading from the left as suggested by the Goodwin–Cohen model.

Curve II shows another type of relation that could arise for example from active transport of a substance from left to right.

Curve III shows a decrease in some property with distance.

Note that the value of α_i for the three relationships may be both qualitatively and quantitatively different.

the ith cell from the end as α_i. The distance is measured in cell number. It is not proposed to discuss in detail here mechanisms whereby the positional information/distance relationship may be generated, but a few examples will be given in order to make the concepts more concrete, by considering a uniaxial array of cells.

A variation in concentration of a substance with distance from the end could be achieved by some form of active transport and other means. The concentration of the substance would thus specify position with respect to the end. An interesting case is to make the end cells a source and sink respectively of some substance. If the concentration of the substance in these cells were regulated to some fixed values then there would be a linear gradient between the cells. The absolute value of the substance and its gradient at any point would specify a cell's position (Wolpert, 1968). A similar mechanism was proposed, by Stumpf (1967).

A particularly interesting and elegant mechanism for the specification of positional information based on the novel principle of wave propagation has been proposed by Goodwin & Cohen (1969). Briefly, they suggest that two periodic signals are propagated from the reference point, the S event and the P event. The P event is propagated from the origin at a definite phase angle difference with respect to the S event, but since it is propagated more slowly the phase angle difference increases with distance from the reference point (Fig. 1).

The other type of specification of positional information involves not a quantitative variation in some cellular parameter, but a *qualitative* one: it is essentially a mechanism for cell counting. With such a mechanism the cell at the origin would be A_0, the next cell A_1 and so on to A_N where $A_{1 \ldots N}$ would represent discrete cellular states. These could, for example, be represented by membrane states (Wolpert & Gingell, 1969); combinations of different genes; or combinations of different enzymes.

Irrespective of the mechanism whereby positional information is specified it is clear that it always involves a sense or direction in which it is measured and it is this sense, direction, or ordering relationship that I here define as the *polarity* of the system. Any system of co-ordinates—and positional information implies a co-ordinate system—requires a direction in which measurement must occur, and this is the polarity. For example, in the examples given above the polarity would determine in which direction the substance was transported in Fig. 1, curve II. The polarity in the phase shift model is the direction in which the S event is propagated. This in turn may be determined by the frequency of the S event in the cells in the system; under appropriate conditions the cell with the highest frequency will become the pacemaker for the system and this pacemaker cell will then be the reference point and will also specify the polarity. It is of the greatest importance to recognize that the specification of polarity may be quite distinct from the specification of positional information, even though, as will be seen, they may be closely related.

(D) RULES FOR THE SPECIFICATION OF POSITIONAL INFORMATION

Consider N units in an axial array. If the polarity of the system is as indicated by the arrow in Fig. 1 the cells will have their positional information specified with respect to the left-hand end, since the polarity determines the direction in which the positional information is measured. If we specify this as the α system, then the left-hand end cell will be α_0 and each cell will have its position specified with respect to it. The ith cell from the end will have positional information α_i. The following rules may then operate. If a cell is α_i then according to the polarity the cell adjacent to it will become α_{i+1}: in Fig. 1 this will be the cell on its right. If a cell does not have its positional information specified then in some systems, especially those capable of pattern regulation, it will become α_0, and thus the reference point. In some situations if a cell does not have its positional information specified it may not become α_0 but α_m: nevertheless, it will become the reference point for the system. It can be seen that with these rules the polarity effectively defines the ends.

As pointed out above, the physical significance of α_i will depend on the mechanism involved in the generation of positional information. It could represent for example the concentration of a substance, the phase difference between two periodic events, a particular state of the membrane or a particular combination of molecules. At this stage it is preferable to keep the concept in its most general form and define α_i with respect to its generation curve, that is how it varies with distance from α_0.

It is important to realize that positional information of a cell may be specified with respect to a number of points, planes or surfaces. Positional information may be multidimensional. The number of dimensions will be defined by the number of axes. An axis is defined as the line at right angles to surfaces of constant positional information. If there is only one reference point (or surface) the axis will be unipolar. If, however, positional information is specified with respect to both 'ends' then the system is bipolar. For example, in a uniaxial system, whether, for example, it be a single line of cells or whether they are arranged in a sphere, if there are two reference points one at each end of the axis then (Fig. 2) these will be termed the α and α' ends. The positional information on this axis will be referred to as α, α'; positional information on other axes will be referred to as β, γ, ... and so on. One may thus, for example, refer to the β' end of the β axis. It will be assumed that only one polarity need be specified for a bipolar system, the polarity for the α', β' or γ' will be measured in the opposite direction.

(E) RULES FOR SPECIFYING POLARITY

Polarity as defined above is that ordering relationship which specifies the sense or direction in which positional information is measured. This definition

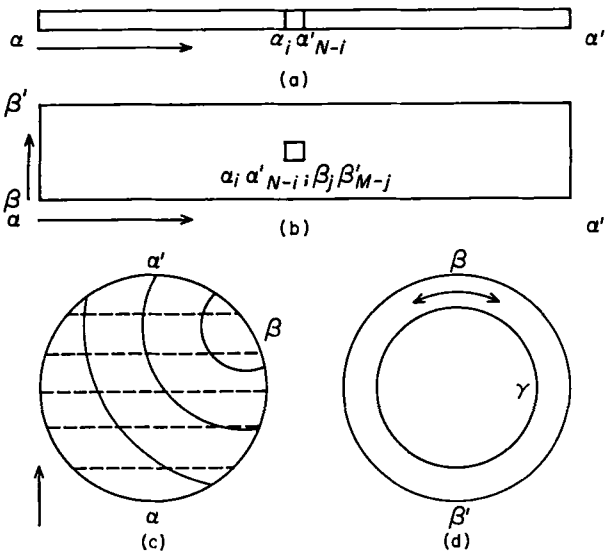

FIG. 2. Diagrams to show some examples of axes and polarities in fields with more than one dimension. In (a) there is a line of N cells and there is one bipolar axis $\alpha\alpha'$. The ith cell, measured in the same direction as the polarity, has positional information $\alpha_i \alpha'_{N-i}$.

(b) This is a sheet of cells, N cells long and M cells wide. It is a two-dimensional field having two bipolar axes, $\alpha\alpha'$ and $\beta\beta'$. Lines of constant positional information for the $\alpha\alpha'$ axis are at right angles to the long axis; for $\beta\beta'$ parallel to the long axis. Thus, for example, $\beta_o\beta'_M$ is the lower edge of the sheet.

(c) This is a spherical sheet of cells having two dimensions: one bipolar axis $\alpha\alpha'$ and one unipolar axis β. The arrow shows the polarity of the $\alpha\alpha'$ axis. Lines of constant positional information with respect to $\alpha\alpha'$ are shown dashed. The solid arcs are the lines of constant positional information with respect to β. Note the radial symmetry of these axes.

(d) This shows the cross-section of a cylindrical sheet of cells. The $\beta\beta'$ axis is bilaterally symmetrical. One could consider a third dimension along the γ axis, positional information being measured at right angles to the inner surface.

should be compared with that of Rose (1957*a*,*b*) who considered polarity in terms of flow of information, namely the direction in which inhibitory information moved. The relationship between positional information and polarity is a very close one and a common mechanism may be involved. However, at this stage it seems preferable to regard them as separate and to treat the rules for determining polarity as different and distinct from those involved in the generation of positional information once polarity has been established.

A very important insight into how one may consider polarity has come from work on the insect epidermis (reviewed by Locke, 1966) and particu-

larly from the ideas of Stumpf (1967) and Lawrence (1966). Certain structures in the insect epidermis are polarized in that they are orientated in a particular direction, and transplantation experiments can lead to alteration in this polarity. The most important idea to come from such studies is that polarity, as a sense, can be regarded as being determined by the gradient of a substance, a reversal in the direction of the gradient corresponding to a reversal in polarity. Lawrence (1966) has suggested a physical analogy in terms of the slope of sand, whose maximum gradient is determined by its angle of friction. In order to account for such gradients in biological terms he has suggested the possibility of active transport in a direction opposite to that of the gradient. Effectively both Lawrence and Stumpf consider the possibility that the absolute value of the concentration of the substance could provide positional information.

This view of polarity appears capable of explaining a wide variety of phenomena. In order to generalize it and formulate some rules for the determination of polarity I will assume that there is a quantitative measure —the polarity potential—the slope of which determines the polarity. It is only whether the slope is positive or negative that matters, not the value of the gradient, the polarity being in the direction of the slope. Since the polarity determines the reference point for positional information, this will always be at a high point in the polarity potential. In Fig. 3(a), for example, the polarity potential of a bipolar uniaxial system is shown. The polarity is from left to right and the reference point, α_0 will be at the left-hand end at the high point of the polarity potential and α'_0 at the low point of the polarity potential. The specification of the reference point at the high point of the polarity potential effectively defines the classic concept of dominance. No further consideration will be given here as to how the polarity potential is maintained but it is probably, as will be seen, related to positional information. Nevertheless one must not confuse the two concepts. Polarity at a point is a unit vector; positional information a scalar quantity.

