Review

Polarity and form regulation in development and reconstitution

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Abstract

In the literature, it is often assumed, for example with respect to Hydra, that several Turing systems coexist and it is also assumed that maintaining the polar profile, even when the system increases in size, is important for the polarity of the final phenotype. It is shown here that in reality there is only one Turing system, Child’s system. To obtain a complete polar individual or organ, whether in reconstitution or development, it is essential that the complete succession of metabolic patterns occurs. Child’s concepts of physiological dominance, subordination and isolation are interpreted in the light of Turing theory and in particular the Turing wavelength. It is emphasised, particularly by pointing to Child’s metabolic patterns in coelenterates, both in development and in reconstitution, that it is the elongation that drives the succession polar metabolic pattern → bipolar metabolic pattern, and this corresponds to the prediction of Turing theory supporting the thesis that Child’s metabolic pattern is a Turing pattern. It is shown that if we assume that ATP is the Turing inhibitor then the many results of Child about the reduction of the scale of organisation with the decrease in the intensity of the energy metabolism correspond to the reduction of the Turing wavelength. The interplay between the Turing wavelength and the length of the form explains the conditions of reconstitution under which partial forms, wholes and form regulation are obtained. It is suggested that higher metabolism is responsible for both larger size and larger Turing wavelength thus securing form regulation. The results could be of importance in modern ‘regenerative biology’. Heteromorphosis, i.e. animals with two heads (or two tails), one at each end, is explained by a bipolar Turing–Child metabolic pattern replacing a polar metabolic pattern. This can be brought about by chemical or by genetic means and indeed the prediction for Drosophila that the transition, wild type → Bicaudal D, occurs because a polar Turing pattern is replaced by bipolar Turing pattern is confirmed, again if we accept that Child’s metabolic pattern is the underlying Turing pattern. Child’s experiments on Drosophila, including the requirement of critical length for metabolic polarity, are explained by Turing theory. Phenocopies and phenotypes are explained by the Turing–Child theory. It is shown that both Child’s results about metabolic patterns and modern results for Hydra about gap junctions, ‘endogeneous inhibitor’ and gene expression, are correlated and explained by (cAMP, ATP) Turing theory.

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It is argued that the double-gradient hypothesis is incorrect in its original formulation and that it is Child's conception of succeeding metabolic patterns that is the correct one and that it corresponds to the prediction of the Turing theory. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Recently, there has been great excitement at the possibility of replacing damaged human tissues and organs (Stocum, 1998; Essays on Tissue Engineering in April 1999, Scientific American; Wright, 1999; Kind and Colman, 1999). Not unrelated is the general interest in regeneration in biology (essays in Ferretti and Géraudie, 1998). It is believed that tissue and organ restoration will be achieved by better understanding how organs develop naturally and then recreating the embryonic (regenerative) environment in an injured or degenerative adult tissue (Stocum, 1998). But the primary events of both differentiation and morphogenesis are not understood. Both cell differentiation and morphogenesis are coherent processes occurring in space and time and it is claimed here that the primary events and driving force for both differentiation and morphogenesis are Child’s (1928, 1929) ‘physiological gradients’ originating through a Turing instability (Schiffmann, 1994, 1997). In a very relevant way to ‘tissue engineering’ and ‘regenerative biology’, Child focussed his interest on both ‘development’ and ‘reconstitution’ in parallel. His and our general conclusion is that both normal development and reconstitution are driven by the occurrence of succeeding physiological patterns which is in turn driven by embryonic growth. This will be demonstrated in particular in relation to head–foot polarity, since the notion of polarity is perhaps the most primitive notion in developmental biology.

Many of the elaborations of the Gierer–Meinhardt models are motivated by the requirement to stabilise a monotonically graded concentration profile in a growing embryo. For example, it is
stated: “Most embryonic fields are growing fields. How can it be that a monotonic gradient is generated at the critical extension of the field and then remains stable during further growth?” (Meinhardt, 1989). The problem as they see it is that an increase in the size of the domain of a reaction–diffusion (Turing) system normally results in the loss of the polar concentration profile in favour of a bipolar (symmetric) profile and a further increase in size results in a spatially periodic profile. See Fig. 1(b)–(e). But it is believed that to obtain a polar phenotype and ‘positional information’ the monotonic gradient should be stabilised even if the size is increased. For discussions of this problem see for example Meinhardt and Gierer (1974) and Meinhardt (1989, 1992, 1995, 1999).

A second characteristic of the Gierer–Meinhardt models is the simultaneous use of a number of Turing systems in one embryo, for example, one for the head, one for the tail (foot), one for the dorsoventral direction, one for the tentacles, etc. For the Hydra, for example, Gierer and Meinhardt (1972) and MacWilliams (1983) envisage a second Turing system for foot formation in addition to the Turing system for head formation. Meinhardt (1993) adds a third Turing system for tentacle formation. Even six Turing systems are considered (Forman and Javois, 1999). In fact, having more than one Turing system is also useful for the “circumvention of the problem of size regulation of gradients” (Meinhardt, 1989), i.e. for dealing with the problem of the “maintenance of a graded concentration profile during growth” highlighted above. Consider for

![Concentration profiles of Turing systems](image)

Fig. 1. Concentration profiles of Turing systems. (a) Two activator–inhibitor systems each manifesting a polar profile. The solid curve represents the profile of the activator or the inhibitor of one system; the dashed curve represents the profile of the activator or the inhibitor of the second system; (a) may also represent the double-gradient hypothesis. (b)–(e) The profile of the activator or inhibitor of one Turing system as the length increases. Only as the length increases beyond a critical value does the homogeneous profile (b) changes to polar profile (c) Further increase in length results in a bipolar profile. An alternative bipolar form with a maximum at the centre is also shown. (e) The continuation of this process resulting in a longer and longer periodic profile which represents segmentation. (f) The possibility of obtaining a bipolar form even without an increase in length but because of a reduction of the Turing wavelength. It also corresponds to Child’s ‘decrease in the scale of organisation’. An increase in the Turing wavelength allows for a polar profile as in (g) even for a length equal to the length in (d). The arrows in (c) and (d) indicate the direction of progress of correlated physiological activities. They correspond to the arrows in the work of Child; in particular they indicate the course of reduction of permanganate and various dyes.
example a one-dimensional system like a *Hydra*. Two Turing (activator–inhibitor) systems that mutually inhibit each other will each ‘dominate’ and ‘organise’ one end of the field and exclude the other from their own end. Each of the two systems manifests a monotonic gradient as required for ‘positional information’ and each helps the other with the suppression of the secondary maxima. The maximum of one gradient provides, for example, an organising region for the head of the *Hydra* and the maximum of the other gradient can provide the ‘organising region’ for the foot (Meinhardt, 1988, Figs. 2 and 4; 1989, Fig. 6; 1992, Fig. 10; 1995, Fig. 3, and Fig. 1(a) here).

We argue that both characteristics, namely the maintenance of a monotonic gradient in a growing embryonic system and the introduction of more than one Turing system, are not necessary and do not correspond to biological reality. In this reality there is only one Turing system manifested in Child’s ‘physiological gradient’ (Schiffmann, 1997), and while the monotonic gradient is lost upon size increase this does not necessarily mean a loss of the overall polarity. To see this consider the statement:

“With increasing field size, a tendency exists to change from a monotonic distribution into a symmetric and ultimately into a periodic distribution. This is inappropriate if the graded concentration should be used in the growing embryo as positional information for the determination of the primary body axes since, for instance, several heads would appear instead of one.” (Meinhardt, 1995). Consider Fig. 1(b)–(e). The statement assumes that the bipolar form shown in (d) will, for example, dictate two heads at the two ends, and this is to be avoided by maintaining the polar profile in (c) even when the system elongates. It ignores the possibility that the earlier monotonic gradient described in (c), although transient, could have left its imprint even later in the longer system so the two ends in the stage (d) will be different, corresponding for example to a head and a foot. The Gierer–Meinhardt point of view constitutes a static picture. It considers the later bipolar stage (d) in isolation without a possible long-lasting effect of the earlier polar stage in (c). As a result it resorts to another static picture as in (a) with two Turing systems. So we see that an alternative to a static picture with several Turing systems is one Turing system manifesting a succession of patterns in a growing embryo and with the proviso that earlier patterns are registered via a memory—a reasonable proviso in view of the many memory mechanisms discovered. Similarly, the requirement of an additional Turing system to provide for the dorsoventral polarity perpendicular to the anterior–posterior axis is unnecessary as discussed in Schiffmann (1991).

The fact that an existing foot has inhibitory effect on the formation of a second foot, just as is the case for the lateral inhibition manifested by the head, is also invoked as a justification for the introduction of an additional Turing system for the foot (Meinhardt, 1989, 1992, 1993). But these inhibitions can be explained by one Turing system. An analogous situation in sea urchin is discussed by Gustafson (1965). He points out that both the animal pole and the ventral side manifest similar and related inhibition, i.e. emit an inhibitory substance that suppresses similar structure in the vicinity of the animal pole or the ventral side. But his Fig. 17 on the reduction gradients of Child in sea urchin shows that both the animal pole and the ventral side are maxima of the metabolic gradients and therefore maxima in the production of the inhibitor = ATP which is the source of the observed ‘lateral inhibition’ around both the animal pole and the ventral side. The results of Child and his colleagues, properly interpreted, correspond to one Turing system with a succession of patterns. When the full set of succeeding physiological (metabolic) patterns occurs, the result is a normal individual and normal organs and this applies to normal
development and reconstitution. But if a metabolic pattern is missing the final phenotype is abnormal in an expected way. In particular, if a polar metabolic pattern, that is present in normal development, is absent, and instead a bipolar pattern is present, then the final phenotype is bipolar (symmetric). In other words, the presence or absence of a metabolic polar pattern makes a difference to the final result. The system remembers the polar pattern. As we shall see, the removal of the polar pattern can be caused in many ways, such as chemical, environmental or mutational; in all cases a bipolar phenotype results.

Just as the absence of the polar metabolic pattern results in expected abnormality (heteromorphosis), the converse is also true: We shall demonstrate for the sea-urchin that a persistent polar metabolic pattern that is not succeeded by a bipolar metabolic pattern is responsible for a fundamentally abnormal embryo; in particular it cannot even gastrulate. The high metabolism at the posterior end, which is absent if a bipolar pattern does not succeed the polar pattern, is essential for the localised release of energy that drives the gastrulation. Thus in contradiction to the Gierer–Meinhardt point of view, the organism does not attempt to preserve the polar Turing pattern for the sake of phenotypic polarity: on the contrary, the succeeding bipolar Turing pattern is essential for the normal phenotypic result in both reconstitution and development. Our conclusion that the complete set of successive Child–Turing patterns is essential in reconstitution and in development points to the solution of the problem of form regulation. An embryo, or a part of an embryo or of an individual, that operates with a higher metabolism will attain a bigger size overall but this will be ‘compensated’ by it also having a bigger Turing wavelength so the resulting bigger individual will share the same localisation of parts, proportion and form as a smaller individual.

It is instructive to describe explicitly the conversion of the Turing picture into the Child picture; and in particular how the gradient in the concentration of the Turing morphogen results in the gradient of the rate of multiple physiological activities and propagating fronts. Child’s “physiological gradient” or “metabolic gradient” is shorthand for a gradient in the rate of many correlated physiological activities. That there is one metabolic gradient with many correlated manifestations was appreciated early on. For example, Hyman (1923) wrote: “The metabolic gradient, according to our conception, consists not merely of a gradation in the rate of one physiological process, such as respiration, but involves axial gradations of a quantitative nature in a large number of processes, and gradations in physical and chemical constitution. . . . We believe that these various aspects of the gradient are interwoven and correlated with each other.”

Consider the leftmost point in Fig. 1(c). Because it is the location of highest [cAMP], it will also be the location of the highest rate of glycogenolysis, lipolysis, oxygen consumption, CO₂ production, electron transfer, ATP synthesis and various other activities including the reduction of external probes such as methylene blue and Janus green. Because at this location the rate of all these activities is the highest, the (visible) manifestation of these activities will start first there, for example, the change of colour due to reduction of an external probe. Consider now a point somewhat to the right of the leftmost point. Here [cAMP] is lower, therefore the rate of all these activities will be lower, and their manifestation will start later. A point even more to the right will be characterised by even lower [cAMP], therefore by lower rate of activities, and the manifestation will start even later. So, if one measures each of these activities, one will get a propagating front moving from left to right. For example, if one measures the change of colour of Janus green from blue to red upon its reduction, the leftmost point will become red first, then the point to its right
will become red, and so on: the further a point is to the right, the later the change of colour will occur. The red territory at the left of the domain in Fig. 1(c) will increase as time goes on. The advance of the boundary between blue and red from left to right can be indicated by an arrow as shown in Fig. 1(c), and it corresponds to Child’s (1941) notation where “arrows indicate direction of progress of reduction”. If we now consider Fig. 1(d) (bold curve), corresponding to a later stage in development, the rate of all the above-mentioned activities will be the highest at the two ends of the domain due to the highest [cAMP] there. Therefore the manifestation of the activities, for example the appearance of the red of the reduced Janus green, start there first. By reasoning similar to the above, we predict two fronts propagating from the ends to the centre, and the direction of propagation is again indicated by the two arrows in Fig. 1(d). It was found experimentally that the organiser is a high point in the concentrations of cAMP and ATP as well as in the rates of oxygen consumption, CO₂ production, glycogen depeletion and probe reduction. The above discussion makes it clear that this is a verification of a prediction of our theory.

2. Physiological gradients in coelenterates and other organisms

2.1. Polarity plus bipolarity can yield polarity

For many animals and plants, and for normal development and reconstitution, Child (1928, 1929) and colleagues were able to show that upon elongation, the sequence, homogeneous → polar → bipolar → multipolar, as described in Fig. 1(b)–(e), occurs with respect to the physiological gradients; one typical example is the annelids. In this section, we focus on Hydrozoa and other Coelenterates. Indeed, development in Coelenterates in general manifests a single apicobasal metabolic gradient in the early planula but later in the development, as the planula elongates, as one expects for the Turing system (activator = cAMP, inhibitor = ATP), see Fig. 1 or Fig. 4.1 in Meinhardt (1982), a second gradient opposite in direction to the first appears in the basal region (Child, 1925, 1926a, 1951) (see also Child, 1947, 1948). This succession of a bipolar metabolic pattern after a polar metabolic pattern upon elongation occurs not only in normal development but also in reconstitution of pieces which give rise to complete individuals—see, e.g., Child (1926a). The succession polar → bipolar upon elongation is central to the argument that the Child system is a Turing system and it is therefore worthwhile to look at it in greater detail.

Consider the development of *Campanularia* (Child, 1925). Using the methods of susceptibility (cytolysis) and reduction of KMnO₄ and other agents, Child discovered the succession of gradients corresponding to the transition, (c) → (d), in Fig. 1. It is to be emphasised that in this case, as in all other cases, the spatial patterns of susceptibility and reduction were isomorphous. The gradients indicated by the reduction of KMnO₄ appear both as gradients in the rate of reduction, indicated by the rate of appearance of the brown colour (due to MnO₂ and other oxides), and as gradients in the amount of reduction, indicated by the depth of colour when the reaction proceeds to completion in excess of KMnO₄. Once again, Child (1925) emphasises that his gradients “are associated with oxidative processes”. He also states “that the gradients indicated by the reduction of KMnO₄ are closely associated with the processes of living in the protoplasm is further shown by the fact that individuals of various stages killed in various ways and then placed in permanganate reduce much less of the agent than living individuals and show,
either no traces of axial gradients, or in some cases slight traces for a short time after the action of
the killing agent”. Results of this type led Child to emphasise frequently, e.g. in Child (1928), that
his ‘physiological gradients’ are an expression of on-going incessant physiological chemical
reactions rather than of a static molecular and crystalline structure. This corresponds to a Turing
dissipative structure and autopoiesis (Schiffmann, 1997).

Child (1925) discovered a physiological gradient along the polar axis from the oocyte to the
planula stage with its high end at the apical end of egg and larva, but “as the planula elongates a
second region of high susceptibility, reducing power and rate of staining gradually appears at the
basal end and from this region a gradient, opposite in direction to the original gradient, develops”
(Child, 1925). This is visualised in Fig. 2. It is a fundamental characteristic of the Turing system
that the transition, polar → bipolar, shown in (c) → (d) of Fig. 1 occurs as a consequence of an
increase in length, and Child (1925) reaches exactly the same conclusion for his physiological
pattern: “The appearance of the second gradient at the basal end as the planula elongates suggests
that the new axis arises through physiological isolation of this basal region from the dominant
apical region in consequence of increase in length... In the course of elongation of the planula the
extreme basal region is the first part to be isolated and to become more active. This precedence
makes it the high end of the new gradient”.

