

## Contribution to the whole (H). Can squids show us anything that we did not know already?

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**Abstract.** For a multicellular organism to proceed from egg to adult it must: (i) undergo cell division, (ii) differentiate, (iii) remain a unified whole ( $H_0$ ). These requirements are at right angles to each other. The first two are achieved through hierarchical processes (vertical control) that are relatively well understood, the third through non-hierarchical processes (horizontal control) physiological evidence for which is abundant, though not widely recognized as a form of control. The essay gives an example of a tissue – the skin of a living squid – whose horizontal network properties come to light when nervous (vertical) control is removed. It offers the name *homeotaxy* or ‘peer conformity’ for the general principle (allied to the community effect, Gurdon 1988) that constrains the parts of the whole to be in the same state within any given layer of the network – where layers correspond to ontogenetic stages in the development of the tissue – and discusses the question of a need and a name for this principle in Biology.

Savouring of an intangible ‘holism’ and of Driesch’s dubious ‘entelechy’, the old question of how it is that the organism behaves as a whole, and not just as a collection of parts, can not be said to be of major concern to today’s biologists. One obstacle is that holistic concepts are notoriously difficult to formulate; the words and concepts available for dealing with wholes are perceived as inadequate; we seem to have advanced little beyond the broad statements of the organismal and *Gestalt* schools. Another is the sheer success of reductionist methodology allied to molecular technologies. While this essay is essentially about the first of these problems, it is heavily influenced by the existence of the second.

As a working ‘whole animal’ biologist, I see it as a question of *controls*, and seek a pragmatic way round the problem by asserting, on general biological grounds and as far as possible in non-technical language, some simple principles amply demonstrable in living things.

To pose the question about wholes and parts in a dynamic form with reasonable chance of being understood by seminar audiences, I have been asking it in a context with which all students and practitioners in the life sciences should be more or less familiar. *What is it that holds the blastomeres together so that*

*they continue to act as one (egg or organism) after the first division(s) of the oocyte?* and – for reasons which will become evident – have been linking it with a second question *what is the opposite of differentiation?*

The answer given to the first question – for simplicity about the blastomeres of a frog's egg and their implicit loss of individuality subsequent to the first division – tends to be in terms of some specific and more or less well-known mechanism holding them together: desmosomes, adherins, gap junctions (see later) or the follicular envelope surrounding the egg, etc.

There is of course nothing new in this appeal to a mechanism, or process, in order to explain the behaviour of the blastomeres, rather than invoking a general rule, or characteristic, of cellular organisation common to all Metazoa and Metaphyta and to all stages of development. But as Woodger points out in his treatment of the 'Mechanical Explanation' in *Biology* (Woodger 1929: 260) 'an explanation of any phenomenon always involves two factors: general laws and a specified set of entities subject to those laws'. He is quoting the physicist C.D. Broad (Broad 1919). Biology, at the level considered, has rather few 'laws' by comparison with physics. But some it needs, if only to enable one to discriminate empirically between the two – between what is general and what is a specific instance.

### **Origins of multicellular organisms**

From the point of view of phylogeny, the importance of questions about the relationship between the 'unit' of life (the cell) and the whole (H), is the light they can throw on the evolutionary step between one-celled creatures (Protista) and many-celled animals and plants (Metazoa and Metaphyta).

Plants and animals have common mechanisms of cell division (mitosis and meiosis) and these are supposed to have evolved only once. Gerhart and Kirschner (1997) write: 'One development of great importance for future metazoan multicellularity was the loss of the cell wall in some unicellular eukaryote ancestor. The lack of a cell wall ... permitted the ancestors of animal cells to interact directly with each other through apposed plasma membranes, to adhere to each other, to crawl on surfaces, to differentiate into complex shapes, to engulf other cells by phagocytosis, and to engage in junctional communication with other cells. Cell adhesion and junctional communication are characteristics of the formation of epithelia and the segregation of an internal milieu, which are found in all metazoa'. (See Mueller 2003 and other contributions to the same number of *Integrative and Comparative Biology*).

The list of attainments mentioned does not include the extracellular matrix (ECM). The matrix is of special interest for the theme of this essay (Oschman 1984). As it is a continuum of polymer fibres and fibrils, it can act as *integrator*. The matrix's complex of large molecules – principally in metazoa the long-chain triple helix collagen fibres – is secreted by the cell and remains in communication with it through its other components: adhesive glycoproteins

attached to integrins spanning the cell membrane (Ingber 1998). In the form of the basal lamina of epithelia, it mediates movements and transformations of cell type during the embryonic development of animals – from the sponges onwards (Morris 1993) – and provides a 3-D microenvironment that helps define tissue specificity during organogenesis by signalling directly to the nucleus (see Bissell et al. 2003).

Billions of years before the appearance of the extracellular matrix of animals, however, a functionally analogous role in constructing and integrating the whole was performed by the matrix of extracellular polymeric substances (exopolymer *biofilm*) still found in the famous ‘living fossil’ stromatolite communities (Reid et al. 2000) of Southern Australian beaches and the Bahamas.

The biofilm of stromatolites is produced by bacteria that inhabit the topmost layer of the multilayered structure<sup>1</sup>. Here it entraps fine particles when covered by the tide, resulting in a sediment which is further colonised by the bacteria, and thus to further layers and growth of the whole – incidentally rendering distinctions between organism and environment singularly difficult to sustain<sup>2</sup>!

### Wholes and parts

By whole (H) I have in mind an individual clearly bounded in space and time – such as an arrow-worm, a squid, a frog or a human being. By parts, I have in mind the differentiated and differentiating cells of such organism. For the purposes of this essay, and for didactic reasons, the ‘whole (H)’ is also intended as the isolated ‘laboratory’ organism  $H_o$  that is still the object of much research. Such ‘H’ is of course an artefact, not only because many, perhaps most, organisms making up the biosphere are not single individuals clearly bounded in space and time. More importantly, as just illustrated, no individual organism is really separable from the community, the environment or other network of relationships constituting the wider whole ( $H_w$ ) – whether or not it be treated as such in the laboratory.