The rules for change in polarity will be that there will be a tendency to maintain the same slope and there will be a flow from regions of high potential to low potential. (It is quite convenient to bear in mind Lawrence's sand model as a general guide.) It is also necessary to assume a threshold effect in that small differences in potential are ironed out without reversal of polarity. These points are illustrated for a variety of cases in Fig. 3. In Fig. 3(b) the change in potential is considered to be too small to cause a change in polarity, there is a threshold effect, whereas a similar graft but with an increased polarity potential, as in Fig. 3(c) does, with time, lead to a reversal of polarity of the left-hand end. Thus a graft with the same polarity as the host could result in a reversal of polarity. In Fig. 3(d) and (e) two similar

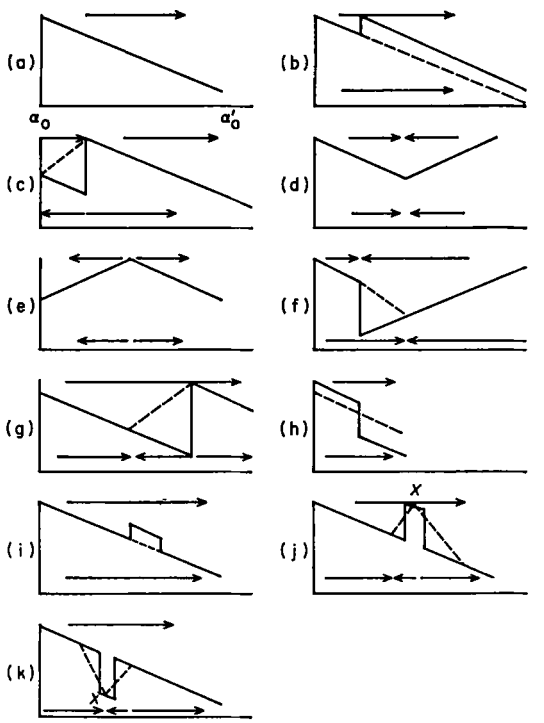

FIG. 3. Diagrams to illustrate changes in polarity potential with different graft combinations. The ordinate is polarity potential and the abscissa distance. The basic system is illustrated in (a) in which the polarity potential of a bipolar axis is shown. The left-hand end is the dominant region. The polarity is determined by the sign of the slope and is shown by an arrow. In (b) to (k) the upper arrow represents the polarity at the time of grafting and the lower arrow the resulting polarities. The dotted line shows changes in polarity potential. Thus in (b) a small increase in polarity potential does not lead to any change in either potential or polarity. However in (c), a larger change leads to polarity reversal. Other changes are discussed in the text.

fields are symmetrically joined together with opposite polarities in mirror symmetry. The fields would not be expected to interact and there would be no changes in polarity. However in Fig. 3(f) where two fields are opposed with opposite polarities, the higher polarity potential in the left-hand one would lead to a portion of the right-hand field becoming within the left-hand one. This would mean that these cells would now have their positional information specified with respect to the left-hand end, instead of the right-hand end of the system. In Fig. 3(h) the graft does not alter the polarity. In Fig. 3(g), which is similar to (c) there is a local reversal of polarity with mirror symmetry about the junction. In Fig. 3(i), (j), (k) the grafts are not

axial ones but involve the insertion of a piece. In Fig. 3(i) the increase in polarity potential is insufficient, but in Fig. 3(j) it results in a localized reversal of polarity. This could have very important implication since in the region of the graft a new field is established, which is effectively separate from the left-hand portion of the original field. A new α_0 could be established at the peak (x) of the polarity potential in the graft. One would also expect to find mirror image symmetry about this point. Figure 3(k) shows an analogous case of grafting, but here a low point of polarity potential has been introduced into the middle of the field.

The interpretation of polarity in terms of polarity potential will be shown to be capable of explaining a wide variety of results. However it is far from being a quantitative theory and three points require immediate comment. The first is that while polarity potential and positional information are treated separately there is little doubt that positional information can effect polarity potential, and in general it will be inversely proportional to i of α_i. The second point relates to the whole question of the validity of the potential concept. It is, for example, not clear that the potential concept would be valid for the phase shift model of Goodwin & Cohen (1969). In their model polarity is determined by the pacemaker cell and its ability to entrain adjacent and distant cells, and since polarity potential would reflect in their model the frequency gradients, may well require considerable modification. Nevertheless, the overall picture as shown in Fig. 3 might still hold. Third, the time required for changes in polarity potential may be very important.

(F) INTERPRETATION OF POSITIONAL INFORMATION

It is tempting to use terms like "translation of positional information" when discussing how the positional information specifies cell behaviour since it can in a sense be regarded as a coding problem, namely, how variation in positional information can specify different cellular activities. In order to avoid the terminology associated with DNA, RNA, protein coding, I propose that the terms "convert" and "interpret" be used to describe the processes. The overall process whereby positional information specifies a particular cellular state or activity or molecular differentiation will be called the interpretation of the positional information. The mechanisms whereby the positional information is "read-out" by the cell and changed into a form that leads to the particular activity will be referred to as the conversion of positional information. For example, the positional information of cell may be specified by the phase difference between two cyclic processes. This phase difference may be converted into the activation of a specific enzyme, which may in turn be converted into a change in internal ionic concentration, which in turn may lead to activation of a gene coding for a structural protein

which is an enzyme which leads to the formation of a red pigment. One would then say that the cell has interpreted the positional information by forming a red pigment. The interpretation of positional information is of course very dependent on the developmental history of the cell and its genome. In fact the terminology allows one to talk about developmental history, or hormones, or mutations, affecting a cell's interpretation of positional information. The concept of conversion than allows one to consider which stage in the process of conversion is affected.

(G) THE FRENCH FLAG PROBLEM AND SIZE INVARIANCE

As an example of the application of the above concepts we can now discuss the French Flag problem. Consider first N cells in a single line. Each cell is capable of molecular differentiation which results in the appearance of blue, white or red pigment. If the system is unipolar with the polarity as indicated in Fig. 4(a) then the cell at the left end will be α_0 and the ith cell will have positional information α_i. For the system to form a French Flag without size invariance one could have the following rules for interpretation.

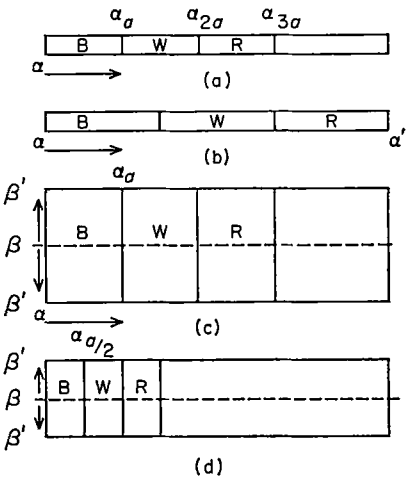

FIG. 4. Diagrams to illustrate the interpretation of positional information so as to make a French Flag.

In (a) a unipolar one-dimensional system, the blue region, for example, is between α_0 and α_a.

In (b) the axis is bipolar and the rules for interpretation are such that the whole axis becomes divided up.

In (c) a sheet of cells has the α axis unipolar and a bilaterally symmetrical bipolar $\beta\beta'$ axis. Blue is between α_0 and α_a and this should be compared with (d) in which, due to $\beta\beta'$ axis being shortened, a is reduced to $a/2$ (B, blue; W, white; R, red).

Between α_0 and α_a, blue; between α_a and α_{2a}, white; between α_{2a} and α_{3a}, red. Removal of regions to the right of α_{3a} would have no effect on the pattern if $N > 3a$. However, if part of the Flag is removed it will regulate provided the remaining N is greater than $3a$. For size invariance it is necessary to have a bipolar system (Wolpert, 1968). Let the reference point at the left-hand end be α_0 and that at the right-hand end be α'_0 [Fig. 4(b)]. Then each cell will have its position specified with respect to α_0 at the left-hand end and α'_0 at the right-hand end: that is each cell will have positional information $\alpha_i \alpha'_{N-i}$. This is a uniaxial bipolar field since it has two reference points. In principle appropriate rules such that the left-hand third becomes blue, the middle third white, and the right-hand third red, can always be formulated. These will depend on the nature of the positional information/distance relationship. If, for example, this relationship were a linear one, and identical for both α and α' then, the rules for interpretation leading, for example, to blue, could be $\alpha_i/\alpha'_{N-i} < \frac{1}{2}$ (see Apter, 1966). It is of great interest to note that α_i and α'_{N-i} can in principle provide each cell with the length of the field. If, for example, the α and α' positional information curves are mirror images then the sum of α_i and α'_{N-i} gives, effectively, the length of the line.

If the line is cut in half, then considering the left-hand side the α_i values would remain unchanged but the cell at $N/2$ will now become α'_0 and the α' value for each cell will change appropriately: α'_{N-i} will become $\alpha'_{N-i-(N/2)}$ which is $\alpha'_{(N/2)-i}$. The rules for interpretation will again lead to the French Flag, provided, of course, the molecular differentiation of the cells can respond to the new positional information.

The system just described is, from a positional information point of view, bipolar since the cells are having their position specified with respect to both ends. The description given involved two values, the α and α' ones, and it is important to realize that even in our effectively two reference point system only one value for positional information need in fact be specified in order to specify the *relative*, as distinct from the absolute, position of the cell from the ends. For example, in the case described above where one end is a source and the other a sink the absolute values of the substance at the one-third points is constant invariant with size (Wolpert, 1968). Thus any system which can fix the value of a parameter at both left- and right-hand ends, and ensures a linear variation between them, would provide a satisfactory solution. It is thus of particular interest that the phase shift model of Goodwin and Cohen can provide just such a mechanism by building into the model the requirement a maximum phase angle difference which occurs at the end opposite the pacemaker. Briefly the mechanism could involve the initiation by a cell, whose phase angle difference is at this critical value, of another wave whose effect is to reduce the phase angle difference in all the

cells. This type of mechanism obviates the necessity for the specification of an α' value independent of α.

Thus far the problem has only been examined in relation to a uniaxial system, and it is now necessary to consider the extension to two axes as would be required if the French Flag were to be formed from a sheet of cells. Consider the unipolar α system Fig. 4(c). Let there be bilateral symmetry such that β_0 is along the midline, and β'_0 at the edge: the β axis is bipolar. Then appropriate rules of interpretation may be specified such that, for example, the value of a which determines α_0 to α_a depends upon the effective sum of $\beta_i+\beta'_{N-i}$: it could be that the greater the sum $\beta_i+\beta'_{N-i}$, the greater a. Compare Fig. 4(c) and Fig. 4(d).

Such rules can provide a solution to the French Flag problem. It is important to realize that with this type of mechanism it is the positional information together with the rules of interpretation that specify the pattern. There is no interaction between the parts of the pattern as such, and it differs in this respect from most other concepts of pattern formation, particularly those that require inductive or inhibitory reactions between differentiated regions. It should be clear that the concepts developed here suggest that pattern formation is at least a two-step process. First, and independent of molecular differentiation, is the specification of positional information. Then the cells differentiate according to the interpretation of the positional information. The nature of the interpretation will depend on the cells' genome and its positional history. Clearly such considerations provide the link between pattern formation and the molecular basis of molecular differentiation.

(H) POSITIONAL INFORMATION AND THE FIELD CONCEPT

As defined above a field is that region in which all cells are having their positional information specified with respect to the same set of points. The concept of field as classically used is not easy to define and is surrounded by a good deal of controversy. Waddington (1954) has pointed out that the term "field" should only be used to refer to the character of the process occurring in a region or district and should not be used simply to refer to the spatial location of, for example, a presumptive region. It is thus now necessary to show that the definition of field in terms of positional information satisfies the classic requirements for the type of process thought to be taking place.

