LIVERSIDGE LECTURE*

On First Looking Into Nature's Chemistry

Part I The Role of Small Molecules and Ions: The Transport of the Elements

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1 Introduction

'Much have I travell'd in the realms of gold,
And many goodly states and kingdoms seen;
Round many western islands have I been
Which bards in fealty to Apollo hold,
Oft of one wide expanse had I been told
That deep-brow'd Homer ruled as his demesne;
Yet did I never breathe its pure serene
Till I heard Chapman speak out loud and bold:
Then felt I like some watcher of the skies
When a new planet swims into his ken;
Or like stout Cortez when with eagle eyes
He star'd at the Pacific – and all his men
Look'd at each other with a wild surmise –
Silent, upon a peak in Darien.'†

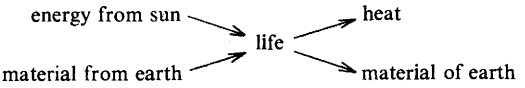
Although as scientists each one of us would like to discover something quite new and to set it down before our colleagues, crying 'Eureka', this moment of triumph is likely to come to but a very few of us. Fortunately there are other satisfactions almost as great as such discoveries but which are more personal. Each of us can experience understanding of the work of others. This is Keats' pleasure on reading Chapman's Homer, and out of that pleasure he creates his own poem. In doing so he generates new insight. The description of a world first sighted and deeply appreciated is quite apart from its first creation, yet it too can be of real value. In this article I would not claim to generate a different view of biological chemistry but I would like to show, particularly to my chemistry col-

*First delivered on 8 November 1979 at a Chemical Society meeting at the University of Leicester.
†The reader is asked to substitute Chemistry for Homer and Nature for Chapman, making Nature the finest expression of Chemistry.

leagues, that there is a new world of chemistry in biology. It is before all of us if only we look. The picture I shall give is a personal one and will only be correct in part, for much of the overall chemistry of biology is not yet known.

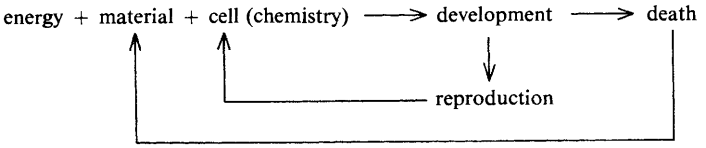
2 The Essence of Biology's Chemistry

The underlying feature of all biological chemistry is its cyclical character, which is only slowly transformed over very long periods of time to make an evolving system. I shall not address myself to evolution. The cyclic reactions of all extant cells (and organisms) originate from the interaction of the sun's energy with matter; this can be described as in Scheme 1. The processes called 'life' in this



Scheme 1

diagram may be elaborated a little at first so that they read in cycles (Scheme 2).



Scheme 2

At this level the steady state of 'life' would be observed in the constant population of cells and species of given kinds. The cell is here shown to have two activities: development, which is the timed sequence of production of macromolecules, and reproduction, which is the timed production of new cells. Neither of these processes is in a true steady state in a given cell, obviously. However, a given cell shows only moderate rates of development and reproduction, while it can maintain itself in chemical balance certainly over medium periods of time. Visual inspection of living systems shows that they develop very slowly, while over the periods of at least minutes and possibly even days or weeks many cells are in a virtual steady state, remaining viable. As a first approximation we can then separate the development and reproductive activity from the chemical steady state of a cell (or organism) and later we can impose as a perturbation both development and reproduction. Finally, we can look at evolution. We now ask what is the so-called steady state of a cell, making it clear that no viable cell comes to equilibrium with its surrounds. (Equilibrium is death.) A living cell has a continuing flow of energy and chemicals (Table 1), and at first we shall analyse, making the gross over-simplification that all final products are passed out of cells as waste. This means that in Figure 1 we do not at first describe any steps labelled under synthesis, while we have a steady state in materials. Moreover, as we wish to describe the complicated activities of metabolism as simply as pos-

Table 1 *Flow in biological cells*

Flow	*Carriers*
Chemical material (free or in particles)	Small organic molecules, *e.g.* co-enzymes, and H^+
Energy (free or in particles)	H^+, e^-, pyrophosphate, and charged ions, elastic structures
Charge (aqueous)	Inorganic ions Na^+, K^+, Mg^{2+}, Ca^{2+}, H^+, Cl^-, PO_4^{3-}
Charge (non-aqueous)	e^-, H^+

sible we shall describe all metabolism as the simple movement of the essential atoms, C, H, N, O, P, and S (in different oxidation states), from food into products (waste).