The way in which this multicellular organism proceeds in time from egg to adult is highly *predictable*. It is alive, and whatever else we may mean by this dynamic state it presupposes intrinsic controls which non-living things do not have. The controls contained in the genome unfold as epigenetic instructions expressed in the processes of cell multiplication and differentiation. Thus the

<sup>1</sup> See below, ‘*quorum sensing*’ by the microbial communities of everyday infections.

<sup>2</sup> ‘Cyanobacteria are the primary producers in this system providing energy, directly or indirectly, for the entire stromatolite microbial community. ... Most of these species are highly motile and can adjust their position and orientation within the sediment matrix in order to optimize their access to irradiance and nutrients. As individual species have different physical and metabolic properties, this motility generally results in segregated distributions of species, which in turn contributes to the laminated textures observed’ (Bebout et al. 2001).

question posed to seminar audiences could be answered by stating that it is epigenesis, and the continuity of the genome from cell to cell through mitosis, that holds the organism together over time as one whole ( $H_0$ ). The answer, in other words, is implicit in the description of the processes of embryogenesis and of differentiation.

For various reasons this will not suffice, and not just because the description of developmental processes is still far from complete or because it leaves out explicit reference to reciprocal relationships between organism and environment.

The question has arisen in a very practical way during my own work on the remarkable *colours* and *colour changes of squids and octopuses* – which are such an important part of their behavioural repertoire and widely known to be under the control of eyes and brain. If a piece of skin is taken from a squid or a cuttlefish (habitually from an individual that is already fully differentiated, and without much regard to its embryology or the ontogenetic dimension) and is placed under the microscope, colours are seen to come and go as tension waxes and wanes in the muscle fibres surrounding its many tiny pigment spots. The optical signal generated by expansion and retraction of a pigment spot were harnessed by A.V. Hill and his collaborator to record the shape of the mechanical response – or *myogram* – (Hill and Solandt 1935) and led the comparative physiologist Ernst Florey (1966) to write of such things as brown twitches and red tetani.

Efforts to understand the colour patterns of these cephalopod animals by studies at the cellular (rather than at the behavioural) level immediately encounter two additional problems, however. First, the elements to which the colours are reducible are not single cells but chromatophore organs: each constructed from several different cell types. The many muscle fibres belonging to any one of these organs exhibit what is called *myogenicity*; they mostly act synchronously because they are intimately coupled<sup>3</sup>, and many chromatophores act simultaneously with other chromatophore organs (whether or not they are under the command of nerves, see below) as if they too were coupled in some way. Second, in isolation or in preparations removed from their original location, the chromatophore *ensemble* does not behave physiologically as it does in the whole squid or octopus. Separation of the piece of skin (or of a piece of gut, see footnote 2) from its whole squid context, damages the network linking the organs and interferes with its rhythms.

On the other hand, attempts to study the intrinsic activity of these *coupled ensembles*, and the contribution that the coupling makes to normal activity in the *intact* animal ( $H_0$ ), meet with other kinds of difficulty. In the squid, the

<sup>3</sup> One early account of electrical coupling between cells was Florey's and Kriebel's (1969) description of junctions between neighbouring muscle fibres on the squid chromatophore. The squid embryo was another (Potter et al. 1966). Familiar examples of coupled ensembles that are 'continuous from within' (Loewenstein, 1981), because the cytoplasm of their cells is linked through gap and other kinds of junctions, are the heart, the gut, the uterus and the bladder (see below).

switching ‘off’ and ‘on’ of colours by nerves and brain during the generation of natural patterns can mask evidence of coupling between cells, evoke it differentially by uncoupling the parts, or can tightly entrain activity, interfering with the underlying rhythms and spontaneity of the ensemble. A classical experimental way round the difficulty is to alter the system by removing only one of its components – in this case removing nervous control from the whole, or a part of it – effectively reducing the individual to ( $H_o-1_n$ ) (see below). Another is to study individuals in which this has occurred naturally or accidentally<sup>4</sup>.

### Two forms of control

In order to proceed from egg to adult as a continuous self-regulating whole, our multicellular organism ( $H_o$ ) must:

1. undergo cell division,
2. differentiate,
3. remain one (organism or individual).

These requirements are of equal status and may be asserted as basic principles. The principles are of cell division and of *differentiation* (here bracketed together) and the principle of *oneness* or unity. The latter does not derive from the former. In analytical terms they are at the same level but scientifically – logically, qualitatively and heuristically – they are very different. They are at right angles to each other. The key to the difference is in the two kinds of control. Control being understood as the set of algorithms fulfilling the two requirements.

The first requirement is fulfilled by processes that are essentially *hierarchical* and can be called ‘vertical’ control (Figure 1). During development it is the series of discontinuous steps in a sequence of causes and effects rich in information and with feedback both between levels, and between organism and environment, that carry it from simple to complex, from undifferentiated to differentiated<sup>5</sup>. Vertical (or hierarchical) control extends beyond embryo- and organo-genesis to physiological control exerted between levels by organ systems that are themselves the product of differentiation – for instance the classical control exerted by the nervous, hormonal and immune systems, etc. The sense of a vertical dimension is reflected in some of the language used to describe their interactions (higher and lower motor control, etc). In the motor system on which this essay is grounded, nerves control muscles both during developmental time (for instance during differentiation of muscle fibre types and during synapse formation, where the control is also reciprocal) and during

<sup>4</sup> For instance in studies of the bladder in hemiplegia, or of the pre-term uterus (which is effectively in a ‘denervated’ condition: see later).

<sup>5</sup> See Davidson et al. 2002 for a recent synthesis of the hierarchical genetic cascades in animal development.

## divide and differentiate

- hierarchical (**vertical**) process
- information-rich
- sequential (cause/effect)
- discontinuous
- feedback between levels in the hierarchy
- simple → complex
- etc.