The term "field" is used to emphasize the co-ordinated and integrated character of the whole complex of processes. When it is used in connection with the formation of a definite organ with a characteristic individual shape the term can be made more precise by qualifying it as an "individuation

field..." (Waddington, 1966). It is clear that this conception of a field implies that the French Flag is an individuation field and the analogy becomes even stronger when some of the operational aspects which define a field are considered. Again following Waddington, these may be listed:

(a) "if a field is cut in two each half may reconstitute a complete field, so that two whole organs are developed";

(b) "if two fields are brought together and allowed to fuse they may rearrange themselves into a single field";

(c) "if part of a field, either central or peripheral, is removed, the remainder may compensate for the defect and become complete again, while the isolated part can often become modified into a small but complete field".

The concepts relating to positional information and regulation, together with the specific example of the French Flag problem, can account for all three operational aspects. It is suggested that it is positional information which provides the co-ordinated and integrated character of fields.

It may well be that the mechanism for specifying positional information is a universal one. If this were true then in principle a cell could not distinguish between fields whose geometrical properties or co-ordinate system were the same. Some operational implications of this are shown in Fig. (5). This conception of field not only provides a firm operational definition for a field and focusses attention on the mechanism involved, but also draws attention to the necessity of identifying the reference points in a field, and considering the specification of boundary values.

The views expressed here should be contrasted with those which invoke specific chemical substances unique to each field.

(I) POSITIONAL INFORMATION AND GROWTH CONTROL

As pointed out in the previous section, every cell in a field which has its position specified with respect to two ends of an axis, effectively could compute the length of this axis by summing α_i and α'_{N-i}. Thus it is possible to provide rules for interpretation of positional information in relation to cell growth and division such that the length of an axis is controlled in that cell division ceases when the axis is of a given absolute length. The idea that growth control may involve the absolute measurement of one or more lengths in a developing system does not seem to have been considered previously. Most theories of growth control suggest a feed back system based on the production by the growing cells of some inhibitor such as a chalone (Bullough, 1967). Those systems then depend on the dilution out of such inhibition in an external pool; they thus only provide a mechanism for proportionate growth control.

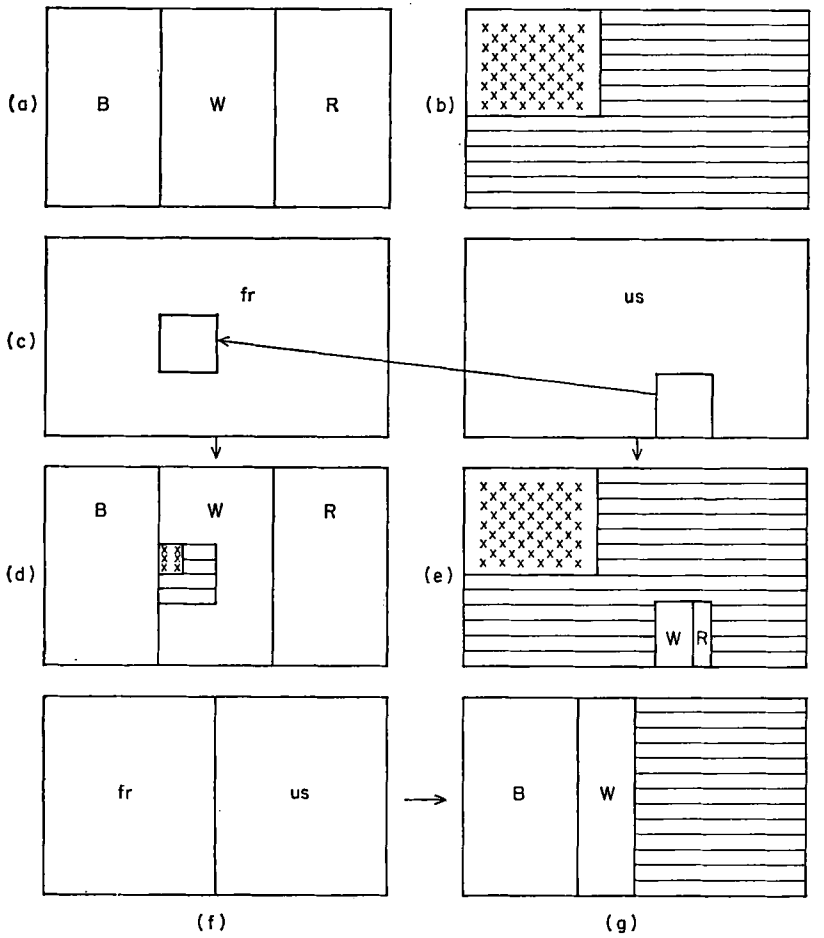

FIG. 5. Some examples to show some possible implications of the universality of positional information. Consider a rectangular field and two different genotypes. Genotype *fr* results in the interpretation of the positional information so that a French Flag is formed (a) while genotype *us* results in the Stars and Stripes (b). If, at an early stage, two pieces are interchanged as in (c), and if positional information in the two fields is the same, then the results shown in (d) and (e) will follow: that is the cells behave according to their genotype and position and are indifferent to the nature of the surrounding tissue. Similarly, if two halves of different genotypes are joined as in (f) a mosaic as in (g) will form (B is blue, W is white, R is red).

2. Specific Examples of Positional Information and Polarity Potential

In this section specific examples of pattern formation will be considered in some detail, though it will be necessary to be relatively selective of the data considered.

(A) EARLY DEVELOPMENT OF THE SEA URCHIN EMBRYO

The early development of the sea urchin embryo involves its subdivision along its animal vegetal axis into mesenchyme, endoderm and ectoderm Fig. 6(a). The effect of operative procedures and chemical agents on the relative proportions of these three regions has been widely studied (Hörstadius, 1939; Gustafson, 1965). The classical studies and review of

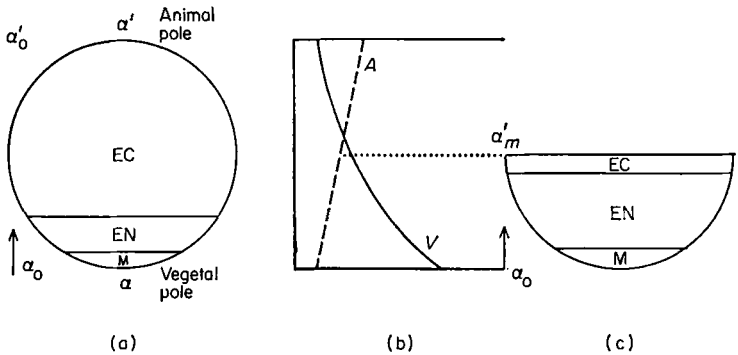

FIG. 6. (a) Diagram of early sea urchin embryo to show the presumptive regions M, mesenchyme, which normally forms from the micromeres; EN, endoderm; EC, ectoderm. α_0 is at the vegetal pole and α'_0 at the animal pole. (b) shows the possible vegetal, V, and animal, A, gradients. (c) is a vegetal half. Because the A/V ratio is below the threshold K_A the initial α' value is not α'_0 but α'_m. This leads to a much reduced ectoderm and an increased endoderm.

Hörstadius (1939) from which all data in this section are drawn unless otherwise stated, using grafting techniques have given support to the double gradient concept as a means for controlling the early development. The characteristic feature of these experiments is that in animalized larvae which result from removal of vegetal material, it is the most vegetal structures that disappear first; and with vegetalized larvae [Fig. 6(c)], which result from the removal of animal material, it is the most animal structures that are progressively lost. In animalized larvae the ectoderm is proportionately too large; in vegetalized the endoderm. While these and other graftings have firmly established the double gradient concept, involving animal and vegetal

gradients, what is completely lacking is a model of how such gradients are established, regulated, or exert their effect, except in the vaguest of terms. The diagrams given, for example, by Hörstadius (1939, Fig. 11) imply that the gradients themselves regulate. Needham's [1942, Fig. 2(b)] diagrams also imply that they change their shape, and that the endoderm-ectoderm border is specified where the two gradients intersect. The interpretation to be proposed here is very different and will suggest that the gradients themselves do not determine the pattern directly, but serve to specify the values of α_0 and α'_0: that is the value of the positional information at the ends which are the points from which the other cells have their positional information specified. The gradients specify the boundary values.

It should be first pointed out that the early development of the sea urchin shows a size invariance of the main pattern over an 8 eightfold size range. That is blastomeres from the four-cell stage give normal larvae, and, of great importance, so do double volume embryos formed by the fusion of two eggs. It is postulated that positional information is specified with respect to both ends of the animal-vegetal axis, the vegetal pole being α_0 and the animal pole α'_0. The embryo may then become divided up along this axis into mesenchyme, endoderm and ectoderm in a manner which is invariant with size if the rules for interpretation are similar to those for the French Flag as discussed above. What is needed here, in addition, is a mechanism to account for the observed failure of proportionality in animalized and vegetalized larvae.

Consider the early embryo to have two gradients represented by A (animal) and V (vegetal) as in Fig. 6. Neither the nature of these gradients nor how they are maintained is known, but they are laid down in the egg. Then it is suggested that the polarity of the α axis is determined by V which determines the polarity potential, the vegetal pole being the dominant region. α'_0 is at the animal pole. One then has a system very similar to that in Fig. 2 and each cell has its position specified with respect to the $\alpha\alpha'$ axis. If the end cells are α_0 and α'_0 then we may expect the systems proportions to be size invariant. It is proposed that the V/A ratio determines the value of the end or first cell for the α axis and the A/V ratio determines the value of the first or end cell of the α' axis. If $V/A > K_v$ then the first cell will be α_0; if however $V/A < K_v$ then the first cell will be some other value α_m and the less V/A is with respect to K_v the greater m will be. This means that positional information distance curve effectively starts m cells along and values between α and α_m are missing (cf. Fig. 1). Similar rules are proposed for α': if A/V is greater than K_A then the first cell is α'_0 but if $A/V < K_A$, then it is α'_m.

One can now attempt to interpret some of the more important grafting results in terms of these concepts.