Analogous gradients and transitions are shown in Figs. 3 and 4 where the gradients were
obtained with redox indicators including intracellular reduction of oxidised methylene blue and
Janus green. Again, the later appearance in the planulae of the second gradient in the basal or
posterior region, which is in the opposite direction to the original gradient as shown by the
direction of the arrows in the figures, is explained by Child (1951) by the physiological isolation
from the dominance of the original anterior end caused by the elongation of the planula. This
again corresponds to the ‘Turing’ transition, (c) → (d), in Fig. 1. Fig. 3 shows the correlation
between elongation and the transition, polar → bipolar, and the fact that the Polyorchis planula

Fig. 2. Susceptibility gradients in the development of the hydrozoan Phialidium. (A) blastula; (B) early planula with
gradient from the apical end; (C) later and longer planula with a second gradient at the original basal end. Arrows show
the direction of progress of disintegration (from Child, 1925, 1941, by permission of the Marine Biological Laboratory,
MA and University of Chicago Press).
becomes greatly elongated, slender, and almost worm-like. Fig. 4 shows how the elongation of the anthozoan planula resulted in the development of a second gradient, with the region of most rapid reduction at the posterior pole. This gradient decrease in rate of reduction was in the opposite direction to the primary gradient (Fig. 4(C)). An invagination appeared and became deeper at the
high end of the new gradient, forming the mouth and stomodeum (Fig. 4(C)). It is the release of energy at the high end of this gradient that drives the invagination and determines its location.

It is the above-highlighted proper succession of metabolic patterns that is responsible for the final polar adult. Indeed: “In pieces [of Corymophora] which give rise to biaxial hydranths without any basal region two gradients, opposite in direction appear (Fig. 12) [corresponding to Fig. 6(a) here]. . . the high ends of these two gradients are the distal and proximal end of the piece. . . Biaxial forms represent simply the simultaneous or nearly simultaneous appearance of two gradients in opposite directions and if the piece is short, only the more apical levels of each gradient are represented and the resulting forms are biapical forms. What shall develop in each gradient or axis depends on its relation to others already existing” (Child, 1926a). Similarly for reconstitution of pieces of Tubularia: “equal or nearly equal [metabolic] gradients in opposite directions arise and hydranths or apical parts arise at both ends with their axes opposed.” (Child, 1915). Thus, if in a reconstitution experiment one (artificially) skips the earlier polar pattern, corresponding to Fig. 1(c), and starts straightaway with a bipolar metabolic pattern, corresponding to Fig. 1(d), a bipolar, two-headed, symmetric adult form results. This situation in Tubularia was generalised very early by Child (1915) for other animals; for example, reconstitution of pieces of Planaria also led to double apical regions and heads when bipolar metabolic pattern occurred without a preceding polar pattern.

Analogous results are obtained in the reconstitution experiments of Child and Watanabe (1935) and Watanabe (1935) on Corymophora palma. Again in pieces which develop as bipolar morphological forms, reduction occurs first at the two ends of the piece and progresses toward the middle. But if a polar reduction gradient precedes the bipolar reduction pattern, a normal polar morphological phenotype results. Thus our general conclusion is that the assumption in Meinhardt (1995) as cited in the introduction is not valid. In reality, as the system elongates, a polar metabolic pattern is succeeded by a bipolar metabolic pattern as is expected with a Turing system. But a bipolar phenotype, for example a hydroid with two heads, does not result, contrary to the assumption in Meinhardt (1995). Also contrary to the Gierer–Meinhardt assumption, there are no special elaborations required to stabilise the monotonic gradient upon an increase in length—a second opposing gradient always appears upon an increase in length. The bipolar phenotype, e.g. a two-headed hydroid, does result when this succession of metabolic patterns fails and we start from a bipolar metabolic pattern. This proves that the earlier polar metabolic pattern makes a difference, contrary to the assumption in Meinhardt (1995) and elsewhere. The situation is exemplified for Corymophora palma in Figs. 5 and 6. Note the parallelism between the geometry of the metabolic patterns and of the resulting macroscopic structures.

It is the case that in the normal development of some hydroids, such as in Corymophora (Child, 1926a), the hydranth (the head) develops from the original apical end, i.e. from the primary gradient, and the second gradient becomes the basal region. But in the normal development of other hydroids, such as in campanularian hydroids (Child, 1925), the hydranth develops from the second gradient and the primary gradient becomes the basal region. But in both these cases, a second gradient appears upon an increase in length as expected from Turing theory and Child’s principle of physiological isolation. Comparative discussions of the two cases can be found in Child (1926a, 1928, 1951).

Central to the possibility of obtaining polarity and spatial diversity in general with only one Turing (metabolic) system is the involvement of memory mechanisms. In addition to those at the
level of gene expression (Schiffmann, 1991; Hagstrom and Schedl, 1997; Laurent and Kellershohn, 1999), a memory mechanism can also occur at the level of the spatial metabolic pattern itself. To see this recall Child’s expression cited above, that ‘‘what shall develop in each gradient or axis depends on its relation to others already present’’. We note that in the normal development of Corymorpha, for example, the second gradient is shorter than the first (Fig. 5 and caption). This second polar gradient, opposite in direction to the first gradient, is less spatially differential and is shorter than the first as indicated by the shorter arrows. At still later stages shown in (c), the high periodic metabolic activity along the ‘‘rings’’ with the greater circumference dictates the primordia of the tentacles. The final phenotypic result is polar and is shown in (d). Adapted from Child (1915, 1926a), Child and Watanabe (1935), and Watanabe (1935), by permission of The University of Chicago Press.

2.2. Physiological dominance, subordination and isolation

According to Child (1928), a region with high ‘‘oxidative metabolism’’/‘‘respiratory metabolism’’ is ‘‘dominant’’: ‘‘any region whose energy-liberating activity [production of the
“inhibitor” ATP] is increased to a certain degree above that of adjoining regions is to some extent dominant over such regions because certain changes resulting from its activity spread to the adjoining regions and influence their physiological activity and conditions” (Child, 1929). Child characterises the spread from the peak to the ‘adjoining regions’—and thus creating ‘subordination’ (‘lateral inhibition’) in the adjoining regions—as ‘transmission of energy’ (Child, 1928, 1929). This corresponds to diffusion of the inhibitor ATP from the metabolic peak to the adjoining regions. The situation described by Child corresponds to the production of the inhibitor ATP at the peak and to its diffusion to the adjoining regions as described in Fig. 1 of Schiffmann (1994), accounting for the ‘instability of the homogeneous’ and for ‘lateral inhibition’.

According to Child (1928, 1929) the ‘range of dominance’ is limited so the possibility of ‘physiological isolation’, i.e. the isolation of a previously subordinated region from the influence of the dominant region, exists. Physiological isolation may result: (a) from an increase in size or length so that a region escapes the range of dominance; (b) from a decrease in activity of the dominant region and consequently a decrease in the range of dominance; (c) by blocking the transmission between the dominant and subordinate regions; (d) from an increase in activity of the subordinate region, making it insensitive to the influence of the dominant region. The computer experiments on the basic (i.e. without the elaborations considered above) Turing–Gierer–Meinhardt models (Gierer and Meinhardt, 1972; Meinhardt, 1982) admirably correspond to Child’s notions of physiological dominance, subordination and isolation, which are equivalent
to the notion of ‘lateral inhibition’ (Meinhardt, 1982), if the ‘activator’ and ‘inhibitor’ are identified with cAMP and ATP.

To understand the experimental results it is useful to interpret Child’s notions of physiological isolation, dominance and subordination according to Turing theory, and in particular the notion of the Turing wavelength. Both Turing theory (Babloyantz and Hiernaux, 1975; Koch and Meinhardt, 1994, Fig. 4.1(a) in Meinhardt, 1982) and Child’s experimental school find that the polar pattern can arise only if the length of the system exceeds a minimum critical length, as demonstrated in the transition, (b) → (c), in Fig. 1. The transition, polar → bipolar, as demonstrated in the transition, (c) → (d), in Fig. 1—see also Fig. 4.1(b) in Meinhardt (1982)—requires further elongation so as to be able to fit two half-waves. This prediction of the Turing theory has been verified by Child’s metabolic patterns for very many organisms and demonstrated in Section 2.1 for coelenterates. As the system elongates further, more half-waves can be fitted as demonstrated in Fig. 1(e) and this is indeed the explanation by Child of the process of segmentation (Child, 1928, 1929). We thus see that criterion (a) above for physiological isolation is the hallmark of the Turing theory—the dependence of the pattern on size.

So far we have assumed that the Turing wavelength, which depends on the rate and diffusion constants only, is constant, and the only way to effect physiological isolation is by increasing the size. But we can also obtain physiological isolation via reduction of the Turing wavelength. This is demonstrated in Fig. 1(f) where for a shorter wavelength a bipolar pattern is obtained without an increase in the length of the system. On the other hand, an increase in the Turing wavelength will result in a polar pattern although the system is longer as is demonstrated in Fig. 1(g). A decrease in gap junction communication can decrease the Turing wavelength, given by

$$\lambda = 2\pi[D_u \cdot D_v / (f_u \cdot g_v - f_v \cdot g_u)]^{1/4}$$

where the quantities are as in Schiffmann (1997). We see that a decrease in both diffusion coefficients, $D_u$ and $D_v$, preserving the ratio $D_u/D_v$, will decrease $\lambda$. Child’s criterion (d) for physiological isolation, which includes ligature, is related to this reduction of $\lambda$. $\lambda$ can also decrease when we decrease the rate of synthesis of the inhibitor corresponding to the decrease in the rate of oxidative phosphorylation. This can be seen from Fig. 1 in Schiffmann (1994) and from Koch and Meinhardt (1994) where the reduction in the rate of inhibitor=ATP synthesis corresponds to a reduction in $\rho_h$ which in turn corresponds to smaller $\lambda$. An increase in the rate of synthesis of the activator=cAMP will also reduce $\lambda$. This can be seen from Fig. 1 in Schiffmann (1994) and analytically from Koch and Meinhardt (1994), where an increase in the rate of synthesis of the activator corresponds to higher $\rho_a$ which in turn corresponds to smaller $\lambda$. These results will enable us to understand experiments which were not understood up to now.

In addition to the above possibilities the Turing theory also allows that sometimes for the same length and value of the parameters, Turing patterns of different wavelengths can exist. For example, both the polar pattern in Fig. 1(c) and the bipolar pattern in Fig. 1(f) can exist; which will be realised in reality will depend on the magnitude and location of the initial perturbations (Babloyantz and Hiernaux, 1975). In this context, it is instructive to quote from Meinhardt (1982):

If the size of the field is large enough to accommodate more than one activator slope, additional maxima can be induced either by a small local increase of activator, realized for instance by the implantation of activated tissue or unspecifically by the removal of inhibitor.
The probability of inducing a secondary maximum with a given stimulus is expected to depend strongly on the distance between the site of manipulation and the natural organizer (activator maximum), since the local stimulation has to overcome the inhibition arising from the maximum. A removal of the existing maximum facilitates the induction of a new activator maximum considerably, since after such removal, the level of inhibition drops in the field. The possible induction of a new maximum is an all-or-nothing event, the final maximum being independent of the mode of stimulation. The concentration of activator increases via autocatalysis only if sufficient activator is present to overcome the inhibition from the existing maximum. This developing maximum will increase until it comes to a steady state with the self-produced inhibitor...

If the activator and inhibitor are identified with cAMP and ATP, this quotation and the related computer experiments correspond to many experiments by Child and others on metabolic patterns. In particular they correspond to characterization (b) and (d) above for physiological isolation, i.e. to perturbing the system so as to favour the shorter wavelength pattern by decreasing the metabolic activity of the dominant region or increasing the metabolic activity of the subordinate region. The ‘all-or-nothing’ event is also amply verified in Child’s experiments.

An example corresponding to the quotation above, where an activation at the subordinate end of a piece can bring about bipolarity, that is a shorter wavelength and smaller scale of organisation, occurs in hydroid reconstitution in particular for *Tubularia* and *Corymophora*: “In short pieces which become unipolar with hydranth at the distal end, the activation at the proximal end following section may be sufficient to shorten the gradient and hydranth primordium at the distal end, that is to decrease scale of organisation there” (Child, 1941). Equal exposure favours high frequency of bipolar forms but often the new proximal gradient is shorter than at the distal end since the gradient at the distal end has the same direction as the original gradient (Child, 1926b). Similar consideration apply, for example, in planarian reconstitution: “In pieces below a certain length, which differs with body-level, head regeneration is partly or completely inhibited by a stimulation from the posterior cut surface and the activation of cells following section; delay of posterior section decreases or abolishes this inhibition” (Child, 1941).

Consider Watanabe’s (1935) results that if the reconstitution is not done in good conditions, then the fraction of both the bipolar reduction patterns and the corresponding bipolar morphological resulting adult forms is greatly increased in relation to the unipolar metabolic and phenotypic forms. But poor conditions mean less oxygen and more accumulation of CO2 and therefore less production of ATP. The increase in the fraction of bipolar forms is precisely what one would expect since we saw that a decrease in the rate of synthesis of the inhibitor = ATP reduces the Turing wavelength $\lambda$, so the chance is increased that the polar pattern will be skipped, which is what is observed experimentally. Reconstitution as in Fig. 5 which simulates development will be displaced in poor conditions in favour of reconstitution as in Fig. 6 with symmetrical stages and symmetrical final result. Also in line with our theory is the fact that in normal development the second opposing gradient in the basal region commonly appear somewhat earlier under slightly depressed conditions (Child, 1925).

Child and colleagues, in many of their works, created conditions of low oxidative phosphorylation, i.e. low ATP production. This was obtained by creating laboratory conditions involving low O2 or high CO2 (for example by overcrowding) and also by using various
“inhibiting or depressing chemical agents”. The above results of Watanabe (1935) are recapitulated with inhibiting agents. “In short [hydroid] pieces bipolar frequency may be increased and scale of organisation decreased by exposure to inhibiting agents for a short time after section” (Child, 1941). Conforming to this are also the results for Corymorpha that at higher temperature the frequency of bipolar forms is lower (Child, 1926b).

The frequency of planarian bipolarity can also be considerably increased by exposure to inhibiting agents for a time following section (Rustia, 1925; Child, 1946). Low temperature also increases the biaxial head frequency (Rustia, 1925). Great care is taken to compare pieces under identical conditions, including equal length, similar state of nutrition and that they are from the same level. The biaxial heads involving the two ends of the piece are “the high ends of gradients, and reorganisation of the remainder of the piece under the influence of these two dominant regions determines the development of post-cephalic regions in relation to each head, so far as the length of the piece permits” (Rustia, 1925). This situation is demonstrated in Fig. 7(a)–(c). The results of Rustia (1925) are a dramatic illustration of our theory that a chemical or physical (temperature) alteration, that depresses metabolism and reduces the rate of synthesis of ATP = Turing inhibitor, reduces the Turing wavelength and allows us to fit two half waves (corresponding to (f) in Fig. 1) instead of one half-wave (corresponding to (c) in Fig. 1) into a piece of the same length. Thus Turing theory provides an explanation to the long-standing mystery of heteromorphosis that has exercised biologists since Bonnet (Rustia, 1925). Corresponding to these results are the results that short pieces taken from near the anterior end of the body develop partial forms that are usually unipolar, whereas pieces from regions at or near the fission zone develop bipolar forms at relatively high frequency (Child (1946) and Fig. 11 therein). Indeed the anterior pieces are characterised by higher metabolism (see Fig. 16) and therefore by higher Turing wavelength and scale or organisation which in turn explains the greater tendency to form unipolar forms.

Bellamy’s results are also of interest in this context (Child, 1929): in the early amphibian the metabolically active and dominant region is the apical pole but later the dorsal lip region, the organiser region, becomes a highly active region, as a “result of physiological isolation from

![Fig. 7. Reconstitution of bipolar forms from planarian pieces. (a)–(c) correspond to bipolar heads and (d) to bipolar tails. The post-cephalic regions develop as far as the length of the piece permits (from Child, 1915, 1941, by permission of The University of Chicago Press).](image)
the dominance of the primary apical region. Under inhibiting conditions... a secondary invagination resembling gastrulation and beginning in the same meridian, but nearer the apical pole” is observed and “such secondary invaginations might result from the physiological isolation of a region nearer the apical pole than the normal dorsal lip region in consequence of the decreased range of dominance of the primary apical region under the inhibiting conditions” (Child, 1929). The coming closer of the two centres of activation is explained by the reduction in the formation of ATP=Turing inhibitor under “inhibiting conditions” resulting in reduced Turing wavelength.