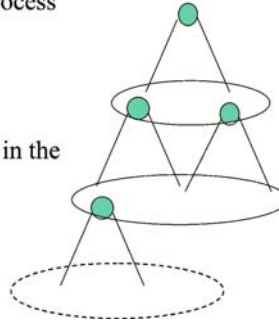


Figure 1.

physiological time as the exercise of nervous commands over the activity of muscle.

The second kind of control – that maintains the physiological oneness of the organism (and fulfils requirement no. 2) – is *non-hierarchical*, is continuous in space and time, is not obviously either cause-and-effect related or information rich; and in ordinary senses of the word it is not generally perceived as a form of control (see Discussion). It is called ‘horizontal’ because, being exerted within levels, it can be thought of as orthogonal to vertical control. As we shall see, within any horizontal level of the conceptual hierarchy – indicated by the ellipses in Figure 2 – the continuity of H maintained in space and time is also a physical continuum.

The ‘organism’ in Figure 2 has a certain polarity (asymmetry), with the head end or animal pole (+) dominating over the tail end or vegetal pole (–). The shaded ellipses are horizontal *continuities of cell type* and of stage of differentiation at two levels along this axis. Within one level, cells are considered, for

## remain **one** (whole organism) = H

- non-hierarchical (**horizontal**) process
- field phenomenon
- information-poor
- continuous in space and time
- conformity within levels

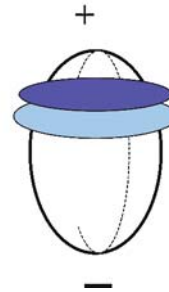


Figure 2.

the purposes of this essay, to be in the same physiological, or dynamic, state. I call this condition *homeotaxy* or *peer conformity*.

### Homeotaxy and horizontal control

To return to the squid and its array of coloured spots. The word *homeotaxy* (meaning 'same arrangement') has been coined to describe the unified state of the array when under 'horizontal control'. It makes no reference to the unifying mechanisms involved.

Horizontal control of the skin in a squid that is fully differentiated asserts itself *pari passu* with removal of the vertical control exercised by the brain and its motor neurones. The condition ( $H_o-l_n$ ) resembles that of a denervated gut or bladder and is achieved experimentally by severing the nerve supplying one side of the animal<sup>6</sup>, whereupon the autonomous activity of different ensembles, lying at lower levels than  $H_o$ , begin to surface<sup>7</sup>. Two days after the operation, a standing wave of myogenic (muscle-generated) darkening shows on the operated side – apparently triggered by some residual influence of nerves (Figure 3). Days later, when the cut nerve has completely degenerated, the picture has changed. Chromatophores remain either quiescent (muscles relaxed, hyperpolarized and therefore minimum colours) or they flip from this baseline condition into an active state *carrying their peers with them*: notably in the form of fast (1 cm/s) waves of coloured twitches that run across the skin in various directions and with broken frequencies, propagating into all denervated regions, but not into neighbouring, intact, skin nor into parts where nerves persist. These phase waves are associated with transient reversing depolarisations. Evidently members of the chromatophore community (ensemble) are now tightly entrained physiologically. Examined closely, there are seen to be several ensembles. Large brown spots, likewise small yellow spots, may all be relaxed, and intermediate-sized red spots all be tonically expanded (muscles contracted) as is the case in Figure 3; fast phase waves may be purely red/brown. From time to time the standing wave of darkening is abolished by a slow wave of relaxation (wave of hyperpolarization?), only to return many minutes later.

The homeotaxy observed in this example has a developmental signature. Each of the different resting-size and colour classes of chromatophore organ is also an *age* class. Each behaves co-operatively as a separate matrix, or network, and sometimes entrains other classes or networks. Put more formally, connectivity between members of a given colour/size/age class of chromatophore is close to

<sup>6</sup> This cuts nerve fibres off from their central cell bodies. Unlike some of the other examples quoted (uterus, bladder, gut), the skin of squids has no independent peripheral network of nerves.

<sup>7</sup> In species less richly innervated than the one illustrated here (*Loligo vulgaris*) autonomous activity of the ensembles occurs without surgical intervention.



*Figure 3. Myogenic control.* The nerve supplying the near side of the body of this squid (*Loligo vulgaris*) was cut two days before the photograph was taken. Contractions of thousands of muscle fibres on the operated side (centre of photograph) are producing a standing wave of darkening amongst certain categories of red spots (chromatophores) just below the surface. Elsewhere, the skin is under nervous control; chromatophores are relaxed and therefore not visible.

100%: i.e. obligatory – whence the name *peer conformity*. Between networks (corresponding to slightly different shades of blue in Figure 2) it is less than 100%. Cooperative activity ceases only when the tissue dies<sup>8</sup>.

The only excuse for taking a liberty with readers of *Biology and Philosophy* to expound this somewhat remote example of a general phenomenon (myogenicity) is its strong illustrative (i.e. heuristic) appeal. Many more familiar examples of muscular structures that behave myogenically temporarily freed from ‘higher’ control are to be found in textbooks of human and of comparative physiology. The beating of the heart, the birth of a baby, the changing size of the pupils, the erection of the penis (Christ 1997), the movements of the gut and bladder, all proceed because the cells making up the organ or tissue concerned are functional syncytia (or coupled ensembles) that generate their own rhythms. But in none of these examples of peripheral *automaticity*<sup>9</sup> can the experimenter see both the small-scale and the large-scale patterns of connectivity within the ensemble with the detail available in Figure 3. None of them combine the spatial and temporal range and resolution supplied by video-recordings of living squids, where every chromatophore is naturally colour-coded and symbolizes a developmental stage

<sup>8</sup> For further details see Packard (2001) and [http://www.gfai.de/www\\_open/perspg/g\\_heinz/bio-model/squids/squids.htm](http://www.gfai.de/www_open/perspg/g_heinz/bio-model/squids/squids.htm). Note that we are not here concerned with the temporal and other characteristics of waves, nor with the pacemaker and triggering conditions. Pacemaker activity is nonetheless a fundamental property of coupled ensembles.