(a) The removal of the micromeres, the most vegetal region, still results in normal development. Doubling the number of micromeres also leads to normal development. This is a somewhat surprising result in terms of classical concepts since the micromeres are the most powerful vegetalizing or organizing region of the embryo. In terms of the new concepts, these results merely reflect the fact that the V/A ratio is greater than K_v both without the micromeres and when their number is doubled.

The organizing power of the micromeres is largely due to their being at the high point of the polarity potential, which is at the vegetal end. The evidence for this is, for example, that four micromeres when implanted in the side of a 32-cell stage blastula induce there a more or less complete secondary larvae. The situation is analogous to the induction of a new axis by the hypostome of hydra [see below, Fig. 3(j)]. The implantation of micromeres at the animal pole of an animal half can lead to a complete reversal of polarity of the embryo. The relative unimportance of the animal half in determining polarity is shown by the experiment in which a saggital half is combined with an animal half and develops normally and in relation to the axis of the saggital half.

(b) While removal of the micromeres, the most vegetal region, does not lead to animalization, further removal of vegetal material does. There is clearly a threshold effect as specified by the model. An animalized larva will result if $V/A < K_v$ since now α_0 to α_m are missing but the positional information given to other cells implies it is there. Effectively the embryo forms animal regions appropriate to a much larger embryo. Moreover the greater m, the greater the degree of animalization.

(c) Removal of animal material again reveals a threshold phenomenon. The behaviour of a vegetal half appears to depend on the position of the third cleavage plane which is at right angles to the animal vegetal axis and divides it into animal and vegetal halves. In vegetalized larvae which develop from vegetal halves, there is a distinct increase in endoderm at the expense of ectoderm [Fig. 6(c)]. Some vegetal halves develop normally and Hörstadius (1939) suggests that this is due to the level of the third cleavage plane being slightly more towards the animal pole. This would suggest that the critical K_A value for the A/V ratio is about the level of this cleavage plane, and in Fig. 6, arbitrarily, has value of one. If the plane of cleavage passes just above it, that is on the animal pole side, then $A/V > K_A$ and the vegetal half will be more or less normal since the α value will start at α'_0. If, however, the plane of cleavage is lower down, then $A/V < K_A$ and α' starts at α'_m. This will lead to a decreased ectodermal region and increased endoderm. Effectively, the embryo is behaving as if the regions α'_0 to α'_m were present [Fig. 6(c)].

(d) A variety of combinations can lead to the development of normal larvae. One of the most instructive is the effect on the most animal region of adding micromeres. The isolated most animal region (called an_1) develops into a hollow ball covered with cilia typical of the animal tuft which is the most animal region of the embryo. The same region to which one micromere is added develops into a ball in which the ciliated tuft is initially reduced to a small region. Four micromeres are required to produce a normal larva. This is in complete accord with the proposed model in which the A/V ratio determines the initial value of α'; four micromeres, which have a high V, make the V/A ratio greater than K_v. Fewer micromeres are needed to make an_2 develop normally since its A value is lower. Practically all the grafting experiments can be interpreted along the above lines: a more quantitative set of conditions and requirements could relatively easily be obtained by simulation studies with a computer.

In these terms the action of vegetalizing and animalizing agents would be to effectively increase the V and A gradients, respectively.

This analysis of early sea urchin development differs markedly from the classical ones in that it assigns a very different role to the animal and vegetal gradients to that usually given. Here these gradients do not determine the pattern of cellular differentiation but are concerned with the establishment of the axes and value of positional information at the ends. In addition, by drawing a distinction between polarity potential and positional information, the organizing properties of the micromeres find an explanation in terms of polarity changes. Their properties in this respect are thus analogous to those of the hypostome of hydra, which will be considered next.

(B) REGENERATION OF HYDROIDS

Discussion on regeneration of hydroids in terms of positional information will be confined to observations on hydra and *Tubularia* which are the best studied systems.

Tubularia is a colonial hydroid with remarkable powers of regeneration. There is a good deal of evidence (e.g. Tardent, 1963) that there is an inhibitory reaction between hydranths, that is, the presence of one hydranth can inhibit the formation of another hydranth nearby. It is not this aspect of pattern, the site of hydranth formation, which I wish to consider here, but rather the pattern within the hydranth itself. The hydranth [Fig. 7(a)] has radial symmetry and the following structures can be identified and appear in a disto-proximal direction: hypostome, distal tentacles, gonophores, proximal tentacles. This hydranth, like the fresh water hydra, is capable of considerable regeneration and pattern regulation (see, e.g. Rose, 1957*a*,*b*; Berrill, 1961).

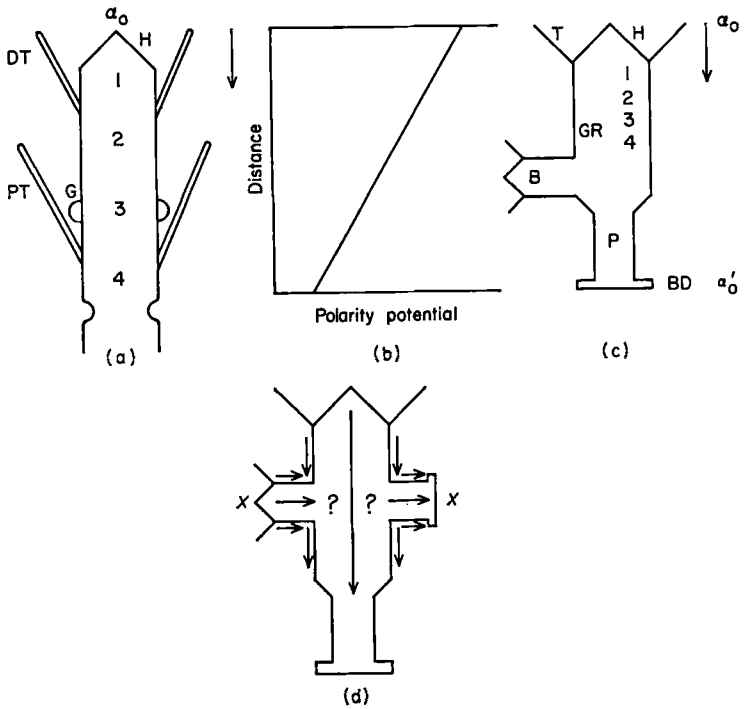

FIG. 7. (a) Diagram of the hydranth of *Tubularia*. DT, Distal tentacles; H, hypostome; G, gonophores; PT, proximal tentacles. The numbers 1 2 3 4 are used to indicate the regions used in the graft experiments, but it should be noted that the grafts are done at an early stage of regeneration when the hydranth is essentially a closed tube.

(b) The suggested polarity potential for both *Tubularia* and hydra.

(c) Diagram of hydra. H, Hypostome; T, tentacles; BD, basal disc; B, bud; GR, gastric region; P, peduncle. The numbers refer to the regions of the gastric region used in grafting.

(d) Diagram to show the polarity of induced distal and proximal regions. Note that the points *X* corresponds to the appropriate point in Fig. 3(j) and (k), respectively. The question marks show the region at the boundary between the primary and secondary axes where the nature or details of the polarity are unknown.

This regulation, unlike the French Flag, is not size invariant and *Tubularia*, along its main axis, appears to be a unipolar system.

It will be assumed that the polarity potential in the hydranth decreases disto-proximally, thus the most distal cells will be the reference point for the α axis, which corresponds to the main axis of the hydranth, and will be α_0 as in, for example, Figs 1(a) and 3(a). Then all the cells will have an α value depending on the positional information/distance curve, and with appropriate rules of interpretation will result in a hydranth: for example,

distal tentacles may form between α_0 and α_a [see Fig. 4(a), (c)]. If the cell that does not have its positional information specified always becomes α_0, that is α_0 is at the high point of the polarity potential, then normal regeneration will occur if the hydranth, or part of it, is removed. The dominant region is thus at the distal end as is well known. It is worth emphasizing that with respect to hydranth form, the rules for interpretation probably involve the specification of cell movements and changes in cell contact at particular α_t and that these give rise to, for example, tentacles. The hydranth may be viewed as comprising a single field and the distinction between, for example, proximal and distal tentacles may be much less than realized since the same cell activities may give rise to both. This view should be contrasted with those of Rose (1957*a*,*b*, 1967).

A large number of different graft combinations have been made between regenerating hydranths particularly by Rose (1957*b*). The graft combinations were made some time after regeneration had begun, at the time when there was already some morphological indication of the pattern, and this should always be borne in mind since it is well established in hydra that crucial changes occur in regenerating pieces long before manifestation of a morphological pattern (Webster & Wolpert, 1966; Webster, 1966*a*). While Rose has interpreted these results in terms of the polarized flow of specific inhibition, each region suppressing a like expression proximal to it, the results can be interpreted in terms of positional information and polarity potential, and with reference to Fig. 3. Consider, for example, three experiments involving grafts in the same polarity. In the first, a distal region, at stage 6, containing the distal tentacle primordium is grafted on to a host, that is a 1 on to a 1 2 3 4 [Fig. 7(a)] and a normal hydranth results [Fig. 3(b)]: in the second, a 1 2 3 4 is grafted on to a 3 4, and a normal hydranth again results: in the third a 1 2 3 4 is grafted on to a 1 2 [see Fig. 3(g)] and each develops more or less independently. These are results that may reasonably be expected in terms of polarity potential if the change in polarity potential at the junction between the grafts in the first and second experiments, is too small to change the sign of the slope. In the third, a larger change is to be expected, a 4 joining a 1, and a local reversal in polarity is to be expected with a new α_0 being established at the graft junction. These results may be viewed in terms of the phase-shift model where the polarity potential corresponds to the frequency gradient: in the first and second experiments entrainment by the pacemaker cell occurs, since the frequency jump at the graft is not great, but in the third case the frequency jump is too great for entrainment and a new pacemaker will develop at the junction. Such ideas also can explain the behaviour of grafts joined with opposite polarity, which, in general, result in each piece behaving as a separate field. However, of particular significance

is the case where a distal region 1 2 is grafted with reversed polarity on to the proximal region of a 1 2 3 4. This is equivalent to the case illustrated in Fig. 3(f), and it is very gratifying that Rose (1957*b*) found that the 1 2 repolarized part of the adjacent piece and interpreted it as such.