In planarian reconstitution one can obtain, via the application of inhibiting agents and in other ways (Child, 1910, 1911c, 1915, 1941), a graded series of reconstitution products, for example from a normal pair of eyes (photoreceptors) to the eyes becoming closer and closer to each other until there is only a single eye (cyclopia). Similarly, the two cephalic sensory lobes (chemoreceptors) become closer and closer to each other until they fuse to one lobe. This can be visualised in Figs. 8 and 9 and also in Figs. 54 and 55 in Child (1915) and Figs. 2–8 in Rustia (1925). Again, these results can be explained by the reduction of the Turing wavelength. Central to

![Diagram of head forms](image)

**Fig. 8.** The inhibition series of head forms of *Dugesia dorotocephala*. (A) The normal head: triangular, with two separate, bilaterally localised “eyes” and two cephalic lobes (auricles) on lateral margins of the head; (B) the teratophthalmic head: slightly smaller and less sharply pointed anteriorly. The eyes are localised nearer the median line than normally, and the pigment cups are more or less connected; (C) eyes of teratophthalmic heads; (D)–(G), the teratomorphic head: rounded, and with cephalic lobes anterior in the median line, with a single median lobe as the extreme, single or apparently single median eyespot; (H)–(J), the anophthalmic head: rounded and rudimentary, with or without a single median cephalic lobe, eyespots absent; (K), (L), the acephalic or headless form: head completely absent (from Child, 1941, by permission of The University of Chicago Press).
the development of Child’s theory (Child, 1915, 1941; Brøndsted, 1969) is the correlation of “head frequency” with the level of metabolism. The “head frequency” is the frequency of occurrence of the various degrees of inhibition of the anterior end in a particular set of pieces. Sets of pieces reconstituting a high percentage of normal or near-normal heads are said to have a high head frequency, while those with a high percentage of strongly inhibited head types are said to have a low head frequency. Child and his co-workers found that a decrease in the head frequency and a shift toward a headless condition (see Fig. 8) can be induced by lower temperature, cyanide, various anaesthetics, CO₂, decreased motor activity, starvation before section and choosing the level of section so that the piece corresponds to a region of lower metabolism. The experimental results can be affected by what Child (1941) calls “physiological inhibition”, to distinguish it from external inhibiting agents such as cyanide. For example, when the pieces are not long enough, an inhibition of head formation in the anterior end of the piece can be caused by a second gradient in the opposite direction resulting from the posterior cut (Child, 1915, 1941). Such a complication, and Child’s explanation of it, correspond exactly to a characteristic of the basic Turing–Gierer–Meinhardt model highlighted above, namely that the Turing pattern that is realised can be a function of the magnitude of the initial perturbations and their location in the system.

The reduction of the Turing wavelength also predicts the possibility of fitting more half-waves in given length, as for example in (f) compared to (c) in Fig. 1. It means we can fit more peaks in a given length. This means more eyes in our context. This is precisely what Child (1910) finds: “After some two weeks or more in the anaesthetic the single-eyed heads give rise to two additional eyes symmetrically placed in the normal position and slightly posterior to the single median eye already present. These three-eyed individuals are a characteristic feature of the experiments with anaesthetics. All the eye-spots persist as long as the animals live, whether they remain in the anaesthetic or are returned to water... The longer pieces in the anaesthetic frequently produce two eyes in the normal position. In many cases such pieces give rise after two weeks or more to a second pair of eye-spots a short distance behind the first pair and, like them, symmetrically placed. Such individuals then possess four eye-spots, all of which persist during life.” Related results were obtained in Child (1911c): alcohol, ether, other anaesthetics, and old culture water crowded with several hundreds planarians and with a high concentration of CO₂, can all result in a single
median eye in the reconstitution of postpharyngeal pieces such as Z in Fig. 20—see Figs. 13, 15, 16 and 19 in Child (1911c). But if after such a stay in alcohol or ether the piece is returned to water, it usually develops two more eyes in the normal position resulting in a three-eyed individual (Fig. 20(J); see also Fig. 54(B) in Child, 1915). Child (1910) concludes: “a very common effect of the anaesthetic [as compared to reconstitution in water] is to increase the number of eye-spots beyond the normal”. Thus, the inhibiting agent can change the relative positions of parts and their size, e.g. “the lower the metabolic rate during development the nearer to the head the mouth and the pharynx arise and the less the length of the new pharyngeal region” (Child, 1915) (visualised in Figs. 65–70 in Child (1915); also Fig. 119 in Child (1941) and Fig. 10 in Child (1946), which are also reflections of the reduction of the Turing wavelength with the decrease in metabolism. But in addition to relative positions, the “number of parts” (Child, 1910) can also change with the change in the Turing wavelength, a change that can be brought about merely by “a change in the [homogeneous] constitution of the medium” (Child, 1910). This can only be explained by an underlying Turing system. Child (1910) notes that in the four-eyed individuals the pigment spots are always distinct and arise separately, not by division of a pigment mass already present. His explanation “that these cases of supplementary eye-formation suggest that the formation of a single eye or of a pair under normal conditions inhibits the formation of further eyes within a certain region of the head, while in anaesthetic media this correlative inhibiting effect is not sufficient to prevent the formation of new eyes” corresponds exactly to the anaesthetic decreasing the range of the ‘lateral inhibition’, or equivalently, a decrease in the Turing wavelength, such as results from the Turing–Gierer–Meinhardt basic model with the specification that the Turing–Gierer–Meinhardt inhibitor is ATP. This possibility of increasing the number of macroscopic parts—e.g. eyes—through the reduction of the Turing wavelength via lowered metabolism should be compared with the possibility of increasing the number of macroscopic parts through the reduction of the Turing wavelength via decreased gap junction permeability, corresponding to smaller $D_u$ and $D_v$ in the expression for $\lambda$ above. Examples of this second possibility, which include genetic mutations, are given in Schiffmann (1997).

These notions can explain many observations. For example, “in general the metabolic rate of these animals decreases when they are transferred from natural to laboratory conditions, and the hydranths which develop in the laboratory possess a lower metabolic rate than those in nature; consequently the range of dominance is less and physiological isolation occurs at shorter distances from the dominant region than in animals in nature.” (Child, 1915). Just as expected from the (cAMP, ATP) Turing theory, Child (1915, 1929) finds that the increase in length, or the decrease in metabolic activity, both cause physiological isolation and bi- or multi-polarity. Thus an increase in length in nature causes asexual reproduction and converts a polar Tubularia to a bipolar Tubularia as seen in the transition, (b) → (a), in Fig. 10.

Note that the polar Tubularia in Fig. 10(b) manifests a bipolar metabolic pattern, but the opposite gradient at the tip of the stolon is shorter and weaker than the gradient at apical end (Child, 1915). It is partially ‘dominated’, ‘isolated’, ‘inhibited’, ‘subordinated’ by the apical gradient (Child, 1929). This partial subordination is removed by the natural increase in length, resulting in bipolar two-hydranth Tubularia as in Fig. 10(a). With still further increase in length, or in consequence of decreased metabolic activity [corresponding to reduction in Turing wavelength], more regions become physiologically isolated and new hydranths appear (Child, 1915, 1929). This example also shows that there are no special mechanisms (corresponding to the
elaborations of the basic Turing–Gierer–Meinhardt models referred to in the introduction) which retain a monotonic Turing gradient upon increase in length, and that there is one Turing system, the Child metabolic system, that behaves as expected of a Turing system, i.e. as the title of Chapter 4 in Meinhardt (1982): “polar, symmetric and periodic patterns—basic properties of an activator–inhibitor system” suggests and as demonstrated in Fig. 1(b)–(f). And in this case of

Fig. 10. Length and metabolic activity determine multi-polarity in *Tubularia*. An increase in length in nature in a process of asexual reproduction converts a polar form shown in (b) to a bipolar form shown in (a). In reconstitution, due to the lower metabolic activity which results in a reduced Turing wavelength, bipolarity is obtained in a piece as in (c) which is shorter than the longer bipolar form in nature as in (a) (redrawn from Child, 1915, 1929, by permission of The University of Chicago Press).
asexual reproduction, Meinhardt’s (1995) concern, cited in the introduction, that “several heads would appear instead of one”, is actually realised, e.g. as in Fig. 10(a).

Reconstitution from pieces mimics reproduction occurring in nature but “differs from it only in that the distance of the second hydranth from the first is less in the pieces than in the animal under natural conditions. This difference indicates that, as might be expected, the range of dominance of the apical region is less in the experimental piece than in the whole animal in nature” (Child, 1915). Thus because of the lower metabolism (resulting in a shorter Turing wavelength) in reconstitution experiments, bipolarity is obtained for pieces shorter than the length required for bipolarity in nature as seen from the comparison of Fig. 10(c) to Fig. 10(a). The development of the second gradient and the second hydranth is delayed, and the delay is greater the shorter the piece. The full-grown metabolically active apical hydranth delays the development of a hydranth at the basal end more than an apical hydranth in an early stage when it is less active, and more than an apical hydranth that is inhibited. Compressing, ligature, bending, or partially crushing the stem at some point between the two ends reduces the influence of the dominant apical region and the establishment of a new gradient and so the development of a hydranth at the basal end is accelerated (Child, 1915, 1929).

It is the case in hydroids that by creating inhibiting conditions, for example, with inhibiting agents, hydranths can be transformed into stolons (Child, 1941). Child makes the analogy between inhibition by external agents such as KCN and internal inhibition by a dominant region. Indeed, in refuting the argument that polarity involves separate Turing systems for the head and for the foot, we note that “In Tubularia the stolon is evidently an axis somewhat inhibited by the dominant hydranth” and Child talks about the “determination of stolon development at proximal levels of an axis by dominance of the hydranth-region” (Child, 1941). In other words, what counts is the memory and effect of the first polar pattern, which is responsible for inhibiting metabolism at the other end, resulting in a smaller peak of metabolism at that end, and therefore producing a foot (stolon), and not another head. Had there not been the first polar metabolic pattern, the inhibition at the other end would be absent. What is important, therefore, is the proper succession of metabolic patterns corresponding to one Turing system, and not having more than one Turing system. But “if oral and aboral hydranth development begin about the same time, neither inhibits the other” (Child, 1941). We then have “heteromorphosis”, i.e. bipolarity and a two-headed animal. These principles for the origin of polarity in Tubularia are also true for other hydroids including Corymorpha and other animals such as planaria and annelids and hold for reconstitution and development (Child, 1941, 1946).

Simple organisms such as the coelenterates maintain their physiological gradients not only through their embryonic development but also throughout life (Child, 1928). This may be because effective memory (hysteresis) mechanisms (for example such as described in Fig. 3 of Schiffmann (1991) (see also Hagstrom and Schedl, 1997; Laurent and Kellershohn, 1999), are not available for the simple organisms, and therefore their macroscopic structure requires a permanent maintenance of the physiological gradients (the dissipative structure). But even here, a different kind of memory exists, in the sense highlighted above that an existing physiological gradient limits a new one, and so it is still true that no more than one Turing system is required for polarity, even in these simple organisms.

Just as there are no different Turing systems for the head formation and for the foot formation, there is no different Turing system for tentacle formation. Indeed, whether in normal development
or in reconstitution the emergence of the tentacles is preceded by spatially periodic metabolic activity along a ring and the metabolic activity is exactly the same as in other regions (Child, 1926a, Figs. 7–11; Child and Watanabe, 1935, Fig. 20; Watanabe, 1935, Figs. 4–6,10,12).

And the same principles of physiological dominance, subordination and isolation, i.e. of lateral inhibition, that we invoked above in relation to the poles (ends) of the system apply to tentacle formation: “the tentacles arise as local regions of greater [metabolic] activity about the circumference” (Child, 1927a). “With the localisation of tentacles certain radii become different from others... each region of growth, whether tentacle or other organ, dominates a certain area so that a similar organ cannot develop within that area. When a particular tentacle is localised, for example, another can develop only outside its range of dominance. Any part of the circumference in the tentacle forming region is undoubtedly capable of giving rise to a tentacle...” (Child, 1927b).

2.3. Modern support for the (cAMP, ATP) metabolic theory

It is interesting to note that the inhibition of bud, head, foot and tentacle formation in *Hydra* by the “endogeneous inhibitor” of Berking (1977, 1979, 1980) and Berking and Gierer (1977), exactly parallels their inhibition with ATP (Newman, 1973). Furthermore, the “endogeneous inhibitor” is not a peptide, it has a molecular weight of about 500 Da, it is hydrophilic and it is not toxic. All these are characteristics of ATP. It would therefore be interesting to check if the “endogeneous inhibitor” is ATP. The concentration of the “endogeneous inhibitor” is highest in the hypostomal region and decreases basally. A second lower peak is in the basal disc. This is precisely the shape of the bipolar metabolic pattern Child finds for various hydroids (Child, 1915, 1925, 1926a, 1951). And we know in general (Schiffmann, 1997) that a peak in Child’s type of metabolic activity also signifies a peak in ATP production and in ATP concentration. The ability ATP has to abolish macroscopic structure even of an intact adult *Hydra* (Newman, 1973) corresponds to the preservation of “physiological gradients” even in the adult forms of the simpler organisms (Child, 1928); and this preservation corresponds to the enhanced ability for regeneration of the adult forms of these simpler organisms compared to higher organisms which do not share this enhanced ability in the adult form and also do not preserve the ‘physiological gradients’ beyond the development stage. This fact is of importance in modern ‘regenerative biology’ (Stocum, 1998), i.e. one can expect to ‘encourage’ regeneration by ‘encouraging’ the formation of ‘physiological gradients’.

The ‘endogeneous inhibitor’ inhibits macroscopic structure formation. “The inhibitor hinders the generation of the pre-patterns or their interpretation... However, only if this inhibitor is involved in the formation of the pre-patterns does a direct relation exist between this inhibitor and the inhibitor involved in the Gierer–Meinhardt model” (Berking, 1979). According to my theory (Schiffmann, 1997) (cAMP, ATP) is the universal and unique (activator, inhibitor) Turing–Gierer–Meinhardt system. ATP is indeed the Turing–Gierer Meinhardt inhibitor and most probably also Berking’s ‘endogeneous inhibitor’. ATP inhibits the formation of cAMP via substrate inhibition. One expects according to this theory that the (cAMP = activator) will activate the downstream processes (differential gene expression). This is precisely what is found experimentally. The transcription factor CREB is activated by cAMP (Galliot, 1997). DNA binding of CREB is enhanced by two types of posttranslational modification: phosphorylation with PKA (Galliot, 1997) and reduction (Klatt et al., 1999). Localised cAMP is responsible for both the localised phosphorylation and the localised reduction (Schiffmann, 1997). It is thus clear
that Child’s metabolic pattern, or equivalently the (cAMP, ATP) Turing system, is the fundamental cause of the downstream differential gene expression: “During regeneration and as a consequence of HA [Head Activator] treatment, an early activation of CREB activity was observed preceding changes in expression of the homeobox containing transcription factors. This may indicate that CREB triggers their expression cascade” (Schaller et al., 1996). Note that cAMP is an ‘activator’ both in the sense of its involvement in the four Turing inequalities and in its activation of the downstream gene expression.

Many of the observations of Child and colleagues tie up with the modern results. For example, they noted that cutting or wounding the hydroids will lead to a localised increase in oxidation and also will enhance regeneration. This is explained by the modern results that cutting and wounding lead to the release of peptides such as “head activator”, “foot activator”, “bud activator” which in turn will increase the cAMP level (Galliot, 1997). And this increase in the cAMP level will explain the observed increase in oxidation activity and also the observed regeneration, since the increase in oxidation activity is equivalent to the activation of the Turing–Child system essential for the regeneration.

Both cAMP and ATP are small, hydrophilic molecules able to pass through gap junction channels and the notion that (cAMP, ATP) is the Turing couple in Hydra corresponds to the existence and importance of gap junctions in Hydra and to the decrease of ‘head inhibition’ upon the disruption of gap junction communication with antibodies (Fraser et al., 1987). It is interesting to note the parallelism between the Hydra patterning and feather formation (Fig. 2 in Schiffmann, 1997). In both systems the Turing morphogen cAMP activates CREB which in turn triggers the downstream expression of homeobox containing transcription factors or adhesion molecules. ATP diffusion provides the lateral inhibition. In feather formation too, and in general for the spatially differential expression of adhesion molecules, gap junctional communication is required (DeHaan, 1994).