<sup>9</sup> See Bozler (1948) (also Hess 1954) for the usage of the term *automaticity* in this essay.





in the history of the tissue. In other wholes ( $H_o$ ), or modified wholes ( $H_o-l_n$ ), such information is simply missing<sup>10</sup>.

The following paragraphs will help the reader place the above in a wider context.

1. In a machine, homeotaxy is ensured by such things as the conductance and compliance of the materials used, as well as by nuts and bolts and links of various kinds. In living systems it is associated with electrical conduction within layers, with conformational spread (see next bullet), with intercellular communication through *gap junctions* (GJIC) and with intercellular *ligands* and their receptors. Gap junctions, of several molecular forms, exist in all tissues and, when open, provide low resistance channels for transfer of charge, clamping members of the ensemble to a common potential (see Loewenstein 1999) and for passage of molecules between cells permitting their 'metabolic cooperation' (Sheridan and Atkinson 1985).
2. At the macromolecular (and subcellular) level, all of those living processes which depend on a dynamic change in the conformation of allosteric proteins (DNA replication, muscle contraction, energy production, etc.) are now believed to do so through *conformational spread* (CS) (Bray and Duke 2004). CS is the, domino-like, free-energy based propagation of the conformational change in these proteins. It 'coordinates the action of large numbers of proteins in extended complexes', within cells, on their surfaces and presumably also in their surroundings. Cited examples (with assembled evidence) of this fundamental form of homeotaxy, are the coupled gating of ryanodine calcium channels in the endoplasmic reticulum of heart muscle (where the change in allosteric state spreads between protein units or sub-units of the receptor lattice), and the switch from one quaternary conformation to another that propagates along linear polymers to bring about the characteristic bending of a bacterial flagellum<sup>11</sup>.
3. The classical example of automaticity and unified action has always been the vertebrate *heart*, which can beat independently of the vertical controls that modulate heart rate. It is usually regarded as a special case, however, because the membranes of the muscle cells forming its walls are incomplete and constitute a structural (as well as functional) syncytium through which a wave of excitation can spread in all directions.
4. In sponges (Leys et al. 1999) and other lower animals (Mackie 1965; Mackie and Passano 1968) the coordination is through electrically conducting *epithelia*. An early metazoan with dramatic defence behaviour

<sup>10</sup> Not excepting, from this statement, the sophisticated and highly successful imaging of normal activity in the brain with the clinical tool known as functional magnetic resonance imaging (fMRI). Its temporal and spatial resolution limits are discussed by Logothetis (2002).

<sup>11</sup> 'To a true believer, there can be no plainer demonstration of conformational spread than the beating of cilia and flagella' (Bray and Duke 2004: 60). CS is a 'theoretical construct', combining many different lines of evidence, for which there can be 'no proof ... that will satisfy all skeptics' since actual visualisation would require a microsecond timescale and spatial resolution less than 1 nm (Bray and Duke 2004: 55).

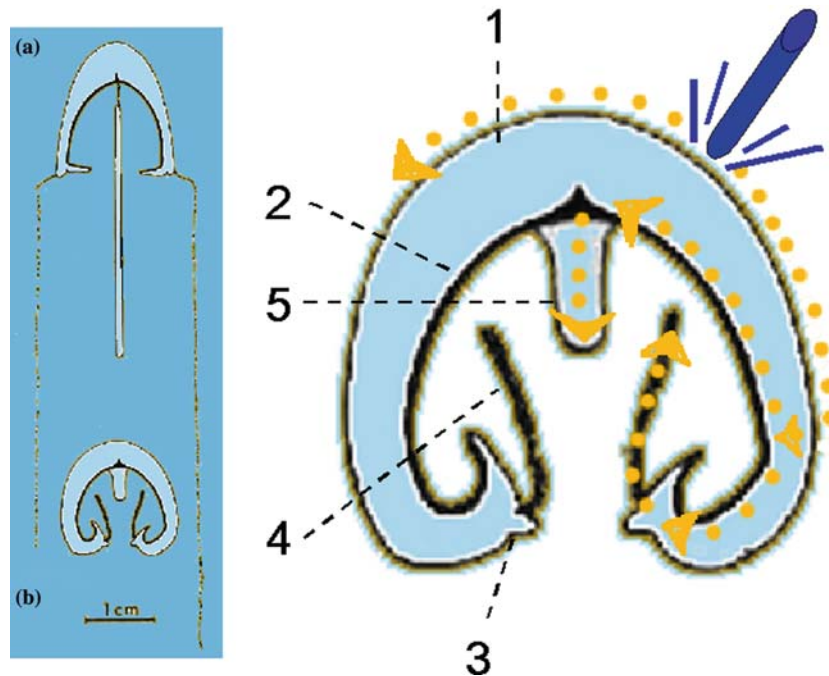


Figure 4. *Non-neural coordination of the whole ( $H_0$ )*. The hydromedusan *Sarsia tubulosa* (a) in fishing and (b) defensive pose after collision with an object. Enlargement of (b) (right) shows routes of electrical waves (arrows) from point of collision through ectoderm (1) and endoderm (2) to epithelio-muscular layers that serve to retract the umbrella margin (3), tentacles (4) and manubrium (5). Response time 200 ms. (Adapted from Mackie et al. 1967).

coordinated by such non-neural mechanism is the jellyfish, *Sarsia tubulosa*. On colliding with an object this essentially diploblastic animal completely involutes (Figure 4). The action is the result of electrical waves spreading along the ectodermal layers (with occasional jumps into nervous layers) to the endodermal and executive epithelio-muscular layers through the mesogloea (Mackie et al. 1967). In many other coelenterates similar behaviour is coordinated by the diffuse neural network<sup>12</sup>. In early chordates, epithelial conduction coordinates the members of a chain of salps. It is found both in early (Bone and Mackie 1975; Mackie and Bone 1976, 1977) and in later chordates (Roberts 1969) running over the surface as *skin impulses* which then interact with the nervous system. Epithelial conduction (electrical and/or calcium waves) survives in advanced vertebrates, for instance in the lung alveolar epithelium (Boitano et al. 1992).