A striking feature of *Tubularia* is the relationship between length, diameter and pattern. Measurements on large and small hydranths of *Tubularia* by Davidson & Berrill (1948) have shown in a particularly persuasive manner the proportional decrease in length of the hydranth with decreased diameter. This aspect of pattern formation has been almost entirely neglected although Morgan (1900) drew attention to it in relation to the regeneration of both hydroids and planaria. In terms of positional information it implies that the interpretation of a cell of its positional information in the axial direction—α axis—is modified by the positional information measured in the radial direction, the $\beta\beta'$ axis as described above (see Figs 2 and 4). For example, the site of distal tentacle formation occurs at increased α values when the diameter is increased so that effectively the sum of β_i and β'_{N-i} is increased [see Fig. 4(c), (d)]. This may account for a variety of phenomena not otherwise easily explained, particularly the behaviour of short isolated pieces. The behaviour of short pieces of hydroids have always been rather troublesome for any theory of pattern formation. In general in such short pieces, as shown by Child's studies on *Coryomorpha*, while there is always a disto-proximal ordering of structures, the more proximal structures are often absent. An examination of Child's (1941) diagrams (Figs 116 and 117) strongly suggest that the scale of organization is related to the radial dimension. In a short piece, if this is relatively large, then according to the concepts given above, only distal structures will be present. The more normal the length/radius ratio, the more complete the scale of organization will be. The results of Davidson & Berrill (1948) who found regional differences in the behaviour of isolates from regenerating primordia may partly depend on similar factors.

In contrast to *Tubularia*, hydra seems to be a bipolar system since the pattern seems size invariant over quite a range, and so the positional information is specified with respect to the two ends, the hypostome and basal disc. The regions in hydra are less clearly defined but proceeding disto-proximally there are: hypostome and tentacles; gastric region; budding region; peduncle; and basal disc [Fig. 7(c)]. While it has not been accurately measured, the ratio of the axial length of hydra to its circumference seems about constant in that large hydra have similar proportions to small hydra. This type of regulation would probably involve the α and β axes for positional information as in Fig. 4(c) but this important problem will not be considered further here. If we designate the distal end α and the proximal end α' then each cell along the axis will have positional information $\alpha_i\alpha'_{N-i}$ where N is the number of

cells along the axis involved in the specification of positional information. Just which cells are in fact involved in specifying positional information is of course, not known. Then the regulation will occur as described above with reference to Fig. 4. It is also possible to interpret a variety of grafting experiments along lines similar to that for *Tubularia* and in accordance with Fig. 3. The cases illustrated in Fig. 3(d) and (e) behave as would be expected. Of particular interest is the case illustrated in Fig. 3(c). This effectively predicts that a proximal portion of a gastric region (3) grafted with the same polarity on to a gastric region (1 2 3 4) might have its polarity reversed, tentacles forming at the graft junction and a foot appearing at the distal end. We have recently shown that this occurs in about 30% of the grafts (Hicklin & Wolpert, unpublished). We have also obtained evidence that polarity determines the ends (Hicklin, Hornbruch & Wolpert, 1969). There is however another type of graft which is particularly relevant to hydra, and which demonstrates the "inducing" properties of the hypostome and which is best considered in relation to budding.

Budding of a new axis from the gastric region of growing hydra requires special consideration. The region of the bud may be regarded as a region of increased polarity potential, the increase being sufficient to effectively establish a new field: it may thus be likened to Fig. 3(j) where there is a large localized increase in polarity potential. In terms of the phase shift model it is a region of increased frequency. This increase appears to be localized on one side of the animal only and leads to reorientation and morphogenesis of the cells in its vicinity, resulting in the formation of a new axis at more or less right angles to the host, though the mechanism is not known (Clarkson & Wolpert, 1967). It is characteristic of the bud axis that it detaches from the host, and a clear understanding of this in terms of polarity potential would be very helpful. The behaviour of the bud axis must now be compared with that of an axis induced by a grafted hypostome. It was Browne (1909) who first showed that a piece of hypostome grafted into the side of the gastric region will induce a new axis. This is effectively the same as a graft as in Fig. 3(j) which will result in a local reversal of polarity as illustrated in Fig. 7(d). The new α_0 at X will be the reference point for a new axis. This results in reorientation of material of the host axis and morphogenesis, which is similar to bud morphogenesis but with the important differences that the axis does not detach, and is shorter. These phenomena raise very important questions concerning the boundary conditions at the junction between two fields, or two axes, which at present we do not know how to draw. In the case of the bud it may be assumed that there is sufficient increase in polarity potential that effectively a new field is set up which does not have time to establish points of common positional information with

that of the host: the independence of budding, once initiated, from the host is well established (Clarkson & Wolpert, 1967). The postulated high polarity potential of the bud is in fact in accord with the observation that any part of a developing bud will, when grafted into a gastric region, induce a new axis (Clarkson, 1967). In the case of the axis induced by the hypostome the situation as regards both polarity and positional information at the junction is again not clear. It might be that at the junction cells receive the same positional information with respect to the α_0 of both host and induced axis, and that this determines the length of the induced axis: it should increase in length when the junction is further from the hypostome. Similar boundary problems arise in relation to the induction of proximal structures by grafts of peduncle (Hicklin, unpublished) [Fig. 7(d)]. This phenomenon may be interpreted in terms of Fig. 3(k), the peduncle region providing, in effect, a sink.

One must now consider the implantation of a subhypostomal region in the gastric region of a host (Webster, 1966*a*). This is absorbed unless the host hypostome is removed, in which case the subhypostomal region does induce an axis. The former case is assumed to correspond to Fig. 3(i) the difference in potential being insufficient to reverse polarity, and it thus takes its positional information from α_0. The induction in the absence of hypostome is less easily explained. One possibility is that in the absence of an α_0 positional information is not generated and both the subhypostomal region of the host and graft will tend to α_0 and will both become this at about the same time. If, as is very likely, there is a relationship between the α_i value and polarity potential, such that polarity potential increases with decreasing i, then the situation will become the same as for a normal hypostomal induction.

The above interpretations of normal regeneration, and grafting experiments, are very different from those usually given (e.g. Webster, 1966*a*,*b*) and do not invoke the flow of specific inhibitors or activators. It is quite misleading, for example, in terms of the above analysis to interpret the failure of an implanted subhypostomal region to induce an axis [as in Fig. 3(b)], as an inhibition of the graft. It should be clear that the concepts of polarity potential and positional information provide quite a powerful tool for analysing a variety of phenomena in hydroids. It is of particular importance that the phenomenon of induction can largely be interpreted in terms of polarity changes.

(c) PATTERN FORMATION IN INSECTS AND THE CONCEPT OF PREPATTERN

For me, the most significant contributions to the study of pattern formation over the last 30 years come from the work of Stern (1956) on genetic mosaics

and the concept of prepattern, together with the experimental work on insects of Kroeger (1959, 1960), Stumpf (1967) and Lawrence (1966). This work provides excellent evidence for the concept of positional information and polarity potential, and the best evidence for the postulate of universality, at least within the same animal. As will be seen, the concept of positional information gets over some of the difficulties associated with the concept of prepattern.

The work on genetic mosaics by Stern and his collaborators has shown that there is complete autonomy of cell differentiation in mosaics of different genotypes. His technique essentially provides experiments of the type shown in Fig. 5(a) to (e). Contiguous areas of different genotypes form their appropriate phenotypes almost regardless of the nature of the neighbouring cells. For example, the sex comb in *Drosophila melanogaster* is located on the first tarsal segment of the foreleg of males but is absent in females. In genetic mosaics comprising male and female genotypes, the behaviour of cells in the region of the sex comb is autonomous: male cells forming teeth of the sex comb even if surrounded by female tissue, and female cells being unable to do so even when surrounded by predominantly male tissue. Similar observations indicate autonomy for the engrailed extra sex comb and sexcombless mutants for sex combs, the achaete theta, and scute mutants for bristle patterns (see Tokunaga & Stern, 1965, for references). These observations have been interpreted in terms of the presence of a prepattern and the competence of cells to respond to singularities in it. This is best explained by reference to sex combs again and quoting Tokunaga & Stern (1965) directly. "The restricted specific area in the male in which a sex comb is formed may be called a regional singularity. An analysis of gynanders (Stern & Hannah, 1950) showed that this singularity exists in both males and females. Its presence evokes a developmental response toward formation of sex comb teeth provided the genotype of the responding cells is male. Female cells lack the competence to form teeth. In so far as the singularity arises during development before formation of the visible pattern of differentiation it constitutes part of a 'prepattern' (Stern, 1954*a*,*b*)."

Stern's interpretation of most mutants affecting pattern that have thus far been studied is that the mutant does not lead to a new pattern by changing the prepattern but by changing the competence of the cells to respond to the invariant prepattern. This is an extremely important concept but has given rise to some difficulties not unlike those associated with the field concept (Waddington, 1962, 1966; Ursprung 1966). The immediate problem concerns the nature of the origin of the prepattern. This presents serious difficulties since the prepattern is envisaged by Stern and others as having itself a well-defined pattern, and the problem then becomes how this pattern

is specified. For example, the prepattern is viewed by Maynard Smith & Sondhi (1961) and Tokunaga & Stern (1965) as possibly being represented by variation in the concentration of some substance along an axis, the concentration curve having well-defined singularities such as peaks at specific points. The specification of this pattern seems no less easy than that of the original pattern, though Maynard Smith & Sondhi (1961) have attempted to account for the origin of such a prepattern in terms of waves generated by a Turing-like (Turing, 1952) system. This is not very satisfactory and all the difficulties disappear when the concept of prepattern is interpreted in terms of positional information.

In terms of positional information there is no prepattern in the sense of a pattern with singularities: there is rather the spatial specification of the cells which the cells' genome can interpret. Cells will behave according to their positional information and genotype, and more or less independently of their neighbours. In these terms the work relating to prepattern comes within the same conceptual framework as that of sea urchin and limb development, and hydroid regeneration.

The work relating to prepattern has particular relevance to the postulated invariance and universality of the means of specifying positional information. In these terms cell behaviour must always be determined by its genotype and position, and it should not be possible for a mutant to locally alter the positional information of a region of just one field, that is, to alter a prepattern. Since, if positional information were universal, all fields would be altered in the same way. For example, if a mutant led to change in the positional information/distance relation such that values α_k to α_l were omitted then all structures in all fields between α_k and α_l would be absent. A single case showing evidence for local alteration of positional information in a single field would throw very serious doubt on the suggestion that positional information is always specified in the same way. Thus far all genetic mosaics are consistent with the postulate of universality and autonomy of expression at a particular position hold true.