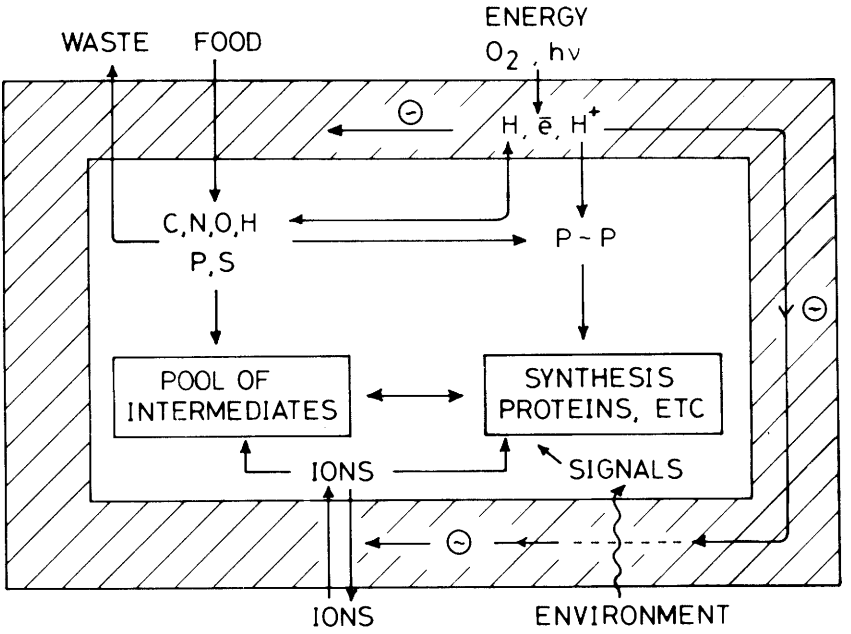


Figure 1 *A basic outline of a cell and cellular activity. The shaded area is the outer membrane and the clear central area the cytoplasmic solution. Only essential connections are shown.* P ~ P *is bound pyrophosphate in ATP.* ~ *is energy transferred by other means,* e.g. *mechanical or electrical in the membrane*

A. The Steady State of Cells.—A cell in a steady state is not of course a rigid apparatus which carries out chemical reactions, *i.e.* it is not just a chemical set of metabolic pathways in a container, although this is a major feature. Exami-

nation of a cell will show that it has, as well as a steady state in chemicals, a steady state in energy input (which is necessary for synthetic activity), a steady state in cell shape, which is motile, and in electrical potential within all kinds of fields although the cell is fluctuating. The cell maintains the three-fold steady state in chemical, mechanical, and electrical properties responding to and counteracting environmental influences, which it senses. (In a multi-cellular organism the environment is composed of other cells and their activities.) We know that the cell returns to its steady state even after an external change causes considerable new fluxes for a short period.

While a chemical steady state can be maintained in test-tube (man's) chemistry by the supply of fuel and chemicals, and this is the basis of a modern continuous industrial plant, the maintenance of shape and electrical potential requires the cell to control its own structure. (In other words, the cell's test-tube is not rigid and is charged.) The cell then needs as well as the flux of chemicals the flux of mechanical and electrical energy to be controlled. We take these points in turn.

In essence, the flux of chemicals through a reaction is controlled by catalysts and controlled barriers (membranes) to diffusion, uptake, and rejection (Figure 1). (Temperature and pressure are not controlled variables for a single cell.) Catalysts (enzymes) restrict the transformation of a chemical to specified pathways. It is essential to note, however, that a cell has very many pathways, each involving many enzymes, and that all must be controlled, and controlled in common, if the steady state is to be maintained. In turn, this must mean that the catalysts themselves are controlled relative to one another. We shall see how this is done largely by the diffusion of a few small molecules between the many pathway catalysts, remembering that there must also be diffusion of metabolites themselves between pathways. There must also be linked control of uptake and rejection.