<sup>12</sup> Evidently there were originally two systems for horizontal control, the second (neural) operating with synapses, became concentrated and centralised to serve vertical control (cf. Mackie 1970).

5. The *uterus* is an organ whose automaticity illustrates the interaction of vertical and horizontal controls. [More instructive than the heart for present purposes since its millions of cells have complete cell membranes]. As many women have cause to know, the muscular walls of the uterus (myometrium) are always capable of spontaneous activity. Normally, in the non-pregnant uterus and in the pregnant uterus before term, such automaticity is actively suppressed by local innervation. At parturition, the nervous control switches off, channels open between adjacent cells of the myometrium and rhythmic waves of contraction start to propagate through it (see Wray 1993; Miyoshi et al. 1996)<sup>13</sup>. The waves are the result of action potentials propagated from one muscle cell (myocyte) to another, and of calcium waves spreading radially from cell to cell within a bundle of myocytes (Young 1997; Young and Hession 1996). The ways in which excitation spreads between the bundles or compartments bounded by the extracellular collagen matrix (ECM) (Young and Hession 1999) is less well understood, however (see below).
6. In the *gut*, emphasis has long been on electrical conduction, for instance on the slow waves of depolarisations coordinating the propagating contractions of peristalsis (Connor 1979; Dahms et al. 1987) which also involve modified muscle cells: the interstitial cells of Cajal and their pacemaker activity (Huizinga et al. 1992; Prosser 1992). Gastroenterologists have long spoken of a ‘myogenic control system’ or MCS (see Sarna 1975; Barakjian and Diamant 1989; Preiksaitis and Diamant 1999)<sup>14</sup>. Based on a coupled oscillator model, this is one of a triumvirate of controls for the gut, the other two being neural and hormonal.
7. More recently, emphasis has been on intercellular calcium waves (rise in free calcium levels within a muscle cell being a prerequisite for its mechanical shortening). In the gut – and in the muscle fibre network of *lymphatic vessels* – the calcium waves are seen to be phase waves resulting from the interaction of short-range (< 10  $\mu\text{m}$ ) calcium events, characterised by coupling and synchrony between locally oscillating calcium stores, and longer range (< 4 mm) electrical ones with the phospholipid membrane having a ‘voltage-accelerating’ role (van Helden and Imtiaz 2003a, b). Phase waves based on calcium pacemaking allied to membrane depolarization are thought by these authors to be generalizable to other systems including brain rhythms.
8. Electrical coupling between cells and intercellular calcium waves appear to be universal also in the non-muscular structures of higher animals: *brain*,



<sup>13</sup> The role of oxytocin in these events is less directly causal than traditionally believed.

<sup>14</sup> The MCS concept qualifies as horizontal control. However it – or similar concept – does not seem to have been adopted by the other physiological sub-disciplines. Perhaps because emphasis has been on the cell-type rather than on the properties of systems, irrespective of cell-type (see Discussion). In mammals, inhibitory nerves (vertical control) modulate the automaticity of this system, reducing the frequency of contraction cycles, by hyperpolarizing the membranes of the muscle coat (Lyster et al. 1995).

*liver, pancreas, salivary glands* (Senseman et al. 1987), *bone, blood-forming tissue* (Rosendaal and Krenacs 2000) and in the *embryo* during the early stages of differentiation. Electrotonic synapses between neurones at a given level of the nervous system are now recognised as a major component of within-level synchronisation (see Draguhn et al. 1998 also Zoidl and Dermietzel 2002).

9. Gap, electrotonic and other kinds of permeable junctions linking the plasma membrane of one cell with that of its neighbours, are not the only mechanisms for coupling within functional syncytia. Equally important for 'horizontal' coordination of dynamic state seems to be the network of connections between the interiors of cells and their *extra* cellular 'microenvironment'. This network is furnished by the *tensegrity* system (Ingber 1997, 1998) and by the *connective tissue continuum* of animals (see ECM and basal lamina above)<sup>15</sup>. Pioneers in the field have pointed out that the extracellular matrix is *liquid crystalline* in nature (see below, *the liquid crystalline continuum*, and Ho and Knight 1998 for a summary), as are the hemicelluloses of plants (Vincent 1999). They therefore possess *electronic* properties via semi-conduction of protons.
10. Some authors have implied that the source of the integrating force is outside the cell in the form of *field potentials* generated by the ensemble. Such potentials guide the growing tips of plant roots. Applied electric fields affect the course of regeneration of a flatworm (Marsh and Beams 1952) and have been used to mimic the injury currents that guide epithelial cells during wound healing (Zhao et al. 1996). (See also Lund 1947 and the series of papers by Lund in the *Journal of Experimental Zoology* 1921 to 1925).

This cursory dip into the enormous literature of primary research reveals that most of the studies of 'functional syncytia' have been carried out on relatively small isolated preparations, and mostly of mammalian origin: notably pieces of organs and tissues, tissue slices or cell cultures rarely more than a few millimetres across. In these preparations it is not really possible to observe the overall pattern of communication (and of homeotaxy) in the various networks and their subdivisions or compartments of  $H_o$  (for related comment see Daniel et al. 1994), nor to see the normal interplay of vertical and horizontal control – e.g. the modifications of gap-junctional intercellular communication (GJIC) that are taking place – for modulation is itself a function of the whole (see Discussion, para. 6). It is likely that several mechanisms are operating in parallel, and doubtless many are still to be discovered. Plants and lower organisms that can be studied whole often pave the way (e.g. Mackie 1965; Mackie and Bone 1977)<sup>16</sup>.

<sup>15</sup> The horizontal control exercised by this system was termed 'dynamic reciprocity' by Bissell et al. 1982.