It is thus important to consider the suggestions of Maynard Smith & Sondhi (1961) and Sondhi (1963) that prepattern can be changed by selection and more important the recent studies of Stern & Tokunaga (1967) suggesting that there is non-autonomy in differentiation of pattern determining genes for sex comb of the mutant eyeless-dominant (ey^D) of *Drosophila*. They found that not-ey^D areas on mosaic basitarsi whose background tissues are ey^D form sex comb structures typical of ey^D and suggest that this "...is the consequence of a mutant prepattern formed under the influence of the mutant genotype. Such a new prepattern would involve an enlargement of an area with a singularity able to evoke sex comb formation. In normal males

the size of this area restricts the activation of the sex-comb-forming genotype towards a single row of teeth." There is however no reason to invoke a change in the specification of positional information but merely to consider the enlargement of a particular area. This is easily interpreted in terms of positional information since one of the effects of ey^D is to broaden the distal region of the basitarsus. The situation may thus be likened to the effect of increasing one of the dimensions of a two-dimensional pattern. This can lead to the extension of a particular part of the pattern or even new features appearing. Examples have been discussed above in relation to changes in axial proportions of the *Tubularia* hydranth with diameter, and another will be given below in relation to the addition of extra mesenchyme to the chick limb leading to the formation of a fully formed fibula. Stern has partly recognized this possibility: "abnormal segmentation and distal broadening of the basitarsus would result in an abnormal enlarged prepattern singularity which results in differentiation of several sex combs all shifted more or less in longitudinal positions".

Sondhi (1963) argues that if all prepatterns are constant and "...if all the constancies in development were to be explained in terms of pre-existing morphological constancies present at an earlier stage of development this would lead by an infinite regression to a preformationist theory of embryology". This argument is not valid since it ascribes to prepattern a structural form, with singularities. The concept of positional information at once gets over this type of argument. Sondhi (1963) pictures a prepattern as the distribution of an inducing substance with high and low concentrations. On this model of a prepattern they find difficulty in explaining the changes in position and number of chaetae and ocelli that occur with selection. For example, continuous selection for an increased number of ocelli and bristles leads to the increase in number of bristles and an irregular arrangement. This required them to postulate a change in the pattern of singularities rather than just a change in threshold response. However, in terms of positional information where there is no singularity such an interpretation is not necessary. What the selection is altering is response to positional information.

Another important piece of evidence for the universality of positional information comes from the work of Kroeger (1960). Kroeger extended Stern's type of prepattern experiment by manipulative means. Whereas Stern's technique could only bring about mosaics of cells with the same developmental history, Kroeger produced mosaics of regions of the insect with different developmental history. For example, early combined forelimb and hindwing imaginal discs of *Ephestia* [grafted together as in Fig. 5(f)] grew together into a uniform complex (Kroeger, 1959). The analysis of the pattern of the hinge parts—sclerites—from such combinations suggested

that the prepattern of both fore and hindwing were identical. This was suggested by the observation that however much the sclerites deviated from their normal configuration the respective parts were always connected in the correct way and tended to form a morphological unit as in Fig. 5(g). There was a clear interaction between the two fields. (One cannot but be struck by the similarity with Kieny's (1964) experiments on chick limbs—see below). Kroeger (1959) even went on to speculate that all prepatterns in an insect could be the same. He also emphasized that a distinction should be drawn between the cellular process whereby the prepattern is established and the cellular processes which determine how the cells will respond to the prepattern. These ideas of Kroeger are very similar to, and quite compatible with, those of positional information which I have put forward here. The concept of positional information puts them in a much more general framework.

Using quite a different approach Stumpf (1966, 1968), from her studies on the insect cuticle, has arrived at conclusions which are similar to the concepts of positional information and provide evidence for its universality. She has suggested that gradients in the insect epidermis are responsible not only for the orientation of certain structures such as hairs (see Locke, 1966; Lawrence, 1966) but that a cell's position in this gradient can determine its behaviour. In the pupa of *Galleria* the cuticle of the first four segments shows no obvious differentiation into a pattern along its axis. In segments 5, 6 and 7 there is by contrast a division of the cuticle into three regions. Stumpf (1968) has shown that the regional character of a piece of the cuticle from one of the posterior segments can be changed not only by altering its position within its own segment but by transplanting it to a suitable place in one of the anterior segments. She has concluded that the gradients in the posterior segments are the same as in the anterior segments; what is different is the competence of the cells to react to it. It is again clear that the gradient concept as here used by Stumpf is equivalent to the specification of positional information, and that the competence depends on developmental history. Her studies are thus in complete accord with those of Stern and Kroeger and the possibility of a universal mechanism for specification of positional information. Stumpf has effectively suggested a mechanism for specifying positional information in terms of a gradient in a substance. It is particularly encouraging that Bohn (1967) has found polarity relationships in the regeneration of the cockroach leg similar to those of Fig. 3, and in particular, Fig. 3(g) and (h).

The concept of positional information appears to be applicable to pattern formation in the insect epidermis and to remove some of the difficulties associated with the concept of prepattern. This is further illustrated in relation to transdetermination and homoiotic mutants. Transdetermination is

the process whereby cells determined to form one type of structure for example, insect anal plates, develop, under certain conditions, into quite different structures such as head parts, or leg, or wings (Hadorn, 1966). In terms of positional information this would be described as a change in the interpretation of the cells of their positional information, though it gives, of course, no insight into the nature of this process. However, if positional information in each field is specified in the same way, then the process is easier to understand since the positional information in an anal disc may be the same as that of a leg: what is different is the process of interpretation. Along similar lines one can interpret homoiotic mutants which involve genes such as *asistapedia* which cause antennae to form legs. Once again the positional information may be the same and only the interpretation different. This is in line with the postulate of universality and it is thus again encouraging to find genetic mosaics of *asistapedia* with normal tissue behaving according to position and genome (Roberts, 1964).

(D) DEVELOPMENT OF THE VERTEBRATE LIMB

The vertebrate limb develops as an outgrowth from the body wall and initially is made up largely of mesenchymal cells covered by an ectodermal layer [Fig. 8(a)]. A very significant characteristic of the ectodermal layer is the thickening that runs anterio-posteriorly along the limb bud's border, which is known as the apical ridge (see reviews by Amprino, 1965; Zwilling, 1961; Saunders & Gasseling, 1968). Numerous studies have confirmed Saunders' (1948) original observations on the importance of this apical ridge in limb bud development. Excision of the ridge as the bud elongates results in terminal limb deficiencies, and grafting an additional ridge to an appendage results in the outgrowth of a double limb. Most studies on the limb have concentrated not on the development of the axial pattern of the wing [Fig. 8(a)], but on the interactions between the apical ectoderm and the underlying mesoderm. This interaction has been mainly viewed in terms of a maintenance inductive activity from mesoderm to ectoderm, and the induction of specific structures by ectoderm acting on the mesoderm.

In order to consider the mechanism of laying down the axial pattern, in terms of positional information (not forgetting the anterior-posterior and dorso-ventral axes) it is necessary to try and identify to the reference points and polarities within the system. One important clue comes from those experiments which show that the main structures in, for example, the wing are laid down in a proximo-distal order. In terms of the French Flag analogy, the development of pattern in the limb is not the growth of a small French Flag but the laying down first of the blue region, then the white, and finally the red, as the region increases in length. This is strikingly illustrated by

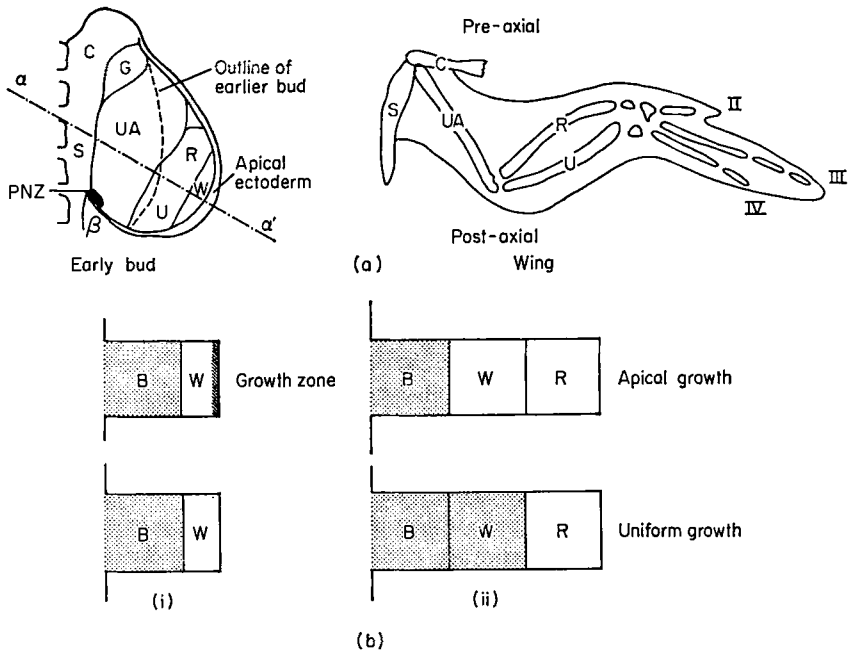

FIG. 8. (a) The presumptive regions (diagrammatic) of an early limb bud and the main skeletal structures that are formed, seen in dorsal view. C, Coracoid; S, scapula; G, glenoid; UA, upper arm; R, radius; U, ulna; W, wrist and hand. The apical ectodermal ridge runs along the edge of the mesenchyme bud. The presumptive map of early stages are congruent, thus the dotted line indicates the apical ectodermal ridge at an earlier stage and the presumptive regions are the same.

(b) Model to illustrate apical and uniform growth in a situation where fate maps are congruent. If during outgrowth the structure forms three equal regions, B, W and R, then if there is apical proliferation the cells that will form B at stage (i) (stippled) do not change their presumptive fate as growth proceeds from (i) to (ii). If, however, growth is uniform, then half the cells in region B at stage (i), the presumptive B cells, will become presumptive W cells.