3. The physiological gradients in insects

The results for insects parallels those for coelenterates. For various insects a small area at the posterior pole is necessary for the development of the embryo. It was found by Seidel that if this area is eliminated by ligation, differentiation fails (Meinhardt, 1982) (see also Wall, 1990). But the ‘activation centre’ of Seidel corresponds to a peak in metabolism in the sense of Child in the posterior pole of the early embryo (Akiyama and Okada, 1992; Ding et al., 1994). We saw above for Hydrozoa that if in reconstitution we replace a polar metabolic pattern that occurs in normal development with a bipolar metabolic pattern a symmetric bipolar morphological phenotype results. The situation is similar in insects. For the same early stage in Drosophila development, in which the wild type manifests high metabolism in the posterior pole (Akiyama and Okada, 1992; Ding et al., 1994), the Bicaudal-D mutant embryos manifest a bipolar metabolic pattern with high metabolism in both anterior and posterior poles (Akiyama and Okada, 1992). It is the bipolar metabolic pattern that is responsible for the symmetric bipolar ‘double abdomen’ phenotype characteristic of the Bicaudal-D mutant.

Nanos is the localised posterior determinant for abdomen formation (Wang and Lehman, 1991) and the localisation of Nanos RNA requires Oskar function but Oskar RNA is localised
independently of Nanos (Ephrussi et al., 1991). The transition, polar → bipolar, in metabolic localisation in the early embryo brought about by the transition, wild type → Bicaudal-D (Akiyama and Okada, 1992), correlates with the transition polar → bipolar in the localisation of Oskar RNA and Nanos RNA upon the same transition, wild-type → Bicaudal-D (Ephrussi et al., 1991). But the Oskar and Nanos mutations do not affect the polar metabolic pattern of the wild type (Akiyama and Okada, 1992). This situation corresponds to our general thesis that the metabolic pattern is the primary cause of the localisation of gene products and not the other way round. The spatial pattern of the caudal protein is polar in the wild type but is bipolar and symmetric in Bicaudal-D (Macdonald and Struhl, 1986) and this too is due to the change in metabolic localisation from polar to bipolar.

The spatial peak in metabolic activity corresponds to a peak in reducing power, i.e. to a peak in the tendency to give off electrons and to charge up an electrode. It is indeed confirmed experimentally that a peak in respiratory activity and redox activity with redox probes also corresponds to a peak in electronegativity and constitutes a basis for ‘endogenous electrophoresis’ (Schiffmann, 1994, 1997). The result is that the transition, wild type → Bicaudal-D, involves a change from a pattern with a negative centre in the posterior pole to one with two negative centres in the anterior and posterior poles. This in turn will contribute to the replacement of a polar localisation of gene products with a bipolar one. For example, Oskar protein may well be involved in the establishment and maintenance of Oskar RNA localisation (Kim-Hal et al., 1991; Ephrussi et al., 1991). But the Oskar protein is a basic protein (Ephrussi et al., 1991) and therefore a positive protein attracted to negative poles, which explains Oskar RNA localisation in the wild type and Bicaudal-D. It is argued in Nusslein-Volhard et al. (1987) and reasons are given that “the posterior activity appears to have more dynamic self-organising properties than the anterior” activity. This prediction is thus correct and the Turing–Child metabolic field provides the self-organisation. There is also a growing recognition that it is the posterior gradient system that is more fundamental and that it establishes the axis of the embryo but “one thing that certainly remains unexplained… is how pattern is set up… in a cellular context, and not in a syncytial environment as in Drosophila” (Patel, 2000). But the Turing morphogens cAMP and ATP can also diffuse through a cellular environment via gap junctions and this explains the question of “pattern formation in a cellular environment” (Patel, 2000).

Nusslein-Volhard (1979) (see also Meinhardt, 1977, 1982) suggests that a dramatic change from a polar phenotype to a bipolar phenotype brought about merely by a single mutation, in particular by Bicaudal-D, is best explained by an underlying Turing system. She argues (Fig. 6 there) that the mutation results in the replacement of a polar gradient by a bipolar and symmetric U-shaped gradient. This is indeed the case where (cAMP, ATP) is the hypothesised Turing system and metabolic polarity is replaced by metabolic bipolarity (Akiyama and Okada, 1992). The situation is summarised in Fig. 11.

If a “double abdomen” mutant is explained by the symmetric U-shape, the inverted U-shape with the maximum at the centre, as Fig. 4.1(c) in Meinhardt (1982) or Fig. 1(d), can explain the double head, the dicephalic mutant, as is suggested in Lohs-Schardin (1982). This too corresponds to the general situation presented by Child for all organisms (Child, 1915, 1941). For example, in reconstitution from pieces of Planaria, when conditions are such that the ends of the piece simultaneously develop high metabolism, a double headed bipolar form develops as shown in Fig. 7(a)–(c); which form develops depends on the length of the piece. But if the two ends
correspond to the low points of metabolism, a symmetrical bipolar “double tail” develops as in Fig. 7(d). Such a Planarian double tail corresponds to pieces of hydroids that produce stolons at both ends, also obtained by Child. Thus the Planarian head, the hydroid hydranth and the Drosophila abdomen each corresponds to a ‘dominant’ region because it is the ‘high’ region of the metabolic gradient.

Child has found for many organisms that normal development involves increase in length and size, and that above a certain length one end becomes metabolically active and then upon further increase in length the opposite end will also become metabolically active. This behaviour is in itself an indication that a Turing system underlies the metabolic activity, as we emphasised in Section 2. Later, in some systems the first pole will dominate, e.g. as we saw for Corymopha and Tubularia, but in others, e.g. as we saw for campanularian hydroids and is also the case for the sea urchin which is discussed in Section 4, the second pole will dominate. The appearance of reduction gradients as described above and the later dominance of the second pole occurs in Drosophila oogenesis and development if the embryo is regarded as a continuation of the egg chamber system (and assuming the identity of D. melanogaster and D. hydei). Indeed, Child (1942, 1943) finds for Drosophila hydei that as the egg chamber (‘follicle’) increases in length, a gradient in the rate of reduction with the maximum in the apical, anterior end, will appear only if the egg chamber is above a certain length. This fulfils the prediction that only beyond a critical extension of the growing insect oocyte will a polar pattern arise (Fig. 3, Meinhardt, 1977). This gradient appears in follicles of length 0.1–0.2 mm, before the oocyte is visibly distinguishable. Child (1942) suggests that “It appears highly probable that the physiological pattern of which the reduction gradient is an expression determines that the basal cell of each follicle shall become oocyte, the other nurse cells”. When the nurse cells become distinguishable from the oocyte, the basal cell of the egg chamber, the nurse cells reduce more rapidly than the oocyte. The higher reduction rate in the nurse cells will result in them being electronegative in relation to the oocyte. The gradient in electric potential will be responsible for electrophoretic transport from the nurse cells to the oocyte as is indeed observed in insect egg chambers (Schiffmann, 1994). This electrophoresis
explains why transport of molecules from nurse cells to oocyte in Drosophila is not inhibited by depolymerising either actin or microtubules.

Later in oogenesis, with further increase in size, in the full-grown egg there is the beginning of a second reduction gradient that extends from the posterior end anteriorly (Child, 1942, 1943). This second posterior gradient dominates the first anterior one later in the embryonic stage of Drosophila melanogaster (Akiyama and Okada, 1992; Ding et al., 1994). The Turing reaction-diffusion theory also predicts a ventral–dorsal gradient (Schiffmann, 1997) and indeed a ventral–dorsal metabolic gradient in Drosophila melanogaster embryos was found (Schiffmann, 1997). But this gradient begins earlier in oogenesis of Drosophila hydei (Child, 1942, 1943), again with the maximum in the ventral side. The ventral–dorsal gradient becomes more distinct in advanced stages of oogenesis. All these results further emphasise that it is the proper sequence of metabolic patterns that drives and is responsible for normal development.

4. Scale of organisation and form regulation

Traditionally, there are two great enigmas in biology (Driesch, 1929; Bertalanffy, 1968): First, the problem of epigenesis and the related problems of increasing differentiation, organisation, localisation, order, heterogeneity, and negative entropy. The fact that a system is an open one only makes the decrease of its entropy possible but not obligatory. Second, the problem of form regulation, i.e. the problem of ‘organic purposiveness’, the so-called equifinality of developmental processes, or the manifestation of foresight towards a goal (Russell, 1945), the goal being the creation of a normal organism—a whole—not only from a normal egg but also from a part of it, or from two fused eggs, or from pieces of hydroids or planarians, etc. These two enigmas can be understood with the combined Child–Turing approach. The solution to “the problem of localisation [which] is simply the problem of the self-production of heterogeneity from homogeneity” (Child, 1908) could not even be conceived before Turing. This problem was a major cause of Driesch’s vitalism. The problem of form regulation was the second cause of his vitalism. The existence of both epigenesis and form regulation was also central in the refutation of Weismann’s theory of “qualitatively unequal nuclear division” as a basis of differentiation, and since a part can yield a proportioned whole, it was referred to as a “Harmonious-equipotential system” (Driesch, 1929). Driesch argued that only a part of an animate whole can give rise to the whole, but an inanimate whole, such as a machine, cannot repair itself into a whole when a part is removed. In this context, Driesch’s vitalism signified to him the autonomy of life, the irreducibility of life to chemistry and physics; “biology, therefore, is not applied physics and chemistry: life is something apart, and biology is an independent science... subjected to laws peculiar to the phenomena in question” (Driesch, 1929), which is the view of the uniqueness of life shared by Schrodinger, Mayr and others (Schiffmann, 1997). As it turned out this vitalistic view of Driesch is correct since, just as the ‘program’ for the coherent decrease in entropy in the macroscopic open system resides in the specific order in the molecular level, so the program for form regulation resides in this order, and this order, in turn, is not contained in the rules of chemistry and physics (Schiffmann, 1997); hence ‘vitalism’ in this sense is justified.

The ‘whole’ that arise from the ‘part’ can be of the normal size for that species or it can be on a smaller scale, but in both cases the appropriate proportions are attained. A classical example is the
restoration of a small or big whole from of a piece of Planaria according to whether the piece was fed or not (Morgan, 1901; Driesch, 1929). A related problem is why in normal development two members of a species could be of different size but the relative spatial proportions and their number of parts is the same. This fact has been raised as an objection to the acceptance of Turing theory (Schiffmann, 1997). One can expect, and below it will be shown that this expectation is correct, that proportion regulation in reconstitution from a piece to a big or small whole, as exemplified for Planaria above, is effected through the same means as in normal development.

It has gone unnoticed that all these problems have in fact been solved by Child who went further and solved related problems. For example, one does not always get a whole from a part and Child developed a unified theory that explains why sometimes a whole results from a part and sometimes only a partial form. As Child (1941) explains, ‘form regulation’ usually refers to the often occurring situation where an isolated part “approaches in some degree or becomes a whole, a normal individual; that is, the isolated part gives rise to more of the individual than if it had remained an integrated part of the original whole”. This will be exemplified below by the basal half of the sea urchin. But Child (1941) emphasises that other results are also possible. In particular, an isolated part can give rise to a partial form that contains fewer structures than if it had remained an integrated part of the original whole. This will be exemplified below in the apical half of the sea urchin. Child also explained how to treat a piece that normally yields a partial form so that it will give rise to a complete form (a whole); and also which of the new parts are formed first, independent of other parts. Child’s explanations and experiments can be interpreted by Turing theory with the correct identification of the Turing morphogens, which lends further support to both the Child theory and the Turing theory. Modern ‘tissue restoration’ and ‘regenerative biology’ as well as in vitro fertilisation and therapeutical cloning, also involves a part giving rise to a whole and may benefit from understanding under what conditions a partial form arises and under what conditions a whole arises.

The historical context is very illuminating. Much was made of the rivalry between Morgan and Child, but it was not realised that much of Child’s life’s work was stimulated by a response to Driesch (Child, 1908). Child argues that “Driesch states the case [of form regulation] as if the formation of the new whole occurred in all cases, but this is very far from being true”. His examples include Tubularia: “in pieces sufficiently short, we may expect to find hydranths alone produced, without any stem, or even distal parts of a hydranth without proximal portions”. “For Driesch these small pieces which give rise to partial structures are simply ‘atypical’, a sort of ‘freak of nature’ and can teach us nothing concerning ‘normal phenomena’” (Child, 1908). Child (1908) emphasises the gradation of partial forms and the importance of understanding the partial form and he proceeds to pose questions that his later work will answer: “According to Driesch’s definition of regulation, it includes only cases where a return or approach to “normal” condition occurs, hence abnormal or atypical results are unimportant. Nevertheless, I believe that these pieces present certain problems of considerable importance: first, why does any localisation and development occur in pieces too short to form wholes? second, why do partial structures of relatively large size occur in some pieces, while others of the same length give rise to wholes of smaller size? third, why do partial structures always represent the distal portions of hydranth, never the proximals?” His observation that there is a minimal size “below which no piece is capable of forming whole” (which is typically a Turing situation) and that the “minimal size-limit” may differ according to region of the body, e.g. in Tubularia the size of minimal pieces is
much less in proximal than in distal regions, will be central to later ideas about the existence of
differential metabolism and scale of organisation. Central to Driesch’s theory is that a sea-urchin
egg is “harmonic equipotential system”, and indeed “meridional halves and even meridional
quarters of early cleavage stages give rise in fact to typical pluteus dwarf larvae” (Horstadius,
1952). But Child (1908) criticises Driesch for ignoring “that the sea-urchin egg is not an
equipotential system in the “animal-vegetative (polar) direction”, and this fact was later central to
his unified theory of form regulation. In fact Driesch (1929) noted that a “blastula, consisting of
about one thousand cells, when cut in two quite at random, in a plane coincident with, or at least
passing near, its polar axis, may form two fully developed organisms out of its halves”. Driesch
(1929), and also in his 1908 edition, notes in a footnote that “If the plane of section passes near
the equator of the germ, two whole larvae may be formed also, but in the majority of cases the
“animal” half does not go beyond the blastula.”

It is instructive to look at an example from the arts: “Imagine that you have a piece of paper
before you and wish to sketch a landscape. After drawing for some time you notice that you have
miscalculated the scale with regard to the size of the paper, and that it will not be possible to bring
upon the paper the whole of the landscape you want. What then can you do? You either may
finish what you have begun to draw, and may afterwards carefully join a new piece of paper to the
original one and use that for the rest of the drawing; or you may rub out all you have drawn and
begin drawing to a new scale.” (Driesch, 1929). Similarly, Child (1941) states: “Reconstitution of
a whole of small size from an isolated part involves decrease in scale of organisation, that is, a
decrease in scale of the gradient system”. If one does not decrease the scale of organisation, the
‘partial form’ that arises corresponds to what is known in art theory as an ‘open picture’. It is
important to realise that Child’s notion of ‘scale of organisation’ does not refer only to the
phenotypic localisation of macroscopic parts but also to the underlying metabolic gradients:
“because with the higher levels of activity the gradient is longer and the localisation of parts takes
place on a larger scale” (Child, 1926b; Rulon and Child, 1937). “An intensely active dominant
region determines, in general, a greater length than one less active” (Child, 1941). “A gradient
established in an isolated part in good condition will, in general, represent a more intense activity
at its high end, will extend over a greater distance...; consequently, the local fields developing
along its course, within which the particular parts develop, will also be longer and the scale of
organisation therefore larger” (Child, 1941). Equivalently, a greater “range of dominance”
obtained with higher metabolism corresponds to a longer metabolic gradient and to a greater
distance between the parts (Child, 1915). In the Turing picture, the longer metabolic gradient, the
greater range of dominance and the larger scale of organisation, correspond to (g) rather than (c)
in Fig. 1.

If the piece is too small for the scale of organisation a ‘partial form’ results and development
always starts from the ‘dominant’ (metabolically active) region. Typically: “The fact that in the
partial forms the more distal parts of the hydranth, even the hypostome alone, develop normally
in the complete absence of other parts, so far as length of piece and scale of organisation permit,
shows that localisation and determination of parts in hydranth development take place from the
apical region basipetally. The region which develops as apical end is the primary dominant region
in development and is determined independently of other parts” (Child, 1931). The general
occurrence in embryology of an anteroposterior developmental gradient has been recognised in
the so-called “law of anteroposterior, cephalocaudal, or craniocaudal development” (Child, 1915,
1941). Morphologically, this gradient is a timetable of events and Child has supplied the physiological basis, namely that development starts from the ‘dominant’, metabolically active region. “But developmental gradients... in other directions—for example, from the ventral region laterally and dorsally in turbellarians, annelids, and arthropods and from the middorsal region laterally and ventrally in vertebrates” (Child, 1941) can also be explained by the physiological gradient and are the basis of dorsal–ventral inversion (Schiffmann, 1994). Child and others varied the length of pieces and/or the scale of organisation and the situation for hydroids that “as much of the hydranth is formed basipetally as length of piece and scale of organisation permit” (Child, 1941) was reproduced for other animals such as Planaria and echinoderms.