<sup>16</sup> In the squid example, the roles of electrical, chemical and mechanical events in the activity of muscle recorded at the cellular level (for references see Lima et al. 2003), allow several inferences to be made about the *intercellular* involvement of these same events from pictures of the skin of living animals recorded at the population level.

Both of the examples of homeotactic behaviour reported below, result from a ‘factor’ produced by the ‘community’ being coordinated.

### **The community effect and quorum sensing**

In 1988, John Gurdon (Gurdon 1988) coined the term *community effect* to account for the observation that embryonic cells within a block of mesoderm (of the clawed toad *Xenopus*) are constrained to be in the same state of differentiation as the group in which they find themselves.

The community effect is a *field effect*, which operates irrespective of the lineage of particular cells within the block, and requires that the ‘community’ reaches a certain size (50–100 cells in the vertebrate mesoderm example). It is found in embryos generally and coordinates genetic programming by way of the *community ‘factor’* (Standley et al. 2001). For example, in future muscle it coordinates the expression of the protein MyoD and inhibits the expression of certain other proteins. ‘All the cells in the group secrete the community factor, and each cell must receive an above-threshold amount of this factor from its neighbours in order to differentiate’ (Standley et al. 2002). Remarkably, there have only been a handful of publications on the community effect in the 17 years following discovery of this form of horizontal control operating in early development. Nevertheless, the underlying community *principle* (see Discussion) is obviously an important one; and not just in embryonic tissues, for it also operates in the self-organization of dissociated cells freed from the hierarchical sequence of genetic cascades (Technau et al. 2000).

In recent years bacteriologists have uncovered somewhat similar arrangements, known as *quorum sensing*, in communities of symbiotic and infective bacteria (Greenberg 2003a, b). Like stromatolites (see above), these communities are usually multispecies and form a biofilm with structural properties that condition their exchange with the environment. The ‘inducing factor’ that operates during quorum sensing allows bacteria ‘to monitor their own population density’. For instance, the luminescent bacterium *Vibrio fischeri* only luminesces when the population has grown large enough for a sufficient amount of the factor (a peptide which diffuses passively out of the cells) to accumulate in the immediate environment and reach threshold (Fuqua et al. 1994).

### **Unification of the whole (H<sub>0</sub>)? – the liquid crystalline continuum**

The various mechanisms noted above, which permit horizontal control within fully differentiated tissues or organs, do not of themselves signify that similar control necessarily extend between tissues or organs: thence throughout the body (H<sub>0</sub>). (The various coloured waves that run across the whole mantle in the skin of squids and octopuses seem to be confined to a particular class, or

classes, of chromatophore organ with lowered probability of entraining other classes or other tissues).

Is there a whole body field that bridges the gap, structurally and functionally – between tissue (or organ) and  $H_2O$ ?

Proof is notoriously difficult. But, Burr and Northrup (1935) in their *electro-dynamic theory of life* proposed that this is the role of the DC (direct current) body field detectable in organisms. Demonstration of the *electronic* properties of biological materials in recent decades – notably of the phospholipid membrane of cells and of the collagen liquid crystal continuum synonymous with the extracellular matrix (ECM) in triploblastic animals – has rekindled interest in the theory. Ho and Knight (1998) argue that the network furnishes the acupuncture system with pathways for ‘rapid inter-communication throughout the body, enabling the organism to function as a coherent whole’<sup>17</sup>. For universality, this should apply equally to plants (Metaphyta). Vincent (1999) proposes that the hemicelluloses of vascular plants, lying below the cell’s cellulose layer, correspond to the liquid crystalline continuum of animals – making a tree the ‘biggest liquid crystal in the world’.

Thus connective tissue, traditionally considered by an older generation as extracellular and non-living, turns out to be unexpectedly well named. As it also guides the growth and repair of bone along lines of mechanical stress (see Ingber 1997), it can be seen in the still wider role of connecting body and environment<sup>18</sup> – relating it in abstract principle to the biofilm of stromatolite communities (see above p.).

### Intersecting controls

It is not the business of this essay to examine the points at which horizontal and vertical control intersect – apart from stating that they do so all the time and at many different points, as with the skin impulses of tadpoles, and in neurogenic/myogenic interactions where one modulates the other (see above). The field that exerts horizontal control in quorum sensing and in the community effect –

<sup>17</sup> The layers of bound water on the collagen fibres of connective tissue, together constitute a continuous liquid crystal supporting rapid semi-conduction of protons (termed *power transmission by proticity* Mitchell (1976)). The DC body field is probably the sum total of the ‘self-reinforcing circuits of proton currents’ in the network (Ho 1998). For Ho, the network constitutes a dynamically distributed memory of what has happened and is happening to the body (Ho 1998: 193 see also chapter 11 and Oschman 2000, for a review of the body electrical).

<sup>18</sup> ‘When a bone or cartilage is compressed, when a tendon or ligament stretches, or when the skin is stretched or bent, as at a joint, minute electric pulsations are set up. These oscillations, and their harmonics, are precisely representative of the forces acting on the tissues involved. In other words, they contain information on the precise nature of the movements taking place. This information is electrically and electronically conducted through the surrounding living matrix. One of the roles of this information is in the control of form.’ (Oschman 2000: 52).

produced by the parts and external to them – must first build up. Manufacture of the ‘signal’ molecules characterizing the field, transcription and activation of target gene circuits, etc. (see Fuqua et al. 1994; Standley et al. 2001, 2002) are all processes that qualify as vertical control. Likewise the control exerted by the enteric nervous system over the ‘horizontal’ layers of the gut; this ‘little brain’ is at the same time a node (or layer) of the vertical conceptual hierarchy (Figure 1), with its own network of repetitive activity qualifying as horizontal control. And so, too, with the cortical layers of the ‘big’ brain (Schiff et al. 1994) – manifested, for instance, in spreading depression.