Saunders (1948) presumptive fate maps at early stages of development which shows that the fate maps are congruent [Fig. 8(a)]. A re-examination of Tschumi's (1957) observations on the developing amphibian limb shows a similar congruence at early stages. This congruence of fate maps was one of the main factors leading to my formulation of the concepts of positional information, for, it seemed to require a measure of the distance of the regions in the limb bud from the body wall. Up to a certain distance the shoulder region was specified, at a greater distance the humerus and so on. This implies that there is an $\alpha\alpha'$ axis extending from the body wall to the apical ridge, the apical ridge corresponding to the origin of α' and it is the positional

information along this axis that largely determines the axial pattern of the limb and also its initial outgrowth. At early stages it would be the α_i value which largely determined the pattern, the proximal region of the limb being specified, for example, between α_k and α_l. The position of the $\alpha\alpha'$ axis is not known but its existence could also account for the considerable regulative capacity at this stage. For example, when an intermediate part of the early limb bud, amounting to one-third of its total length, is removed (Hampé, 1958) the limb undergoes an almost complete regulation and develops almost normally. This and other examples (see Zwilling, 1961; Amprino, 1965) suggest that the behaviour of the cells is determined by their position along the $\alpha\alpha'$ axis and any theory based on a strict temporal sequence of events related to growth would seem to be inadequate. One of the best pieces of evidence that it is position within the limb bud that determines cellular behaviour, comes from the transplantation experiments of Searle (1967). He showed that cartilage formation by the mesenchyme cells depends on their being in the central area: cells from this area will, when transplanted to more peripheral areas differentiate into muscle. It is worth pointing out that the limb bud also has mosaic characteristics and an incision dividing it into anterior and posterior halves may result in each half developing as part of a mosaic (Warren, 1934).

The mechanism of limb outgrowth has some important implications for any theory of pattern formation, in view of the congruence of presumptive maps at early stages of development, which have not been fully appreciated. The outgrowth of the limb involves cell growth and cell division and it is crucial to know the pattern of mitoses. For if growth occurs mainly beneath the apical ridge, in the distal region of the limb, then it is not too difficult to reconcile growth with a proximo-distal laying-down of the axial pattern [Fig. 8(b)]. Amprino (1965) has suggested that "the gradual elongation of the limb bud and the individuation of mesodermal territories in an ordered temporal sequence from the base toward the apex of the bud requires (1) more rapid proximo-distal growth than craniocaudal and dorsoventral, and (2) a comparatively higher rate of proliferation in the distal than in the proximal mesoderm of the bud during the period of territory individuation. Both these requirements are fulfilled during limb bud elongation." He claims that a higher percentage of mitoses has been found in the apical portion of the bud than in the rest of the bud mesoderm. Our own investigation (Hornbruch & Wolpert, unpublished) has failed to reveal such a difference, and our observations would be consistent with a more or less uniform growth in the mesoderm with other factors determining the form of the bud. More important, uniform growth requires that cells which are initially presumptive proximal regions must become more distal regions as shown

in Fig. 8(b). It thus requires that cells change their presumptive fate according to their position on the $\alpha\alpha'$ axis.

It is now necessary to consider the anterior-posterior axis of the limb and those experiments that involve reversal of this axis or duplications. A typical experiment involves the rotation of the wing apex through 180° which results in duplicate wing tips, reversed dorso-ventrally, one from the originally postaxial (posterior) part which is behaving as it normally would have, and another from the originally preaxial part (anterior) which would not normally have formed distal wing structures. It was this type of experiment that originally gave rise to the idea of an asymmetric distribution of some mesodermal maintenance factor and its inductive influence on the ectoderm: in general preaxial materials regulate their anteroposterior axes to conform with the polarity of experimentally associated postaxial tissue (Saunders & Gasseling, 1968). However, recent experiments by Saunders & Gasseling (1968) make a different interpretation possible and one which is much more in line with the concepts of positional information and polarity potential. The crucial discovery is that tissues from the posterior junction of limb bud and body wall (sometimes referred to, for other reasons, as the P N Z) has the unique property, when grafted to the apical ridge region, of inducing the preaxial tissues to form an additional appendage. The wing parts tend to form with the anteroposterior axis directed towards this region, whether or not it is in an abnormal position. In terms of positional information this is interpreted as showing that this region is the high point of the polarity potential for the anteroposterior axis (β) and is one of the reference points. A suggested interpretation of this situation and the corresponding polarity potential is shown in Fig. 9. For example, grafting a piece of tissue from the high point, that is region P N Z, to the region of the anterior region of the bud would be expected to lead to a polarity potential as shown [Fig. 9(b)]. The experimental result is a double appendage which in the clearest cases showed a digital formula in anteroposterior order IV, III, II, III, IV. It is also possible to interpret the 180° rotation experiment, described above, in similar terms. Of particular interest is the ability of these models to interpret the 180° rotation of a tip severed by a diagonal cut [Fig. 9(c)]. This effectively implants a high point in the preaxial region of the wing, and is similar to Fig. 3(j). A triple wing results. The most anterior one is normally orientated and is of stump origin; the other two are mirror twins reversed dorso-ventrally. The β axis appears to be unipolar.

That the specification of positional information along the $\alpha\alpha'$ axis is the same in both wings and legs is shown by the transplantation of parts of limb buds between wing and leg. For example, the grafting of a distal portion of a wing bud on to the proximal portion of a leg bud results in chimaeric

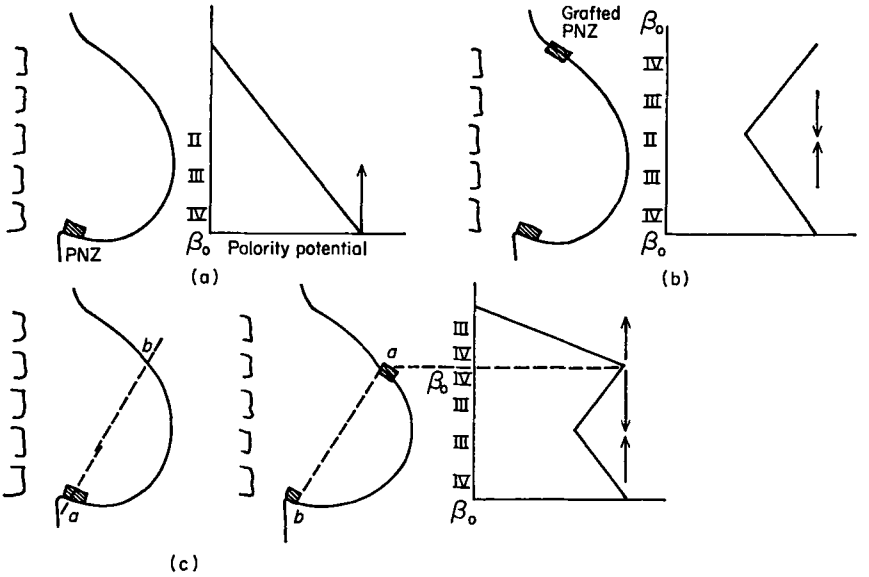

FIG. 9. (a) The polarity potential of the β axis of an early bud, and the position of the digits which form.

(b) A PNZ zone, the region of highest polarity potential, is grafted to the most anterior region of the limb bud. A new β_0 would be established there and the resulting digits are shown.

(c) Rotation of the tip 180 Å anteriorly posteriorly along the oblique dotted line *a b*, results in a change in polarity potential and a new β_0. The resulting digits formed are shown. Because of the geometry a new set of digits are also formed in the preaxial portion of the stump.

limbs, more or less along the lines of Fig. 5(f) and (g) (Kieny, 1964), the regions behaving according to their position and developmental history. There is no real structural discontinuity at the junction, and it is clear that the specification of positional information is the same for both fore and hind limbs. The difference between them is their developmental history which changes their interpretation of the positional information. This is strikingly brought out by the experiment (Saunders, Cairns & Gasseling, 1957) in which a block of proximal leg mesoderm was placed at the distal tip of the wing bud. In this position it formed typical distal element but retained its leg characteristics; that is, it formed toes at the wing tip.

It is of interest to consider Hampé's (1958) observations on the fibula, as it has some evolutionary implications. In general he found that the development of the fibula, a small bone in the chick leg, depended on the amount of material in the limb bud. A reduction in limb bud size leading to absence of the bone; addition of material to the bud leads to a fibula of

increased size, in fact it forms a complete fibular such as existed in the ancestors of the birds. These results can be interpreted in terms of the cells in the fibula region interpreting their positional information partly in terms of the width of the bud. This would mean that the cells in the fibula region only interpreted their positional information so as to form a complete fibula, when their positional information with respect to the β axis was appropriate. The evolutionary change has been to reduce the length of the β axis rather than to alter the ability to react to a particular set of values of positional information. These observations again emphasize the importance of considering positional information along more than one axis.

(E) OTHER SYSTEMS

The above examples should suffice to show how the concepts of polarity potential and positional information can be applied. There is no reason to believe that they cannot usefully be applied to other regulative systems such as early amphibian development and the regeneration of the vertebrate limbs and planaria, all well-studied systems. These will not be considered here and will certainly require detailed analysis. Nevertheless a few brief comments on these other systems may be helpful.

(1) Early amphibian development (Spemann, 1938; Holtfreter & Hamburger, 1955) should probably be analysed in a manner similar to early sea urchin development. The animal and vegetal gradients, usually referred to as the cortical and yolk gradients, are probably involved in the specification of the animal-vegetal and dorso-ventral axes. The region around the dorsal lip of the blastopore is one of high polarity potential and this would account for its 'inducing' and 'organizing' power. The neuralizing and mesodermalizing gradients (Saxen & Toivonen, 1962) are probably, like the A and V gradients of Fig. 6, involved in specifying boundary values for positional information. The classical experiments of Schotté, showing induction of anuran mouthparts in a urodele mouth field (Spemann, 1938) correspond to the principle of autonomy illustrated in Fig. 5(a) to (e).

The development of the axial systems of both neuroectoderm and mesoderm illustrate very well how difficult it is to alter the order of the regions in the pattern. It is usually assumed that the pattern within the nervous system is determined by induction by the underlying mesoderm, communication within the developing nervous system rather neglected. An investigation of the mechanism whereby positional information is specified in the developing nervous system would be very useful.

(2) The development of the pattern of retinotectal connections in the amphibian embryo could probably be accounted for, particularly since reduction of size of the retina at early stages leads to regulation such that a

whole retinal field is re-established, suggesting it is a bipolar system. Jacobson (1966) has discussed such phenomena in terms of each cell acquiring a unique value in a gradient system.