We now provide the fundamental argument for ‘form regulation’, or equivalently for ‘proportion regulation’, or ‘size invariance’, whether in reconstitution or in development. We first note that Child (1941, 1946) invariably decreased the scale of organisation by a decrease in the rate of metabolism. This can be achieved by many external chemical agents, by overcrowding, by lower temperature and by starvation. In reconstitution it can also be achieved by the choice of the level from which the piece is taken: a piece taken from the low part of the metabolic gradient will have a lower metabolism than a piece taken from a high part of the metabolic gradient. But a decrease in metabolism corresponds to inhibited ATP synthesis. And if we identify ATP as the ‘Turing inhibitor’, then Turing’s theory predicts that lower metabolism corresponds to smaller Turing wavelength. Therefore, if we identify ATP as the ‘Turing morphogen’, smaller scale of organisation in the senses used by Child, corresponds to smaller Turing wavelength.

Child (1941) and others (Oldham et al., 2000) have found that growth rate and the final size of an animal increase with the rate of metabolism. Thus for an individual whose development is characterised by higher metabolism, the growth rate and the final size of the animal, as well as the Turing wavelength (scale of organisation), will be greater, and this explains ‘proportion regulation’. The same is true for the reconstitution of a piece into a whole; if it occurs with higher metabolism, the resulting ‘whole’ will be a proportionately bigger individual. The ‘wisdom of the body’ is reflected here in the fact that the same cause (the level of metabolism) is responsible for an individual’s higher growth rate and final size, and for this individual developing with a larger Turing wavelength (scale of organisation). The larger size is balanced by a larger scale of organisation, so a well-proportioned individual can result from reconstitution or from development; the proportion is invariant to the difference in size.

We are now in a position to explain Driesch’s famous 1892 experiment following Roux’s 1888 experiment. An English translation of Roux’s 1888 paper and of the abridged Driesch 1892 paper is to be found in Willier and Oppenheimer (1964). In this experiment, also described in Driesch (1929), he separated the two blastomeres of the sea-urchin embryo and expected to obtain “half”-blastula, “half”-gastrula and “half”-larva according to the conception of Weismann and Roux as shown in A2 and B2 of Fig. 12. But instead, he obtained half-sized blastula, gastrula and larva as shown in A3 and B3 of Fig. 12. On the basis of these results he argued that a complete nucleus was inherited by the two blastomeres and that the protoplasm of the two isolated blastomeres was also equivalent. This means that normal development beyond the two-cell stage is bound to involve epigenesis. This led him to refute a mosaic-preformation-type theory of development of the kind suggested by Weismann and Roux.

The two main conclusions revealed by Driesch’s 1892 experiment [and by many related experiments, some described in Driesch (1929)], namely the existence of epigenesis and form
regulation, have not been explained even today. Epigenesis can be explained by the Turing instability in the (cAMP, ATP) system. The form regulation, the obtaining of two small proportionate wholes from each of the isolated blastomeres, is explained by noting that the first cleavage divides the nutrients into the two daughter blastomeres. As a result the two isolated blastomeres will develop with a lower metabolism as compared with normal development. It is this lower metabolism that will be responsible for both the smaller final size of the pluteus larva and for the smaller Turing wavelength characterising the development of each of the separated blastomeres, and thus resulting in a smaller but proportionate whole from the development of each isolated blastomere.

Morgan (1901) notes: “If a planarian is kept for several months without food, it will decrease very much in size. In fact, the volume of a starved worm of Planaria lugubris compared with that of a fully fed individual may be only one-thirteenth of the latter” (see Fig. 13). Form regulation is again explained by the decrease in the rate of metabolism due to the starvation which decreases both the overall size of the Planaria and the Turing wavelength. The same explanation of form regulation applies in lateral reconstitution (Morgan, 1901; Russell, 1945). If a well-fed planaria is cut in two along the middle line of the body as indicated in Fig. 14, a full-sized proportionate whole is reconstituted, involving regeneration of new tissue if food from outside is available; or a reduced-size proportionate whole is reconstituted, more by redifferentiation and reorganisation of the isolated piece, if food from outside is lacking.

Overcrowding and starvation of Drosophila can result in perfectly proportioned microflies five times smaller than those reared with optimal care. These microflies are in fact phenocopies of genetic mutants where the mutations, as expected from the above, are equivalent to nutrient limitation (Edgar, 1999; Lehner, 1999; Oldham et al., 2000). Thus the genetic cause of proportion invariance in the face of size variation is “equivalent” to the environmental cause, and both correspond to the above Child–Turing explanation of proportion regulation.

“Redifferentiation or reorganisation is reconstitution of an isolated part into something else without outgrowth of new tissue from surfaces of section... Regeneration is reconstitution by outgrowth of new tissue from the cut surface and its differentiation into a part of an individual,
often, but by no means always, more or less similar to the part removed. . . Reconstitution in stem pieces of the hydroids *Tubularia* and *Corymorpha* is wholly redifferentiation; there is no replacement of parts removed, but transformation of the piece. In most planarians both regeneration and redifferentiation occur in reconstitution, and the proportion of each differs in

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**Fig. 13.** (A) Large well-fed individual of *Planaria lugubris*. (B) Same after being kept without food for 4 months, 13 d. Both drawn to same scale (from Morgan, 1901).

**Fig. 14.** *Planaria lugubris* reconstitution. Dotted line in (A) indicates where the worm was cut in two lengthwise. Upper three figures (B)–(D) show the regeneration of a half which is being fed. Lower three figures (E)–(G) show regeneration of other half kept without food. Note the equifinality of regulation: both the small and big reconstituted wholes are well-proportioned (from Morgan, 1901).
pieces from different body-levels of the same individual and can also be altered experimentally” (Child, 1941). According to Child (1911a), “if we decrease the rate of metabolism in Planaria by extreme starvation or by the use of anaesthetics, then parts which under the usual conditions are formed chiefly by regeneration, e.g. the head, may be formed largely by redifferentiation”. These results (Child, 1910, 1911a, 1941) correspond to what we would expect from an harmonious-equipotential piece intent on form regulation. Indeed, if the reconstitution is done under conditions of higher metabolism, both the final size and the Turing wavelength are greater than when the reconstitution is done under conditions of lower metabolism. This situation will contribute to form regulation whether the reconstitution occurs via regeneration or via redifferentiation.

Child and others found that the ‘head frequency’ (the percentage of pieces yielding heads) and the rate of head regeneration in planarian reconstitution (see Fig. 15) depended on the level (position) along the longitudinal axis of the planarian body from which the piece was isolated. This phenomenon was the basis of Child’s theory of metabolic gradients (Brøndsted, 1969). The monotonic gradient in Fig. 15, typical of species without a posterior zooid, is underlied by a monotonic metabolic gradient. But when new zooids are formed in the posterior region of the body, monotonicity is replaced by spatial oscillation (Child, 1929; Hyman, 1923). In the shorter animal there is only one of these zooids. Child (1915) describes the corresponding metabolic gradient thus: “The apical region of this [metabolic] gradient is the head of the animal, and from the head the metabolic rate decreases to the level where separation occurs in fission; there a sudden rise in rate occurs, and then again a downward gradient toward the posterior end”. As the length increases more than one posterior zooid is possible. This is reflected in the longitudinal metabolic profile: “But as the length increases the basal body region may show two, three, or more of these distinct [metabolic] gradients. Represented graphically the metabolic gradient in such an animal is like the curve in Fig. 45 [Fig. 16 here]” (Child, 1915). “These zooids are the result of successive physiological isolations of the basal region as the animal grows in length” (Child, 1915). This formation of new zooids (individuals), resulting from successive physiological isolations of the posterior regions of the body from the dominant head region, corresponds exactly to the Turing process described in the sequence, (c) → (d) → (e) → . . . , in Fig. 1 and is the prototype for all spatial periodicity, whether in somitogenesis or stripes and segmentation in the fly.

Fig. 15. Regeneration rate, in days, of head from various body levels of Dugesia lugubris (from Dubois, 1949, reproduced in Brøndsted, 1969, by permission of Les Presses Universitaires de France, and Elsevier Science).
If form formation is governed by one universal Turing system, one would expect it to manifest self-similarity across scales. Such a Turing-type ‘self-similarity’ was found by Child (1911b): “As a matter of fact there is good reason for believing that the second zooid actually consist of two zooids in a great many cases, perhaps always, after it has attained a certain size; if, for example, this second zooid is cut into very small pieces we commonly find that the regional distribution of the regulatory capacities resembles that in the whole individual from which this zooid was taken. A decrease in the power of whole formation occurs with increasing distance of the level of section from the zone of fission to the posterior quarter of fifth or the zooid where it increases again. In short, if we cut the second zooid into sufficiently small pieces it appears as a miniature of the whole animal [our emphasis].” In general, the increase in size is the driving force for form formation in, and subdivision of, first the whole system, in particular the whole embryo, and later a subsystem, in particular an organ such as an imaginal disc.

Child finds that the scale of organisation in the products of planarian reconstitution reflects the metabolic curve in Fig. 16: “Pieces of the same length from different body-levels differ as regards relative size or length of parts. Size of head and distance between head and pharynx, that is, length of prepharyngeal region, decrease from anterior to posterior levels of origin of piece, or in species with fission zone, to this zone, and both increase again in the posterior zooid” (Child, 1941). Fig. 17
is a homologous series with respect to the metabolic curve in Fig. 16. In greater detail Child (1941) describes the situation thus: “Marked differences in scale of organisation of the preoral region occur in planarian reconstitution in relation to the level of origin of the piece. These consist of a gradual decrease in relative length of the preoral region and more anterior localisation of the pharynx in successive pieces of equal length from the most anterior level posteriorly to the fission zone... In the region of the posterior zoid length of preoral region is again greater. In species without posterior zoid region decrease in preoral length continues to the posterior end... Scale of organisation of the new head in earlier stages shows a similar relation to level of origin of the piece. It decreases from the anterior level to the fission zone and is again larger in the posterior zoid region in the species with posterior zoid. In those without such zooids the decrease continues to the posterior end” (Child, 1941).

The frequency of occurrence of the head forms shown in the graded inhibition series of Fig. 8, i.e. the ‘head frequency’, is often plotted by Child as ordinates against body-levels as abscissa—see for example Fig. 66 in Child (1941). The general result is that the head frequency decreases from the anterior level to the region of the fission zone where it increases posteriorly, and in species without posterior zooids it decreases from anterior to posterior levels over the whole body length (Child, 1941; Brøndsted, 1969). Child’s basic claim is that the head-frequency gradient reflects the metabolic gradient. Of particular interest is to focus on his fundamental series of papers in 1911 (Brøndsted, 1969) and in particular on the first in the series (Child, 1911b) since this paper includes experiments and discussions that can quintessentially only be understood and interpreted by Turing theory and metabolism-dependent Turing wavelength. It also includes fundamental simultaneous discussions of the problems of ‘polarity’ and ‘form regulation”—the two problems of major concern in the present paper. As we saw, the interplay between the size (length) and the Turing wavelength is central in development and form regulation. Corresponding to this is Child’s (1911b) varying the size of the reconstituting (regenerating) pieces and the region (level) of the pieces; the level of the piece reflects the rate of metabolism (Child, 1915) and therefore the Turing wavelength. The results of these experiments were used by Child (1911b) both to claim that there is a metabolic gradient in the parent animal from which the pieces were taken, and also to focus on how the length of a piece and the region of the body (= rate of metabolism = Turing wavelength) determine the products of the reconstitution of the pieces. The only explanation for all his findings is the metabolic Turing theory. The whole body of the _Planaria dorotocephala_, posterior to the head, was cut into two, four, six, eight, etc. pieces of equal length. The products of the reconstitution are displayed in Figs. 18 and 19. The halves ai and iq shown in Fig. 18(A) produce the normal wholes shown in Fig. 18(B) and (C). “The anterior half (B) possesses a somewhat larger head than the posterior half (C), and the change in shape and the increase in length are also somewhat more rapid in the anterior than in the posterior half.” The position of the pharynx in (B) is posterior to its position in (C). The quarters ae, ei, im, mq shown in Fig. 18(A) reconstitute into products shown in Fig. 18(D)–(G). “The head is the largest in the anterior one fourth and decreases to the third (D)–(F), while in the posterior fourth it is again large (G) ... the eyes are normal in the first, almost always normal in the second and third, and normal in the posterior fourth.” The position of the pharynx moves anteriorly upon the transitions, D → E → F, but then returns posteriorly for F → G.

Of the eighths, ac, ce, eg, gi, ik, km, mo, oq, shown in Fig. 18(A), only the alternate products of reconstitution are shown. The first (A), the third (B and C), the fifth (D) and the seventh (E), are
shown in Fig. 19(A)–(E). “The first eighth (A) is usually a normal whole with large head... and with pharynx far posterior to the middle. The second eighth is intermediate in character between the first and the third. The third eighth varies in character: it may be a teratopthalmic whole of the character of (B), or very rarely it may be a normal whole, but commonly it is a headless like (C) ... The fourth and the fifth eighths are practically always headless (D) ... In the fifth eighth the new pharynx is anterior to the middle (D). The seventh eighth (E) is, on the other hand usually a normal whole... with pharynx in the middle of the body. The sixth eighth is most commonly headless like the fifth, but sometimes forms a teratopthalmic whole, or more rarely a normal whole like the seventh. The most posterior eighth almost always forms a normal whole like the
seventh (E), rarely it is teratopthalmic but never headless. In these eighths we see the results of regulation running from normal wholes at the anterior end to headless forms in the middle region and then in the posterior fourth suddenly becoming normal wholes again. The differences in size of head, shape of body, amount of regeneration and position of the pharynx are similar to those in the fourths, but more extreme” (Child, 1911b).

The sixteenths ab, bc, cd, de, etc. yield the reconstitution products shown in Fig. 19(F)–(P). “The first sixteenth after removal of the head is commonly tailless with relatively very large head (F), though it may sometimes produce a normal whole of the type of (G). The second sixteenth produces either a normal or teratopthalmic whole of the general type of (G) and (H).” The ‘partial form’ (tailless) in (F) is explained by the combination of a particularly small size of the piece and a
large Turing wavelength (corresponding to large “scale of organisation” in the terminology of Child, 1941, or large “range of dominance” in the terminology of Child, 1915). The transition, partial form → whole, (F) → (G) or (H), occurring for the same length of a piece, is explained by the smaller Turing wavelength due to the lower metabolism in the more posterior piece. Below it will be shown that a whole such as (G) or (H) can be obtained from the first sixteenth by experimental reduction of the Turing wavelength in this piece—further pointing to the consistency of the predictions of the Turing–Child theory. “The third sixteenth may produce a normal whole (G), a teratopthalmic whole (H) or even a headless tail (I): like the third, the fourth sixteenth may produce normal or teratopthalmic wholes or headless tails, but usually the percentage of headless tails is higher than in the third. The fifth (J), sixth, seventh (K), eighth, ninth (L), tenth and eleventh (M), sixteenths are practically always headless or else do not live long enough to undergo regulation. Moreover, they show an increasing degree of headlessness, as indicated by (J)–(M); the length of the prepharyngeal region decreases in successive pieces and the size of the pharynx becomes less, until in the tenth and eleventh sixteenths the pharynx often fails to appear at all, i.e., these pieces do not succeed in producing a prepharyngeal region or even a pharyngeal region in regulation but remain postpharyngeal. The twelfth sixteenth may produce either a normal or a teratopthalmic whole or a headless tail. The thirteenth and fourteenth sixteenths likewise range from headless tails (N) to normal wholes, but the fourteenth produces normal wholes more frequently than the thirteenth. The fifteenth and the posterior sixteenths rarely produce anything except normal wholes” (Child, 1911b). So we see that for sixteenth pieces also, the frequency of head formation as well as the frequency of normal eyes when plotted against body-levels manifest the decreasing and then increasing profile (Figs. 40 and 41 in Child, 1911b) corresponding to the profile of the metabolic gradient. The series:

\[
\text{tailless} \rightarrow \text{normal whole} \rightarrow \text{teratopthalmic whole} \rightarrow \text{headless}
\]

corresponding to decreasing metabolism and increasing ‘headlessness’ is understood if we recall that decreasing metabolism corresponds to decreasing size and scale of organisation of the head, and that the reconstitution starts from the head. The first sixteenth piece forms a large percentage of tailless heads instead of wholes since the very large head that forms first does not leave enough space within the length of the piece for the more posterior part. The other ‘partial form’ is the opposite extreme to ‘tailless’—the ‘headless’—corresponds to the lowest metabolism.