### Discussion

Why should one need a new word, let alone the announcement of a new principle, for something already well established and perhaps only too obvious to the layman? There are several reasons.

1. Surprisingly, there is still no generally accepted fundamental principle of *cooperation* in biology – matching that of valency in chemistry or gravitation in physics – to account for the simple observation that most cells act collectively if they act at all.
2. Living things are incomprehensible if one tries to proceed without inferring some such concept. It is logically necessary to have something to set against the principle of differentiation and separateness implied by the cell as unit. My task has been to state, on general biological grounds, that acting in unison is a fundamental principle of living things equal and opposite to the principle of differentiation and separateness. (The concepts of competition and of selfishness dominating much of biology in the last century had no adequate countering ideas to balance them, even though biologists needed only to look around them to see that the cooperative and the social are just as strong as the competitive and the self-assertive).
3. One does not have to be complicated about this, but it has to have a name<sup>19</sup>. Whether one calls it integration, coordination, coherence (Ho 1998)<sup>20</sup>, cooperativeness or sociality – or love! – it should stand for a property that can not be left out of the equation. To illustrate: *the integrated activity of a coupled ensemble*, as seen in the coloured waves running across the skin of squids and their relatives, continues for days long after sacrifice of the animal and *for as long as the component cells show any other signs of life*. If,

<sup>19</sup> Cf. the intellectual awkwardness created by omission of an appropriate name, and associated concept, from an influential piece of writing in an allied field. In E.O. Wilson’s *Sociobiology* (1975) the word ‘sociality’ – name for the principle underlying social relationships – appears nowhere in the book. Interestingly, the absence is also reflected in the illustrations. Waddington (1975) commented that the drawings of the various groupings of animals are peculiarly unaesthetic.

<sup>20</sup> In her book *The Rainbow and the Worm*, Ho (1998) traces the coherence of organisms back to that of quantum coherence and the physics of coherent energy storage cycles.

then, the process of acting as your neighbours do, or peer conformity, is found to be equivalent to being *alive*<sup>21</sup> then it certainly warrants adoption of a suitable name and the necessary importance attaching to that name.

4. *Cooperativity*<sup>22</sup>, as used by protein chemists, is the bedrock concept in the theory of Conformational Spread (see above), which its originators say 'may teach us something about the integrated behavior of living cells' (Bray and Duke: 54). CS has obvious parallels with the concept of homeotaxy and peer conformity.
5. The usual physiologists' description of homeotactic phenomena is in terms of *intercellular communication* and more particularly in terms of the mechanisms (electrical, mechanical, chemical) involved in conduction and/or in *signalling*: nowadays usually calcium signalling (and more recently ligand signalling, Freeman and Gurdon 2002). Apart from being too general, however – for all biological activity depends on communication between cells – employment of the word 'communication' somehow misses the point. If one places electrodes into the collective (or ensemble), or illuminates it with dyes recording free calcium levels, its component cells are found to be following each other so closely that they must be 'in communication' in some way. But it is debatable whether or not a signal can be said to be passing<sup>23</sup>. Secondly, the local 'signals' of experimenters concentrating on a single cell, or on neighbouring cells, do not necessarily contribute to 'communication' in the whole (whether compartment, tissue, organ or organism) of which they are a part.
6. Something approaching a general law to which the parts (cells) are subject is expressed by Werner Loewenstein (1981: 889), the main pioneer of cell-to-cell channel research.

'Probably the most basic physiological role of the channel is homeostatic: a buffering of individual variations in channel-permeant molecules in tissue cells .... The action of the channel here is *a coordination toward uniformity*; that is, toward *equilibration of chemical and electrical potentials in the cell system* [my italics]<sup>24</sup>.

<sup>21</sup> N.B. a mammalian embryonic cell that finds itself accidentally separated from the blastoderm is genetically programmed to end its life: i.e. undergo apoptosis (genetically programmed cell death) (Maurizio Zuccotti, personal communication).

<sup>22</sup> COOPERATIVITY (definition): an interaction of the constituent subunits of a protein causing a conformational change in one subunit to be transmitted to all others.

<sup>23</sup> Plieth has drawn attention to cell biologists' habit of calling a 'signal' any sharp change in intracellular free-calcium level, whether or not it has a role in signalling (see Plieth, for review).

<sup>24</sup> In his next sentence, Loewenstein uses the German word *Gleichschaltung*. In the present context, the word could be supposed to have the same meaning as homeotaxy, if it were not that it refers to the early 20th century German policy (and, under the Nazis, attendant legislation) of rendering society uniform: i.e. to a policy imposed upon the collective rather than to something which arises from within it.



7. This brings us back to the question of whether one is dealing with a *field phenomenon*. In studies of the kind of auto-associative, self-regulatory, activity that is increasingly attracting the attention of physiologists – and also of psychologists and sociologists – the primary datum is a pattern of collective activity in a given area or field and/or the repeating, usually chaotic, iterations of a network. Many field phenomena express themselves as gradients.
8. Both the ‘community effect’ and ‘quorum-sensing’ are field phenomena: basically chemical. The unifying ‘signal’ is extracellular, is *generated by the collective* and individual cells are subject to it. The biofilm of bacterial communities, the extracellular matrix and the connective tissue continuum are also extracellular and generated by the collective. They unite structurally and have both mechanical and ‘electrical’ properties (see above). What is still not known, except in a few instances, is whether cells or other parts of the whole are subject to an external electrical field generated by the collective and to what extent (see Bullock 1997 and Burr and Northrup 1935; see above).
9. The assertion that horizontal control is ‘information-poor’ (see above) is a ‘soft’ one reflecting the difficulty of seeing much ‘information’ passing through parts that are evidently in the same state (phase-locked, coupled, or in communion), whether they be cells, muscular structures, members of a fish shoal or of a flock of starlings manoeuvring at sunset<sup>25</sup>. Obviously the process of differentiation – instructing entities to be different – must, in any technical sense of the word, require more information than telling them to be the same.
10. The proposal that a way out of the intricacies of the whole (H) is to pose the problem as a question of *control*, lends itself to pictorial presentation. Figure 5 illustrates the two kinds – horizontal and vertical – as a play on the derivation of the word<sup>26</sup>. One is the hierarchical operation of checking the roll, the other is the persons forming the community of workers or soldiers or inhabitants of a city who are linked passively by the fact of being on a single roll and dynamically as members of one community. In Microsoft Power Point the pencil checking the roll can be made to agitate up and down, and there may well be a function in the programme for ripples (of feeling) passing between the entries on the register – especially when the pencil is removed!