(3) The pattern of cell division in the crypt of Lieberkühn (Lamerton & Steele, 1968) suggests that division may be determined by positional information. Dividing cells are restricted to a specific region along the axis that runs from the base of the crypt, to the tip of the villus.

(4) Regeneration in systems, such as the vertebrate limb and planaria which form a blastema will require special consideration since they involve growth of a blastema and pattern formation within it such that it becomes integrated with the stump. How this integration is achieved in terms of the concepts of positional information is not clear. Nevertheless it is of interest that transplantations as in Fig. 3(j) in planaria give rise to the expected reversals of polarity (Okada & Sugino, 1937).

3. The Mechanism for the Generation of Positional Information

Very little, if anything, is known about the physiological basis of positional information and polarity potential, largely because appropriate experiments have not been designed with this end in view. For example, an extremely important question is whether or not the specification of positional information, as distinct from its interpretation, involves transcription of the genome or protein synthesis. We are currently investigating this in hydra by inhibiting for example RNA synthesis in one part of the field and observing its effect on polarity and positional information in another. However, apart from such an approach, one can ask some general questions to which one may reasonably expect to find some answers in the available literature, or which could be relatively easily obtained by experiment. How quickly is positional information generated? how far can it be transmitted? how accurate need positional information be? and what is the physical basis of the signals?

(A) HOW FAST IS POSITIONAL INFORMATION CHANGED OR TRANSMITTED?

Our studies on the determination of the hypostome in regenerating hydra suggest that after removal of the hypostome, the subhypostomal region takes about four to six hours before hypostomal properties, such as the ability to induce a new axis on transplantation into the gastric region, appear (Webster & Wolpert, 1966). This probably involves a change in both positional information and polarity potential. On the other hand a subhypostomal region requires about 14 to 16 hours to become a hypostome when tested by grafting on to the subhypostomal region in the same axial

polarity relation, that is a 1 2 on to a 1 2 3 4 (Hicklin & Wolpert, unpublished results). In sea urchin development the primary mesenchyme enter the blastocoel about 14 hours after micromere formation: thus regulation after micromere removal which involves change in positional information must take place within about 12 hours. In a careful and very important study on the time required to produce twinning of distal structures following temporary reversal of the distal region of the chick wing, Saunders & Gasseling (1963) found that about 12 hours was required. Jacobson (1968) has suggested that specification of the central connexions of the retinal ganglion cells in *Xenopus* development takes about seven to ten hours, on the basis of experiments involving inversion of the eyecup.

While it is striking that these observations from a variety of systems point to a time of about 10 hours for a change in positional information they should be interpreted with care, since one does not know whether it is only the polarity potential that has been altered during this time. For example, in Jacobson's experiments, the conclusion as to the time required for the specification of the ganglion cells is based on inversion experiments, and determining when inversion of the retina resulted in inversion of the retinotectal projection. This result only shows the time at which the polarity and reference points of the retina were fixed; the detailed specification of the cells may only occur much later on. Similar considerations apply to Saunders' experiments on the limb and our own on hydra. Nevertheless it seems reasonable to assume that significant changes in polarity potential and positional information take place within a time interval of five to ten hours.

(B) HOW FAR CAN POSITIONAL INFORMATION BE TRANSMITTED AND HOW ACCURATELY CAN IT BE SPECIFIED?

This is a central problem for deciding the requirements of any mechanism for it determines not only the 'length' of the positional information/distance relationship but also how reliable and precise this relationship must be. The problem of distance can be rephrased by asking how big embryonic fields are. 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. The definition of a field and the delineation of its boundaries is not easy, nevertheless some very persuasive data may be obtained. Some of this data is summarized in Table 1. The distances in cell numbers are taken where possible, as the maximum overall linear dimension. One may conjecture that positional information need not be transmitted over distances greater than about 100 cells. If this unexpected conclusion is correct one may ask how larger organs arise or can regulate. This might be

TABLE I

Linear size of positional fields in terms of cell numbers

System	Approximate size
Axial length of *Hydra littoralis* ectoderm	60
Early amphibian gastrula—animal pole to dorsal lip	30
Early sea urchin gastrula—animal pole to vegetal pole	30
Early starfish gastrula—animal pole to vegetal pole	50
Larval insect segment—epidermal cells from front edge to back	50–100
Diameter of retina at stage 29	30
Mesenchyme of chick limb from trunk to apical ridge at stage 24	<100
Width of amphibian meduallary plate	40
Imaginal disc of leg of *Drosophila* before determination occurs (Bryant & Schneiderman, 1969)	<100

accounted for in terms of repeated subdivision of a field (Maynard Smith, 1960); growth of a field after it has lost its field properties, in the manner of mosaic eggs in which cell lineage is so important, and other mechanisms. This is a central problem and will require detailed analysis.

The problem of precision is much more difficult and deserves much more attention at the cellular level than it has received (cf. Maynard Smith, 1960). We have pointed out that in morphogenesis of the sea urchin embryo the precision required of some of the mechanisms is less than might be thought, and that there is considerable variability from embryo to embryo (Gustafson & Wolpert, 1963). Almost no information on the precision of pattern forming processes is easily available, though the serial order of structures seems very reliable (Wolpert, 1969). One clue comes from studies on the number of primary mesenchyme cells formed in early sea urchin development (Hörstadius, 1936). These vary from about 50 to 60 out of a total of about 1000 cells. Thus, along the $\alpha\alpha'$ axis (Fig. 6) there is an error of about 1 in 30. As a first guess we may take this as the degree of accuracy required by the mechanism for specifying positional information. (The requirements for polarity potential are very probably very much less stringent.) This means that in a positional information/distance relationship, 30 cells long, the ith cell could have positional information α_i or α_{i+1}. It will be of importance to know whether any patterns which arise from fields have a precision better than this, particularly when they grow to give large numbers of cells in the pattern. For example, is the re-establishment of optic nerve connections to the retina in amphibian regeneration (Jacobson, 1966) any better than this along any axis? An examination of his diagrams does not obviously reveal a requirement for a greater specificity.

At this stage we may thus consider that the mechanism for positional information should be able to specify up to 50 cells, with an error of only +2, within about 10 hours.

(c) THE PHYSICAL BASIS

Positional information and polarity potential involve intercellular communication and it will be crucial to determine how this occurs. One question relates the channel of communication and particularly whether cell to cell contact is necessary. This is particularly important in view of the recent discovery of low ionic resistance junction between embryonic cells in contact (Furshpan & Potter, 1968). These so-called functionally coupled junctions would be an extremely attractive candidate for the channel for intercellular communication. However, Saunders & Gasseling (1963) experiments seem to indicate that for polarity potential, at least, cell to cell contact is not necessary. It is one of the great virtues of the phase-shift model of Goodwin & Cohen (1969) that it makes use of functional coupling and that communication between cells involves the movement of small molecules only between the cells. In their model nothing in fact physically passes along the axis, rather it is a wave of activity that is transmitted. Another attractive feature of this model is that polarity potential could have a metabolic basis, since it corresponds to a frequency of oscillation. This would be in line with Child's (1941) ideas on the existence of some relationship between polarity and metabolic gradients. Other models could involve the transmission of numerous informational macromolecules between cells and yet others could rely on membrane interaction (e.g. Wolpert & Gingell, 1969). It is also necessary to consider the relationship between the specification of positional information along different axes. In this connection it is worth remembering that the polarity of the different axes in a system are almost always determined at different times.

What is required is experiments designed with these problems in mind. In general terms one may anticipate if positional information and polarity potential are universal features of field systems they will make use of very basic cellular properties and one would not be surprised to find, for example, that they make use of respiratory pathways or cell structures associated with cell division.

4. Conclusions

The concepts of positional information and polarity potential seem capable of providing a conceptual framework within which a wide variety of patterns formed from fields can be discussed, and at a relatively crude level explained. It is perhaps encouraging that similar diagrams and concepts can be used

for pattern problems in four organisms from as many phyla. A universal mechanism for pattern formation, whereby genetic information is translated into spatial patterns of molecular differentiation remains a real possibility.

The type of analysis used here requires, surprisingly, quite an intellectual reorientation for those brought up on classical concepts of induction, organizers, and the rather uncritical use of gradient concepts. While the idea of position determining cell behaviour is well known the implications of this and the possibility of effectively establishing a co-ordinate system have not previously been explored. In a sense, the ideas put forward are relatively simple and do little more than redescribe known phenomena in new terms. Their value lies in providing a general conceptual framework and in defining more clearly the problems involved. While it would be possible to design experiments to test the concepts and invalidate them, by, for example, demonstrating an absence of universality or polarity changes not consistent with the concept of polarity potential, this is probably not the most useful approach. Of far greater importance is the design of experiments and theories to determine how positional information is specified. The development of the phase shift model of Goodwin & Cohen (1969) is the outstanding example of the latter. On the experimental side a variety of questions as to mechanism can be posed, such as, for example, the involvement of the genome in the specification of positional information, and the nature of intercellular communication. That one can discuss the problem in terms of specifying 50 cells relatively reliably in 10 hours, is in itself surprising and encouraging. It also becomes necessary, for example, to design experiments to determine the nature of the boundaries between fields and the site of reference points.

It is of interest to note that a universal mechanism for specifying positional information would have considerably evolutionary advantages since it would be possible to locally alter a pattern without affecting other cells. The provision of a universal co-ordinate system to which the cells' genome can respond is probably the most effective way of exploiting the fact that each cell has a full complement of genetic information, and it also enables a tremendous variety of patterns to be formed. This also has experimental implications since the mechanism for positional information will have been selected for its stability, and the possibility of viable mutants seems extremely remote. Any change in its specification would drastically affect all systems. Thus a genetic approach to the problem of specifying positional information, as distinct from the problem of interpretation which has not been dealt with here, is not very promising.

The analysis presented here is still crude and some awkward problems have been omitted or glossed over. Nevertheless it has been possible to interpret a wide variety of phenomena and to give new meaning to some classical

concepts such as field, dominance, and the induction and organization of new developmental axes. One is acutely conscious of the absence of the physiological and molecular basis of positional information and polarity. But unless the correct questions are asked one has little hope of finding out how genetic information is interpreted in terms of spatial patterns.

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