With the Turing picture in mind, it is instructive to present Child’s (1911c) explanation as to why, “if we isolate very short pieces from just behind the old head they give rise in most cases ... to tailless heads”, and why this occurs less frequently as we move posteriorly: “The occurrence of ‘tailless heads’ in very short pieces from extreme anterior regions, in other words, the failure of such pieces to form pharyngeal and postpharyngeal regions depends upon the fact ... that a higher rate of metabolism is concerned in the formation of a head than in that of a posterior part: in these short pieces the posterior regions do not form because the process of head formation with its higher rate of reaction uses up the available material so rapidly and to such an extent that the formation of a posterior end is inhibited or rather prevented”. Child (1911c) emphasises the analogous situation for Tubularia where, like head formation in Planaria, hydranth formation is dominant and furthermore, in Tubularia the distal hydranth regions are dominant over proximal ones. Therefore: “In short pieces of the Tubularia stem, particularly in those from the more distal regions, the product of regulation is more and more exclusively distal in character, the shorter the
piece. Similarly, in the anterior regions of *Planaria* the product of regulation becomes more and more exclusively anterior as the length of the piece decreases” (Child, 1911c). The results for *Planaria* (Child, 1911b, c) that “at more posterior levels the pharyngeal and postpharyngeal regions are formed [whereas tailless heads develop from pieces taken from the extreme anterior regions] and as the level of the piece in the original body becomes more and more posterior they become larger and the new head becomes smaller” (Child, 1911c), have analogues in *Tubularia* and *Corymorpha*: “if a tubularian stem is cut into a series of pieces of equal length the oral hydranth develops most rapidly and is largest in the most distal piece and its size and rate of development decrease with each successive piece from the distal end proximally. These differences are similar in character to the differences in head formation at different levels of the first zooid in *Planaria*” (Child, 1911c). These analogous results are explained via Child’s (1911c) emphasis that it is the head in *Planaria* and the distal region of the hydranth in *Tubularia* and *Corymorpha* that form first in the process of formation of a new individual. Therefore for more posterior pieces the lower metabolism results in a smaller size and scale of organisation of the planarian head or the distal region of the hydranth, thus leaving more room for parts posterior to the head or to the distal region of the hydranth.

We see from Figs. 18 and 19, that within each series of pieces of equal length, the size and scale of organisation of the head, and also the distance of the pharynx from the head, increase with the metabolic rate of the piece. Ignoring the first sixteenth (Fig. 19(F)) where the ability to produce a head is so overwhelming, that the head is so big that it does not leave space for other parts and therefore a ‘partial form’ results, the ability to produce wholes is determined in all pieces by the ability to produce heads. And the capacity for head formation decreases with increasing distance of the level of section from the original head until at some level in the postpharyngeal region it increases again. Child (1911b) starts from an attempt to understand the central problems of ‘form regulation’, namely how the size of the piece and the region of the body from which the piece was cut determine the result of reconstitution, e.g., whether the result will be a ‘partial form’ or a ‘whole’ and whether the whole will be normal or one with abnormal eyes (teratopthalmic whole). His results can only be understood if the “region of the body” is correlated with the level of metabolism and the corresponding Turing wavelength, and then the interplay of the ‘size’ and the ‘Turing wavelength’ is interpreted in the context of Turing theory. Thus Child’s (1911b) general statement that “the capacity to produce a ‘whole’ decreases with decreasing length of the piece, until in pieces below a certain length, which differs in different regions of the body [corresponding to different Turing wavelengths], partial structures instead of wholes arise. There is no sharp limit between ‘wholes’ and partial structures (e.g., tailless heads or headless tails)”, corresponds to our Turing picture in Fig. 1 where it is precisely the increase in length that drives the pattern formation. Of particular interest to us is Child’s connection between the problems of ‘form regulation’ and ‘polarity’: “The size limit in the formation of wholes and the appearance of partial structures in pieces below this size limit indicate that polarity consists essentially in a dynamic gradient or gradients along an axis,. . . . wholes arise only from pieces which include a certain fraction of the gradient or gradients. That the dynamic gradient is not uniform is indicated by the fact that longer pieces are necessary for the formation of wholes…” (Child, 1911b). And it is the “dynamic gradation along the axis” that is simultaneously responsible for regulation into a ‘whole’ and for polarity: “polarity is not a condition of molecular orientation, but is essentially a dynamic gradient in one direction or different gradients in opposite directions along an axis,. . .
The change from the production of wholes to partial structures with decreasing length of piece constitutes strong evidence in favour of this view, while on the basis of a molecular hypothesis of polarity it is difficult to understand why short pieces should not produce wholes as well as long pieces, if they produce anything. The results of these experiments also indicate clearly that the dynamic gradient is not uniform but differs at least quantitatively in different regions of the body. A molecular hypothesis of polarity affords us no basis for understanding why the minimal piece for the formation of a whole must be larger in some regions than in others (Child, 1911b).

This 1911 discussion on the nature of polarity is pursued by Child (1941) in a section entitled “Progressive determination of polarity”: “the reconstitution of pieces shows that the new polar pattern is not established all at once but begins with the dominant region and extends progressively from it, as far as range of dominance, size of isolated piece in relation to scale of organization, or presence of other dominant regions and polarities permit.” The arrows in (c) and (d) of Fig. 1, and the analogous arrows in Child’s work, also correspond to the direction of progress of the successive development of parts in a polar pattern, starting from the dominant regions, i.e. they correspond to the “progressive determination of polarity”. Note, however, that these arrows represent the direction of the propagating fronts of, for example, the change in the redox state of a chemical probe; and these propagating fronts in turn indicate the existence of spatial gradients in the rate of energy release. It is such a spatial gradient in the rate of energy release that is responsible for the progressive (polar) determination of parts from the apical (dominant) region posteriorly. Note also that the location where the planarian head or hydranth starts to form is the location where the redox or susceptibility front starts to move in a typical Child-type experiment on metabolic patterns. Child (1941) continues: “That a new polarity may originate gradually and progressively from a dominant region in nonembryonic forms of development which involve determination of new pattern seems evident from the available data. Moreover, the evidence concerning origin and progressive extension of a new gradient from a dominant region is in accord with this view. And, finally, the evidence pointing to this conclusion has a very important bearing on the question of the nature of polarity and developmental pattern in general. If polarity results from a molecular orientation or an “intimate structure” of some sort, it is not easy to account for the gradual, progressive origin of a polar axis and pattern, beginning at one end.” Child (1941) then states: “If gradual progressive determination of a polarity, beginning at a dominant region, does take place, the dominant region must originate independently of other parts of the polar pattern.” And he draws attention to the fact that this is indeed the case for partial forms in hydroid, planarian and annelid reconstitution. Head development starts before that of pharyngeal, oral and prepharyngeal levels in a post-oral planarian piece. And it is never the case that head development occurs last, following the successive development of oral, pharyngeal, and prepharyngeal regions, as we would expect if reconstitution were determined anteriorly from more posterior levels. The same relations hold in annelid reconstitution, where the head also regenerates first. The head or hydranth, both of which can develop when other parts are not present, are, in his words, “self-determining and self-differentiating systems”, corresponding to the ‘self-organization’ residing in the Turing system in Fig. 1. When short pieces of Tubularia or Corymorpha develop into partial forms, these include the distal (apical) and not the proximal region of the normal polar pattern. The part of the normal polar pattern included in the partial form is determined by the length of the piece, the Turing wavelength (the scale of organization), and the presence or absence of other partial polarities; the
situation can be visualised in Figs. 22 and 23. Thus if the scale of organization in reconstitution is the same as in normal development, i.e. reconstitution does not occur under inhibiting conditions, the parts of the axis formed appear normal in pattern and fully developed, even though they may consist of nothing but a hypostome or a hypostome with distal tentacles; the extreme apical region is never absent—the situation can be visualised in Fig. 22. The ‘progressive determination of polarity’ in reconstitution is similar to ‘the law of anteroposterior development’ in normal embryonic development. Both are underlined by a similar ‘metabolic gradient’.

A century ago, Driesch (1929) had already pointed out the actual fate of an element of a biological system (e.g. an element of an embryo or a piece thereof) depends on its relative position in the system and on the size of the system. I have emphasised in Schiffmann (1997) that one major reason why various current theories of development cannot be right is because these theories and their artificial experimental counterparts ignore the requirement of holism and size. This requirement is fulfilled by the combined Child–Turing approach: “‘Potence’ is not necessarily inherent in the part involved. The head-forming potence of certain pieces, for example is shown to depend, not merely upon the parts directly involved in the development of a head, but upon the piece as a whole. We can even determine whether a head shall or shall not form at the anterior end of a piece by including in the piece or removing from it a certain amount of tissue at its posterior end” (Child, 1911b). To see this, consider Child’s (1910) statement: “If we compare pieces of different lengths with anterior ends at the same level of the body we find that with decreasing length of piece single eye-spots appear more frequently, until in pieces of a certain length perhaps only single eye-spots are formed. With still further decrease in the length of the pieces the heads with single eye-spots give way to a headless condition.” More specifically, consider for example the isolated second fourth of the body (ei, Fig. 18); we find from Table 1 in Child (1911b) that “it produces a head at its anterior end and this head is commonly normal. If, however, we cut it [i.e. the isolated second fourth of the body] transversely in half, both of its halves produce heads only rarely and these heads show abnormal eyes” (Table 1 in Child, 1911b). Furthermore, “if we cut the original quarter into fourths, none of these produces a new head” (Table 1 in Child, 1911b). Evidently, the potence of the cells at the anterior end of the original piece to form a head is dependent “not merely upon these cells alone, but upon the cells of all levels of the piece, for we find that with the successive removal of cells from the posterior end of the piece, the ability of the cells at the anterior end to form a head decreases and disappears” (Child, 1911b). Thus, “the morphogenetic reactions of a given part, depend within certain very wide limits upon its physiological correlation with other parts. Planaria is not then an equipotential system in Driesch’s sense” (Child, 1911b). It is Child’s notion of “physiological correlation” between the cells that perhaps corresponds the best to our modern interpretation in terms of ‘holism’ and Turing-type reaction–diffusion systems defined on a spatial domain where physiological correlation and spatial correlation between cells in the spatial domain is achieved because of the existence of gap junctions through which the Turing morphogens can diffuse. In general, Child (1911b) states: “The apparent equipotentiality in some eggs and in some of the lower organisms is undoubtedly merely apparent, in so far as these forms are capable of development or regulation. As in the case of Planaria, it will disappear as soon as isolation of parts and analysis of results is extended sufficiently far”.

Child (1911b) contains some very good discussions of his general philosophy of the problem of ‘form regulation’. In particular, we note: “In the interest aroused by the remarkable capacity of
planarians as well as various other simple forms for reconstitution of wholes from parts, but little attention has been paid to the factors which limit this capacity. As a matter of fact the limiting and determining factors are perhaps of even greater interest and importance than the great capacity for regulation, for it is these factors which enable us to learn something of the character and relations of the processes involved. The existence of such limits of regulation is totally ignored by Driesch in his definition of the equipotential system. It is certainly not true, either for Planaria or for any other form that any part is capable of producing any other part, or that it is capable of producing a whole. Only pieces of a certain character possess such capacities, and we are already able to determine some of the factors which go to make up this character” (Child, 1911b). This paper also contains a good discussion on why understanding the development of isolated pieces from adult organisms is informative for the understanding of both asexual and sexual reproduction in nature. Indeed, the interplay of ‘size’ and ‘Turing wavelength’ governs all three forms of reproduction and development. Child (1911b) explains that to understand the more complex asexual and sexual reproduction, it is advantageous to learn from the simpler reconstitution of pieces because of the possibility of controlling experimentally the size of the reproductive element, the region of the body from which it is taken, the nutritional and physiological condition of the parent and other conditions of development of the isolated reproductive element not easily controllable in sexual reproduction in higher animals. But if we also have in mind the modern interest in ‘regenerative biology’ and ‘therapeutic cloning’, then the findings of Child (1911b) and his other works about the reconstitution of pieces of lower organisms such as hydroids and planarians and their interpretation according to Turing theory, have an additional interest and practical significance.

Later work on planarian reconstitution (Child, 1941) further confirms that in pieces of equal length, the size of the head, the rate of head regeneration, and the length of prepharyngeal region, decrease posteriorly to the fission zone, or in the species without posterior zooid, to the posterior end or as far posteriorly as head regeneration occurs. One can get graded series homologous to those shown in Figs. 17–19, but instead of moving posteriorly along the principal axis of the Planaria, and thus obtaining pieces of equal length from different levels with different metabolic rate during reconstitution, a piece is cut from the same level, such as the piece Z in Fig. 20, but is then subject to reconstitution in a solution of increasing concentration of inhibiting agents or increasing intensity of other inhibiting conditions; examples of these include alcohol, ether and other anaesthetics and toxic agents, products of metabolism (CO$_2$ in particular), KCN, low temperature and insufficient nutrition (Child, 1911c, 1941). An example of a homologous series thus obtained is shown in (B)–(D) in Fig. 20. It is seen that the distance between the regenerated planarian head and pharynx, that is, the scale of organisation of the prepharyngeal regions, as well as the scale of organisation of the pharyngeal regions and the size of the head, decrease with increasing inhibition of the metabolism. In extreme cases no pharynx develops, and there is no reorganisation of a prepharyngeal region—see Fig. 69 in Child (1915). On the other hand, “Reconstitution at higher temperatures (26–28°C) is more rapid; heads are larger; and scale of organisation of prepharyngeal and pharyngeal regions is increased” (Child, 1941)—see Fig. 70 in Child (1915). “The metabolic gradient associated with the new head shows a corresponding decrease or increase in length in such pieces. The influence of the new head-region extends to a greater or less distance according as its metabolic rate is high or low, and the position of the various organs is altered correspondingly, or, as in the extreme case of Fig. 69, no new organs are
formed except the head. When the metabolic rate is high, as in Figs. 66 and 70, dominance extends nearly or quite to the basal end of the piece” (Child, 1915, p. 140). But in inhibited forms like (C) and (D) of Fig. 20, “most of the piece represents one or more posterior zooids, and fission often occurs anterior to the middle [and not posterior to the middle as is shown in Fig. 44 of Child (1915) which is the situation for uninhibited forms]; that is, the dominance of the inhibited head does not extend over the whole length of the piece, often not over the anterior half” (Child, 1941). The reversibility of inhibition is of interest. When pieces reconstituting under inhibiting conditions

Fig. 20. (A)–(G) scale of organisation in reconstitution of Dugesia (= Euplanaria) dorotocephala. (A) outline, indicating body-levels of pieces X, Y, Z; (B) reconstitution of piece Z in normal environment; (C), (D), decrease in scale of organisation of preoral region of Z-pieces under inhibiting conditions. (E)–(G) physiological inhibition of posterior development in pieces shorter than scale of organisation; (E) head + X; (F) and (G), X or Y. (H–J). Regulation in ether followed by return to water in Planaria dorotocephala. Postpharyngeal pieces (piece Z in (A)) regulate in extreme cases to the type shown in (H) lacking pharynx and auricles and with a single eye only. Upon return to water, during the first 4 or 5 days the head region enlarges and a pharynx appears near the anterior end of the piece (I). After 8 days in water, the pieces resemble J, having 3 eyes (from Child, 1911c (by permission of John Wiley & Sons, Inc.), 1941 (by permission of The University of Chicago Press)).
(Fig. 20(C)), e.g. in the presence of anaesthetics, “are returned to water the development of the head proceeds, it becomes larger, the prepharyngeal region becomes longer…” (Child, 1911c). This can be visualised in the transition, Fig. 12 → Fig. 14, in Child (1911c).

It is important not to confuse the increased size of a larger individual compared with a smaller individual in the context of form regulation in development (or reconstitution)—and which is ‘compensated’ by an increased Turing wavelength, thus resulting in the preservation of proportion—with the increase in size in the development (or reconstitution) of any single individual, which involves one fixed Turing wavelength and is characterised by the sequence, (b) → (c) → (d) → (e) → … in Fig. 1. This sequence of patterns, which is the hallmark of a Turing system, was indeed found experimentally for all organisms by the sequence of Child’s physiological patterns obtained upon size increase in development, as discussed in Section 2.1.