<sup>25</sup> It is only fair to their authors, to point out that this assertion fits ill with the ideas quoted in Notes 17 and 18: namely that the role of the extracellular matrix in the control of form implies that it transmits precise ‘information’. Nor would it stand up to examination by a student of embryology or of pattern generation, used to thinking in terms of map-like positional information in a gradient or field.

<sup>26</sup> The word is from Old French combining ‘*contre*’ meaning ‘against’ – as in ‘over against’ – and ‘*rôle*’ from medieval Latin *rotulus* roll: i.e. a roll of parchment containing a list of articles or persons making up a register as in ‘payroll’. The controller was the person who checked the register.

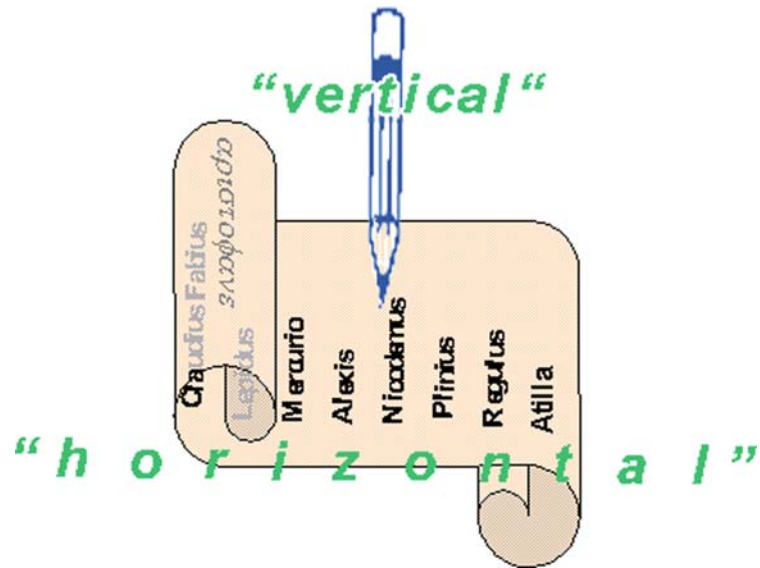
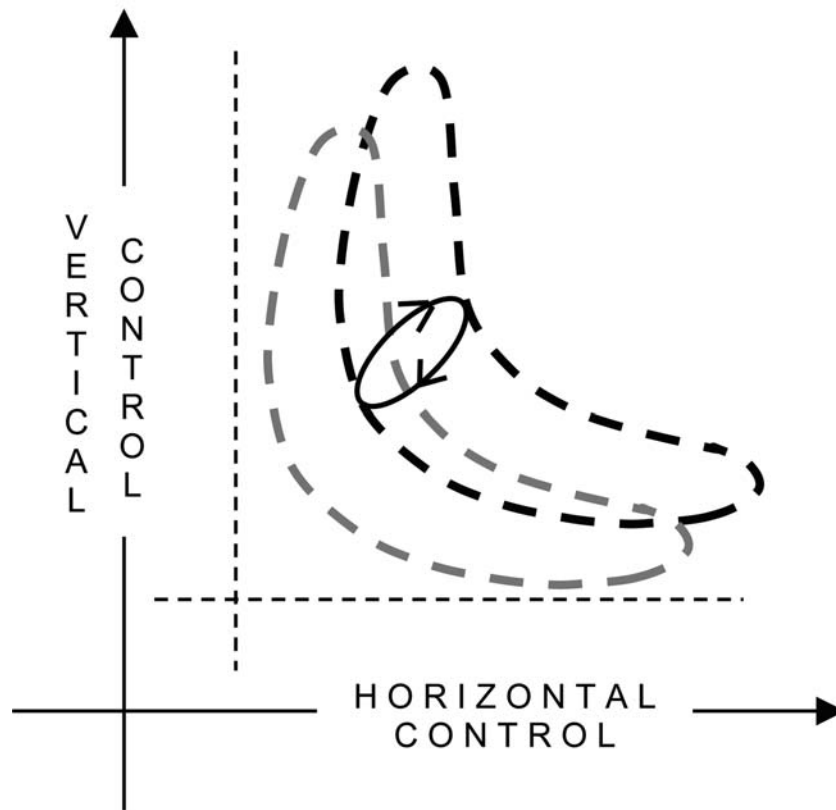


Figure 5.



Figure 6. *Triumphal entry of Championnet into Naples.* Copper engraving in the archive of the Museo del Risorgimento, Rome. [see Text].



*Figure 7.* Different locations within the space enclosed by these curves correspond to variations in the amounts of vertical and horizontal control of  $H_o$  during different stages of the life history (two separate areas) and different seasons or times of day (arrowed ellipse). The minimum values for the persistence of  $H_o$  are indicated by dashed lines, the maxima are arbitrary. The shape of the closed curves assumes a certain reciprocity: that assertion of one form of control generally relaxes the other. (The width probably corresponds to the notion of 'vitality' in Yates's homeodynamic stability model of a human life-span: Yates 1993).

Readers with an Arts background may prefer an alternative image. Figure 6 depicts a moment in which authority is reasserted over the autonomous behaviour of a crowd as French forces enter the gates of Naples to quell the 1799 revolution.

More formally, the two-dimensional space occupied by  $H_o$  operating under a combination of vertical and horizontal control is seen in Figure 7.

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