Below, we consider homologous experiments involving hydroids, Planaria and echinoderms and the interplay between size and metabolism-dependent scale of organisation (Turing wavelength), demonstrating the unified theory that explains whether a whole or a partial form arises. Driesch and Morgan found a century ago that “short transverse pieces of Tubularia will develop into certain partial forms (unipolar or bipolar partial hydranths) and that the production of such forms is dependent upon the length of piece and level of the stem from which the piece was taken. The frequency of these partial forms has been found, in general, to be greater in pieces from the distal than the proximal regions. Also, pieces from the more proximal regions of the stem must be shorter than more distal pieces to produce partial forms” (Rulon and Child, 1937). “In pieces of a given length the scale of organisation of unipolar partial forms is of course larger than that of unipolar wholes. Bipolar partial forms possess the distal parts of a hydranth at each end and other parts in the usual sequence from each end so far as scale of organisation and length of piece permit. In pieces of a given length the scale of organisation of bipolar partial forms is larger than that of bipolar wholes” (Child, 1931). Pieces characterised by higher metabolism, whether because they originate from more distal levels, or are in “good conditions”, or because of higher temperature, manifest an increase in the length of the hydranth primordium, which is a measure of the scale of organisation. Of particular interest is the decrease in primordium length with inhibiting agents, anaesthetics and KCN (Child, 1915, 1931, 1941)—see Fig. 21. But it is also the case that “the higher concentrations of KCN which give the greatest decreases in primordium length also give the greatest increases in frequency of wholes. The 2–3 mm piece which develops as a partial form when the scale of organisation is large develops as a whole when the scale is sufficiently decreased, so that hydranth, stalk, and stem are all localised within the length of the piece. The data on primordium length and on frequency of wholes supplement and confirm each other” (Child, 1931). This parallelism between the data on primordium length and the data on increase in frequency of wholes is explained by KCN being an effective inhibitor of the respiratory metabolism and the resulting lower production of the ‘Turing inhibitor’ ATP, leading to a reduction in the Turing wavelength and the scale of organisation.

It is worth noting the general philosophy of Child with respect to the value of reconstitution of pieces as providing different starting points (expressed in the context of planarian reconstitution but of general validity): “In these pieces it is possible to determine the differential effects not only of external factors but also of certain physiological conditions. Pieces of different lengths from different body-levels, from individuals of different physiological age, nutritive conditions, previous conditioning, etc. provide somewhat different starting-points for physiological analysis.”
The effect of varying the ‘starting-point’ from the point of view of the length of the piece, in the reconstitution of Tubularia, is of particular interest to us. Just as it was found for Planaria that “with decreasing size the piece gradually loses its capacity to form a whole and forms a part instead” (Child, 1911b), so it is the case that “as the length of the piece cut from the stem of Tubularia decreases the parts to which the piece gives rise become more and more exclusively distal: the longer pieces produce hydranths and stems, somewhat shorter produce hydranths alone, still shorter only manubrium and distal tentacles and so on, until finally the shortest pieces give rise only to the distal region of the manubrium with the distal tentacles [visualised in Fig. 22]. These facts demonstrate that the distal region is able to form without correlation with other parts, but the more proximal regions have never been seen to arise except in connection with considerable regions distal to them” (Child, 1911c). In general “all the longer pieces developed complete hydranth primordia” (Child, 1931). Also for reconstitution in seawater “the 10–12 mm and 6–7 mm pieces always gave rise to wholes, that is, the length of piece was always greater than that of the hydranth primordia and stalks so that some part of the piece remained as stem... The 4–5 mm pieces very rarely gave rise to partial forms... In the pieces 2–3 mm in length, however, ... partial forms appeared much more frequently” (Child, 1931).
Correspondingly, it was found that the effect of the experimental conditions (KCN) on the form frequencies in the longer pieces were absent or very slight but in the shorter pieces (2–3 mm in length) the occurrence of wholes was much more frequent in the experimental lots than in the controls (sea-water). These results can be explained by the interplay of length and Turing wavelength as expected from Turing theory: In the longer pieces, wholes could be obtained in the controls since the piece was long enough to accommodate even the longer Turing wavelength of the controls, but the 2–3 mm pieces were too short to accommodate the longer Turing wavelength in sea-water and yielded partial forms. However, with the experimental reduction of the Turing wavelength, these smaller pieces too could be reconstituted into wholes. These experiments together with Turing theory provide the answer to Child’s (1908) question as to “why do partial structures of relatively large size occur in some pieces, while others of the same length give rise to wholes of smaller size?” It depends on the relative length of the piece and the Turing wavelength.

The experimental conditions decrease the length of the primordium in pieces of all lengths used. But only in the shortest pieces of 2–3 mm does it occur with an increase in the frequency of wholes. For pieces of all lengths the decrease in the length of the hydranth primordium under the experimental conditions alters the scale of organisation, and the parts of the primordium are

Fig. 22. Apical partial forms of Tubularia or Corymorpha from pieces of different length. Degree of wholeness of polar forms decreases along the series, A → B → C → D. For bipolar forms, degree of wholeness decreases along the series, E → F → G → H → I (from Child, 1941, by permission of The University of Chicago Press).
differently localised with respect to the cut end from the parts of the control primordium. The primordium does not develop by growth in size and the differentiation of embryonic tissue arising from the cut end, but by the transformation of a region of the stem without outgrowth. “The fact that in the partial forms the more distal parts of the hydranth, even the hypostome alone, develop normally in the complete absence of other parts, so far as the length of piece and scale of organisation permit, shows that localisation and determination of parts in hydranth development take place from the apical region basipetally. The region which develops as apical end is the primary dominant region in development and is determined independently of other parts” (Child, 1931). Here Child deals with yet another fundamental question he posed in Child (1908): “Why do partial structures always represent the distal portions of hydranth, never the proximals?” Our combined Child–Turing approach explains this in a particular intuitive manner: structure starts at the apical region because there, at the high point of the metabolic gradient, energy needed for the formation of structure is first released. Structures are then formed from the apical region basipetally since energy is released in succession as we move basipetally down the metabolic gradient. This process continues as far as the length of the piece permits. If it is too short some parts will be missing. For a smaller scale of organisation (Turing wavelength) more parts will be included in a piece of a given length.

Similar considerations and experimental results apply in the reconstitution of other hydroids and the “development in the partial forms includes as much of the hydranth from the apical end basipetally as scale of organisation and available length permit”. (Child, 1941). For Corymorpha, for example, Child says: “Among short pieces of a given length a less active piece may give rise, for example, to a complete individual or to complete bipolar hydranths, while a more active piece of the same length may develop only a hydranth without stem or base, or bipolar manubria because with the higher levels of activity the gradient is longer and the localisation of parts takes place on a larger scale” (Child, 1926b). Or: “Thirds or quarters of pieces, 1 mm or less in length from Corymorpha stems, develop into small unipolar or bipolar partial hydranths or into extremely small complete individuals if slightly inhibiting conditions determine a small scale of organisation” (Child, 1946).

In both Corymorpha and Tubularia, primordium determination very often occurs from both cut ends of a piece, and bipolar forms result. The development of each axis is determined by the scale of organisation and the portion of the piece occupied by the other axis. Each axis is complete from its apical end basipetally, as far as determination and development extend. Thus short pieces of the same length can give unipolar and bipolar partial forms and complete individuals depending on the scale of organisation, which may be affected by metabolically inhibiting conditions, and also by an activation at the proximal end following section which may be sufficient to shorten the gradient at the distal end where hydranth is forming and thus decrease the scale of organisation. On the other hand, one can increase the scale of organisation in a short piece by inhibiting development at one end, e.g. by closing the end with paraffin, sticking it in sand, by contact with the glass of the container, or by differential oxygen supply. Driesch regarded a decrease in the length of the distal hydranth primordium in pieces below a certain length, occurring in correlation with a decrease in the length of the piece, as strong evidence in support of his vitalistic conception. So, it is of interest to note that Child (1931) explains this decrease in the primordium length as resulting from the effect of the proximal cut end on development at the distal end. All these experimental facts (Child, 1931, 1941) are explained by the Turing (cAMP, ATP) theory and by the basic Gierer-Meinhardt...
computer experiments when activator = cAMP and inhibitor = ATP. The range of forms obtained from short pieces of *Corymorpha* of the same length is shown in Fig. 23.

The interplay between the length and the Turing wavelength can also explain whether a reconstituting piece of planarian tissue results in a partial form or a whole. Indeed the data and interpretation for *Tubularia* (Child, 1931) and *Corymorpha* (Child, 1926b) are analogous to those obtained in *Planaria* where “short transverse pieces from the anterior region of the first zoid of *Euplanaria* may develop a perfectly normal head but lack entirely, or develop to only a limited degree, a posterior end or tail [See Fig. 20(E)–(G)]. These forms can aptly be compared to certain partial forms which develop from the animal portion of an echinoderm egg.” (Rulon and Child, 1937.) This is explained by Rulon and Child (1937) within a generalised theory: “It seems probable from the data that the situation in *Euplanaria* is to be interpreted much the same way that Child (1926) [here, 1926b] interprets his data on *Corymorpha*. In other words, short pieces from the anterior region are from a region of high [metabolic] activity [see Fig. 16], and, because of this activity, reorganisation takes place on a larger scale. Since the size of the piece is limited, the only part of the individual forming is that part which is the most active in the complete animal”. In this research on the effect of the length and the level of a piece on the reconstitution of partial forms in *Euplanaria*, Rulon and Child (1937) considered short anterior pieces from levels indicated in Fig. 20: X pieces—anterior transverse pieces, one-sixteenth of the length of the animal, from the region immediately posterior to the head; Y-pieces—pieces of the same length but immediately posterior to X one-sixteenth level; X + Y pieces—anterior transverse pieces, one-eighth the length of the animal and comprising the same region as X and Y. It is found (Rulon
and Child, 1937; Child, 1941) that X pieces reconstitute into a relatively high percentage of tailless and near-tailless forms, while pieces of the same length from the Y level reach a lower degree of taillessness. Typically, posterior development is absent or inhibited in 64 per cent of X-pieces and 30 per cent of Y-pieces. We explain these results by the X-pieces reconstituting with higher metabolism than the Y-pieces and therefore with larger Turing wavelength, resulting in a larger percentage of partial forms, for which, to use Child’s language, ‘the scale of organization is too large in relation to the length of the piece’. It was also found that 100 per cent of the X + Y pieces reconstituted into normal wholes with no posterior development absent or inhibited. This is explained by the greater length, which allows all parts to be localised within the available length, even though the “scale of organization” (the Turing wavelength) of these anterior pieces is large and comparable to that of the X-pieces and Y-pieces.

When some of the X and Y pieces were exposed to KCN for a time after section before being returned to water for reconstitution, the percentage of the partial forms, the tailless and near-tailless forms, decreased: posterior development is absent or inhibited in 71 per cent of the X-controls (water) and in 41 per cent after KCN exposure. In Y-controls it is absent or inhibited in 31 per cent, and after KCN is absent in 21 per cent. These results recapitulate the experiments with KCN in *Tubularia* (Child, 1931) discussed above. By lowering the synthesis of ATP, and therefore reducing the Turing wavelength and the scale of organisation in reconstitution, it was possible to increase the percentage of ‘wholes’ with tail and pharynx present, even with these short pieces.

The increase in the frequency of posterior development after KCN is accompanied by a slight decrease in head frequency in the X-pieces and a marked decrease in head frequency in the Y-pieces (Rulon and Child, 1937; Child, 1941). This inhibition of head development together with the decrease in the scale of organisation, resulting in a higher proportion of ‘wholes’ in the reconstitution of these short anterior pieces, parallels the inhibition of head development and the decrease of the scale of organisation of the prepharyngeal and pharyngeal regions under inhibiting conditions in the reconstitution of long posterior pieces (Fig. 20(A)–(D)).

It is emphasised in Rulon and Child (1937) and in many places in Child (1941) that this theory for hydroids and *Planaria* is more general and holds for other animals, for example for annelids and echinoderms. Indeed the sea urchin provides a particularly interesting analogy. The basal (vegetal) half of the echinoderm embryo can gastrulate and form all tissues, so it can reconstitute into complete or nearly complete larva; it can ‘regulate’ towards a normal larva so as to produce more structures than it would within a whole embryo. (Wall, 1990; Child, 1941). Again the explanation is that it corresponds to lower metabolism and consequently to a smaller scale of organisation (Child, 1941). But an apical (animal) half cannot gastrulate and is arrested as blastulae; it produces less than it would within a whole embryo (Wall, 1990). In the words of Child (1941): “The scale of organisation determined in water is apparently too large for the apical half”. So Child (1941) says that just like pieces of *Tubularia* and *Corymorpha* that give “rise to apical partial forms in well-aerated water may develop as complete individuals after certain degrees of inhibition”, so too for the sea urchin the use of “slightly inhibiting conditions producing lower levels of metabolism and consequently smaller scale of organisation” will turn the apical half into a whole individual. This was proved in Hörsstadius (1953). Child argues that within the smaller scale of organisation the lower level of the primary gradient will decrease the dominance of the apical region of the apical half, and this will allow a secondary activation in the basal region of the apical half, resulting in mesenchyme formation and gastrulation (indeed this secondary activation
will provide the localised release of energy necessary to drive gastrulation) much as in normal individual. The proof of this in Hörstadius (1953) is particularly interesting since it is done with dinitrophenol (DNP) replacing KCN in Tubularia (Child, 1931) and Planaria (Rulon and Child, 1937), but yielding analogous results.

We obtain a decrease of scale of organisation here, “so that a piece of a given length develops a larger part, or a whole, of the polar pattern, instead of only the apical portion” (Child, 1941). But instead of using cyanide (KCN) which inhibits both respiration and ATP synthesis, an uncoupler (DNP) is used which allows respiration to continue without ATP synthesis. This is yet another direct proof that ATP is the ‘Turing inhibitor’ as well as a further strong support for our universal conceptual framework, in particular that a decrease in the synthesis of ATP (the Turing inhibitor) decreases the Turing wavelength (scale of organisation). And this in turn can result in ‘whole individual’ in a piece that is too small for a complete individual without the uncoupler. It is interesting to note that lithium can also yield a complete individual when applied to the apical half of the sea-urchin embryo. Child (1941) explains this by arguing that lithium reduces the scale of organisation. But lithium too hinders the formation of ATP (Lallier, 1964).

It is worthwhile to note explicitly how the experimental results of Child and HörstADIUS on sea urchins correspond to the above general Child–Turing conceptual framework. Hörstadius (1952) isolated the vegetal and animal halves in the early cleavage stages (the 16-cell stage). But in the early cleavage and blastula stages the animal region has a stronger reducing activity than the vegetal region; see the animal-vegetal reduction gradient in Figs. 44 and 45 in Child (1941) or Fig. 17 in Gustafson (1965). This is confirmed by Bäckström (1959) and others (Lallier, 1975). Therefore we expect that the isolated animal half will manifest a higher metabolism and consequently a larger scale of organisation (Turing wavelength) than the vegetal half. Indeed, some vegetal halves manifest a bipolar reduction pattern (see Fig. 2(B) in Hörstadius (1952) corresponding to Fig. 1(f), which is the expression of a smaller Turing wavelength), whereas the animal halves never manifest a bipolar reduction pattern but only a polar reduction pattern as shown in Fig. 3 of Hörstadius (1952), corresponding to Fig. 1(c). It is this lower metabolism responsible for a smaller Turing wavelength that results in a complete or nearly complete individual from the vegetal half but not from the animal half. In order to get a ‘complete individual’ from the animal half we have to use a chemical such as DNP as discussed above in order to reduce the Turing wavelength and thus to reduce the scale of organisation in the animal half. Note that Driesch’s footnote (1929) highlighted above, namely his difficulty to obtain a whole larvae from the animal half when the section is done at the blastula stage, is also explained by the higher metabolism in the animal half.

The sea urchin provides a good example of the interplay between size (length) and Turing wavelength (scale of organisation). In normal development, it is the increase in length that is responsible for the transition, polar → bipolar reduction pattern, as in (c)→(d) in Fig. 1, a transition which is expected from Turing theory. This expectation is fulfilled for the reduction pattern of sea urchin; see the transition from Figs. 44 and 45 to Fig. 46 in Child (1941) or to Fig. 1 in Hörstadius (1952). But if the size that is available is not sufficient, then even at the same stage (time) after fertilisation, the normal bipolar reduction pattern will be absent and replaced by a polar pattern, corresponding to the transition, (d)→(c), in Fig. 1 and to Turing theory. And indeed the animal halves, which naturally are shorter than the entire embryo, and many (but not all) vegetal halves, manifest only one single reduction gradient, i.e. a polar reduction pattern, as in
Fig. 2, A₁–A₅, Fig. 3 and Fig. 6 in Hörsstadius (1952). But if one increases the size of the animal half and stimulates it by implantation of micromeres, the basic Turing–Gierer–Meinhardt model will predict the possibility of a bipolar reduction pattern, which is indeed observed; see Fig. 5 in Hörsstadius (1952). This reduction pattern resembles that of a normal egg as in Fig. 1 of Hörsstadius (1952). Thus the implantation of micromeres into animal halves results in both a normal reduction pattern and in whole larva (Hörsstadius, 1952), and the former is responsible for the latter.

It is significant that the different situations described in Hörsstadius (1952) in his Figs. 1, 2(B) and 5—all of them resulting in complete or nearly complete animals—also have in common that in their later stages, i.e. in the advanced blastula and during gastrulation, they also manifest a (similar) bipolar reduction pattern. By contrast, when these later stages manifest only a polar reduction pattern as in Fig. 2, A₁–A₅, Fig. 3 and Fig. 6 of Hörsstadius (1952), no normal complete animal is obtained. This constitute a further proof that succession of a bipolar Turing pattern after a polar pattern is required for normal development and that the later bipolar pattern does not jeopardise the polarity of the final product, contradicting Gierer–Meinhardt opinion as discussed above. In fact the succeeding bipolar pattern is responsible for the occurrence of gastrulation. In the words of Hörsstadius (1975): “In animal halves which never gastrulate there is logically no vegetal reduction centre. Instead, reduction begins at the animal pole and continues around the blastula”. This corresponds to our general theory that the invagination involved in gastrulation requires a localised release of energy to power the localised morphogenetic movement.

We saw above that the application of an uncoupler to an isolated animal half can result in reduction of the Turing wavelength and therefore in a whole individual. If an uncoupler is applied to the entire egg we can expect that the reduction of the Turing wavelength for this longer system will result in a bipolar pattern, corresponding to (f) rather than (c) in Fig. 1. This means that we skip the polar metabolic pattern which occurs in normal early development (Figs. 44 and 45 in Child, 1941). The situation is then like that in Fig. 6 for Corymorpha where the metabolic bipolarity resulted in Janus heads heteromorphoses. Indeed, the application by Lindahl (1936) of the uncoupler NaSCN before fertilisation, with subsequent fertilisation and development in natural sea water, can yield a bipolar form with an animal centre at both the animal pole and the vegetal pole (Fig. 24). Hörsstadius (1973) notes: “The animalisation did not spread gradually

![Fig. 24. Bipolar sea urchin larvae are obtained by the application of NaSCN before fertilisation, followed by fertilisation and development in sea water (from Lindahl, 1936, by permission of The Royal Swedish Academy of Sciences).](image-url)
vegetal-wards but instead created a new animal centre at the presumptive vegetal pole’. Again our theory not only explains this old experimental result but is a further strong support for our thesis that ATP is the ‘Turing inhibitor’, in particular in view of the fact that it was obtained not with inhibitor of the respiratory activity that also inhibits ATP synthesis, but directly with an uncoupler that inhibits ATP synthesis only.

Child’s explanation of the bipolarity of sea urchin larvae is based on his principles of physiological isolation, dominance and subordination, and is equivalent to our explanation involving the reduction of the Turing wavelength due to reduced ATP synthesis: “thiocyanate inhibits differentially the slight apicobasal gradient present in the unfertilized egg, so that any dominance of higher gradient-levels or any definite relation between parts is almost or quite abolished; that is, in extreme cases axiate pattern is virtually obliterated. After return to water and fertilization, with the accompanying activation, the regions which would have been the lower gradient-levels and would, therefore, have developed as entoderm are more or less physiologically isolated, since they are in the same, or almost the same, physiological condition as more apical levels. Under these conditions they develop as higher gradient-levels. This may be regarded as essentially a reconstitution similar in principle to... reconstitution of apical regions by physically isolated pieces of hydroids and planarians.” (Child, 1941). Child has in mind works such as Watanabe (1935) and Rustia (1925) where another type of manipulation also results in a bipolar metabolic pattern instead of a polar metabolic pattern, which in turn is responsible for the bipolar phenotype in the hydroid or planarian.

Application of lithium to the complete egg results in a single acropetal reduction gradient (Figs. 1 and 3 in Hörstadius, 1955) instead of both acropetal and basipetal gradients for undisturbed development in water (Hörstadius, 1952). This replacement of the normal bipolar pattern as in Fig. 1(d) by a polar pattern as in Fig. 1(g) could be explained by an increase in the Turing wavelength. In Schiffmann (1991) experimental results are given showing that lithium reduces cAMP production. But we saw above that a reduction in the rate of synthesis of the Turing activator results in an increased Turing wavelength. Lithium can affect many biochemical reactions and it may be difficult to know which of its effects is of greater consequence in any particular case. Lithium’s effects often appear opposing and contradictory (Jope, 1999), and therefore all we would like to suggests here is that the effect of lithium on development may be explained by its leading to a modification of the Turing wavelength.

Hörstadius (1955, 1973) has emphasised the “parallelism of morphogenetic and metabolic processes”. That is, for normal development and for all modifications, whether brought about by isolation of animal and vegetal halves or implantation of micromeres (Hörstadius, 1952) or by chemical means for example with lithium and trypsin (Hörstadius, 1955), there is a parallelism between the spatial metabolic pattern and the spatial pattern of differentiation and morphogenesis. Also, the shift from normality in differentiation and morphogenesis corresponds to the shift in the reduction pattern. This is reminiscent of the strong emphasis on similar parallelism for Corymophra in Watanabe (1935) and Child and Watanabe (1935) discussed above. With respect to these results on Corymophra, Child emphasises that the dye reduction gradient “is established... long before any other evidence of hydranth development appears” (Child, 1946). His recurring phrases such as “the marked increase in rate of oxidation-reduction in the hydranth region preceding morphogenesis” (Child, 1946) or in general: “Gradients appear to precede sharply defined and bounded morphogenetic differentiation” (Child, 1941) convey Child’s
conviction that this parallelism is a reflection of the fact that the metabolic pattern is the cause and driving force of differentiation and morphogenesis.

The parallelism in sea urchin also includes the spatial isomorphism between the gradients in the rate of reduction and the gradients in the rate of RNA and protein synthesis (Gustafson, 1965; Shostak, 1991; Wall, 1990, Lallier, 1964). And as argued in Schiffmann (1997), localised release of energy, as reflected in Child’s gradients, is indeed the primary event for RNA and protein synthesis. Lallier (1975) adopts a similar approach: “The existence of reduction gradients is a particularly interesting phenomenon. Their evolution during development, and their chemically or operatively provoked modifications show that they develop in a direction parallel with the morphogenetic gradients... they reflect, at least for a large part, the mitochondrial activity. [Lallier (1964, 1975) argues that there are no gradients in the number of mitochondria and give other reasons for gradients in mitochondria activity.] These gradients should express the activity of the mitochondrial enzymatic systems which supply, in the form of ATP molecules, the energy necessary for the synthesis of specific proteins. This synthesis would be distributed in gradients corresponding to morphogenetic gradients”.

5. The double-gradient hypothesis

One proposed mechanism by which one end of a developing system becomes a head and the other, a tail, i.e. the mechanism by which polarity is created, is the idea of a (monotonous) gradient first advanced by Boveri: the two poles are opposite ends of a single gradient (Wall, 1990). Morgan (1905) suggested that there is a head-forming substance that grades off from head to tail and a tail-forming substance that grades off from tail to head, as in Fig. 1(a). Later Runnström advanced a similar idea, the ‘double-gradient hypothesis’, according to which two overlapping gradients decreasing from the opposite poles are present from the beginning of development throughout the whole egg. They were first regarded as gradients of concentration of specifically different substances but later they have been discussed in terms of qualitatively different kinds of metabolism (Child, 1941, 1944; Shostak, 1991; Wall, 1990). Lindahl has suggested that the animal gradient is based on carbohydrate metabolism and the vegetal gradient on protein metabolism (Hörstadius, 1952). Child (1941) criticises the double gradient hypothesis but its Swedish proponents went on supporting it well into the seventies. In a later criticism of this hypothesis, Child (1944) argues for the absence of qualitative difference between the two poles in the beginning of development. Furthermore, he points out that in the beginning of development only one gradient is present; the second gradient with the high point in the second pole suddenly (this sudden appearance corresponds to analytical bifurcation theory and to Meinhardt’s (1982) computer experiments) appears only later in development. Furthermore he points out that the gradients do not overlap but in fact oppose each other; if one metabolic gradient is experimentally amplified at the expense of the other, the phenotype in this pole is also amplified at the expense of the phenotype of the second pole. He also points out that the two gradients are qualitatively the same, e.g. “Also, there is a basipetal reduction gradient which has the same characteristics, so far as indophenol, methylene blue, and Janus green are concerned, as the acropetal gradient” (Child, 1944). Lallier (1975) is led to a similar conclusion: “The first hypothesis, made on the
differentiation of the sea urchin egg, implied the existence of different types of metabolism in the animal and vegetal regions. It has not been possible to demonstrate any obvious difference in metabolism up to now, between animal and vegetal blastomeres."

Thus according to Child the poles become different, as is reflected by later downstream different gene expression, not because the two opposing gradients are qualitatively different but because one gradient appears first and the opposing gradient appears only later. If this condition is not fulfilled, for example as we saw above in some experiments on the reconstitution of pieces of Corymormpha palma, the distinction between ‘head’ and ‘tail’ fails: “The course of differential reduction is definitely related to regions where hydranths will develop. In pieces which develop as bipolar forms, reduction occurs first at the two ends of the piece and progresses toward the middle” (Watanabe, 1935). In all cases the details of the reduction gradients dictate the details of the phenotypic development. Thus the pieces that develop hydranths at both distal and proximal ends—and the hydranth at the proximal end develops slightly later than the distal hydranth—will also manifest a reduction gradient that appears at both ends of the piece, but the proximal gradient, which represents a reversal of the normal stem gradient, appears a little later and is a little shorter than the distal gradient (Child and Watanabe, 1935; Watanabe, 1935). This situation corresponds to the explanation of asymmetrical bicaudal embryos when one gradient dominates the opposing gradient (Fig. 6 in Nusselin-Volhard, 1979). The pieces that develop as phenotypically polar also manifest the close correlation with the reduction gradients; hydranth development can occur in the distal end or in the proximal end but it is always the end where the hydranth will develop that will manifest a reduction gradient before hydranth development, the other end will only later manifest a small reduction gradient (Child and Watanabe, 1935; Watanabe, 1935). The details of the phenotype trace the details of the metabolic gradients so closely because the latter are the primary cause of the former.

The correlation between the succession of reduction gradients and hydranth development in reconstitution, in normal and in modified development, is typical of all other biological systems and applies whether the modification is brought about by chemical means (Shostak, 1991; Wall, 1990) or genetically such as in Akiyama and Okada (1992).

The final conclusion is that a double-gradient hypothesis is correct but more in the sense of Child (1944) than in the original sense of Runnström. Runnström’s approach is akin to Meinhardt’s as discussed in the introduction, where each of the opposite poles has a different Turing system and the Turing morphogens of the different systems are localised in the different poles. Fig. 1(a) can represent the conceptions of both Runnström and Meinhardt characterised by a static view but with two systems: two different substances or types of metabolism and two different Turing systems, respectively. But in real biological systems there is only one Turing system that manifests one type of metabolism and with successive prepatterns in time. Spatial diversity is obtained by virtue of this succession in time and not by virtue of many different systems existing at the same moment. The ideas of the ‘double-gradient hypothesis’ about balance, competition and opposition between the gradients (Shostak, 1991; Wall, 1990) are essentially correct if we keep in mind that the two opposing gradients are of the same type—the type expressed in the various manifestations of Child’s ‘physiological gradient’. The ‘double-gradients hypothesis’ is still invoked today, e.g. in Angerer and Angerer (1999), but it was originally conceived in terms of primary events whereas the gradients considered in Angerer and Angerer (1999) are not primary events according to our theory.
6. Conclusion

Three observations are immediately obvious and striking from the large volume of work of Child and colleagues. The first is that the elaboration of a spatial pattern is invariably preceded by a spatially isomorphic physiological pattern. The second is that the complication of the physiological pattern, for example the later appearance of a second opposing gradient and the tentacle primordia, is driven by an increase in size and length. Thus we immediately know that in all cases the physiological spatial patterns are created by a chemical Turing system even before we identify the Turing morphogens. That Child’s physiological pattern was discovered a century ago, while for the last half a century there has been a very intensive search to identify the Turing pattern (the biological dissipative structure) without realising that Child’s physiological pattern is the elusive dissipative structure, is extraordinary from the point of view of the history of science. It is particularly so in view of the fact that many have recognised that to explain the ‘form’, the macroscopic structure, the phenotype, the molecular-genetic level is not enough. For example, Sir Vincent Wigglesworth acknowledged that “‘to say… the body form is controlled by the genes is hardly more illuminating scientifically than to say that it is controlled by God’. It is evident, however, that, if the theory of dissipative structures is universally valid, it can conceivably fill in all the blanks in our understanding in this area. Thus it should be the focus of most contemporary research in such disciplines as embryology and regeneration studies” (Scott and McMillin, 1980).

The normal succession of physiological patterns is the source of spatial diversity and there is no great effort on the part of the organism to maintain the polar monotonic Turing pattern upon size increase. Third, reconstitution and normal development are driven by spatially similar physiological patterns. In fact asexual reproduction is also driven by the Turing–Child physiological patterns.

It is important to bear in mind in the context of modern ‘regenerative biology’, tissue restoration and engineering, that if in ‘artificial’ reconstitution we simulate ‘normal’ development, namely if we create the same physiological spatial patterns and the same succession in time of these patterns, then we could reconstruct ‘normal’ development from ‘pieces’. It is best to quote here from Child (1926a):

“The similarity of the gradients in the earlier stages of embryonic development and of reconstitution is of interest as indicating that reconstitution is essentially the same developmental process and follows the same general laws as embryonic development, but takes place under somewhat different conditions. In both embryonic development and reconstitution a single gradient appears first, corresponding to the polar axis in direction. This gradient becomes the hydranth stem gradient. Then in the embryo and in pieces which give rise to complete individuals a secondary gradient opposite in direction to the first appears in the region which becomes basal and the various later modifications and complications are the same in both.”

This quotation should be read while comparing the evolution of physiological patterns in the developmental stages of Corymorpha as shown in Figs. 2–9 in Child (1926a) with the evolution of physiological patterns in the stages of reconstitution of pieces as shown in Figs. 10 and 11 in Child (1926a). The parallelism between the ‘reconstitution’ and ‘development’ is evident, including the spatially periodic metabolic activity along a ring that precedes the appearance of the tentacles.

The ability to understand a phenocopy, i.e. a morphological change that is not inherited and is brought about by chemical or physical agents, that copies a change in a phenotype brought about
by a genetic change that is inherited, could be of importance in the understanding the roles of genes; a timely goal in view of the rapidly accumulating data on the sequences and structures of nucleic acids and proteins. For example, an increase of temperature was found to decrease the number of facets in the eyes of flies such as *Drosophila*, which imitates the effect of genetic mutations such as *bar*. Similarly, higher temperature decreases the number of bristles, imitating the effect of genetic mutations of, for example, the fruit-fly gene *dichaete* (Needham, 1966). But we saw that higher temperature could result in a larger Turing wavelength, which can explain why higher temperature reduces the number of facets and bristles. We expect the relevant genetic mutations also to increase the Turing wavelength. The unifying power of the Child–Turing explanation is also seen in the fact that a bipolar phenotype can result from inheritable genetic mutations or from chemical and physical alterations, but in both cases a polar metabolic pattern is replaced by a bipolar metabolic pattern, as we saw for example for *Drosophila* (Akiyama and Okada, 1992) and *Corymorpha* (Watanabe, 1935). We also saw that over-crowding and starvation of *Drosophila*, which are not inherited factors, can result in microflies, also obtainable with genetic mutations. The Turing–Child explanation for form regulation highlights the equivalence of genetic changes and environmental changes in form regulation.

The Child–Turing periodic prepattern is the basis of all segmentation. Consider, for example, vertebrate segmentation. Successive physiological isolations resulting from the elongation of the posterior end of the presomitic mesoderm (PSM) results in the continuous formation of spatially periodic reduction centers in the chick PSM (Spratt, 1958). The existence of gap junctional communication between cells in the PSM has already been verified for the amphibian embryo (Warner, 1986), meaning that the Turing morphogens are able to diffuse along the PSM. Form regulation as explicated here explains why individuals of different size within the same species will tend to have the same number of somites.

Finally, one cannot but marvel at the vitalistic cleverness that has seen to it that, whether in reconstitution or development, it is the same factor (energy metabolism) that is responsible for size and the Turing wavelength, thereby securing proportion regulation. The ability to modulate the scale of organisation via the tuning of the level of metabolism may well be useful in the context of modern ‘regenerative biology’.

**References**