An electrolytic potential can be maintained only by restricted diffusion of ions to and from a surface – here, to and through a membrane (Figure 1). Control over the potential is control over diffusion, which is a change in a *pump* for ions from or through a membrane, *i.e.* a catalyst for transference, or by making a controlled *gate* in the membrane. The terminology here is that of mechanical devices (*pump*, *gate*) and we see immediately that the dynamics of interest are those of large structural components of part of the cell in the membrane. Similarly, control over the cell shape must be by mechanical adjustment of large organized cross-links connecting the outer membrane. The tension in the structure must be adjustable (see Figure 3).

If there is to be a steady state in all the characteristics of the cell then we must connect together all these dynamics under a central control. Let us ask about each one in turn. That is, we shall ask how it is possible to connect through dynamic features:

(i) metabolism itself in one pathway and then to connect it to another pathway, *i.e.* the catalysis by enzymes, proteins, and the transfer of information between the enzymes,

(ii) the strain in structural units, *i.e.* other proteins,

(iii) charge flow through structural units, *i.e.* yet other proteins.

We shall need to look at diffusion control of small molecules and at the dynamics of non-diffusing, especially protein, structures. To maintain these activities the cell needs to control:

(iv) the supply of energy, and

(v) the uptake of food (C, H, N, O, P, S) and rejection of waste.

As is clear from this description of the cell as being in a steady state (not equilibrium) and from Scheme 1 the cell apparatus dissipates energy. To stay in the steady state it must then produce energy continuously (Figure 1). The energy must be distributed to the various parts of a cell, in a controlled manner, just as material is distributed. Energy production and energy distribution must then be 'catalysed' and directed specifically by enzymes and diffusion barriers. The diffusion of energy then becomes under kinetic control and we shall see that this control uses both small-molecule diffusion and transfer within polymer structural frames much after the manner in which cells handle material. Finally, the uptake of food and rejection of waste must be a controlled process very like that of the uptake and rejection of ions (Figure 1).

B. Flow in Biology.—The analogy with an industrial plant is quite close for the organization of a large animal. There are obviously many pipes (flows of fluids in blood vessels) and many electronic wires (electrolytic circuits of nerves) that connect to a variety of control points. The mechanical devices (muscles) which drive the flow of materials are also obvious enough. It is not the principle of such a multi-cellular machine which is confusing but the complexity. By way of contrast at the level of single cells we find that the principles of organization are hard to visualize. The simplest cells have but one membrane as an exterior diffusion barrier (Figure 1). They have no pipes and at first sight there are no circuits. More thoughtful inspection shows, however, that within the membrane and along its surface there could be organized networks and that although there are no other membranes (in the simplest cells) there are many very large particles, molecular weight $> 10^6$ daltons, and in many cells there are networks of fibrillar proteins, tubules (see Figure 3). Now let us consider the possibility that the large-scale multi-cellular assemblies of the larger animals and plants are replaced by miniature units, large particles in each cell where a unit is equivalent to a functional separate phase or domain.[1] Flow is not now in a pipe but from chemical centre to chemical centre within the particles or microphases. What devices of this kind can be made? If there are such particle devices, then inside the cell they can only communicate with one another through free diffusion of chemicals in the bulk aqueous phase or, if several different units are placed together say in the outer membrane, by the diffusion of chemical or electrical messages within or along the surfaces of the membrane (Figure 1) or (they could communicate) using mech-

[1] R. J. P. Williams, *Biochim. Biophys. Acta*, 1975, **416**, 237.

anical devices made from the fibrillar proteins (Figure 3). Let us look at some large particles which exist in cells and then try to picture control devices. (Remember that instead of using any conventional metabolic charts we shall just describe the whole of metabolism as the flow of the chemical elements H, C, O, N, P, S.) I hope it is clear that the intention in this article is to treat the single cell as a machine of complexity parallel and equal to that of a whole organism.

C. Organized Systems of Cellular Reactions.—Some of the very large particles which have been found in cells are described in Table 2; see also Figure 3. The

Table 2 *Organized particles*

Particle	*Flow*	*Function*	*Site*
Ribosome	Amino-acids	Protein synthesis	Membrane
Nucleus	Nucleotides	DNA + RNA synthesis	Membrane
Large free particle	CH_3CO-	Fatty-acid synthesis	'Free' in cell
Glycogen particle	Glucose	Polysaccharide synthesis	'Free' in cell
Oxidative metabolism	e^-	Energy generation	Membrane
ATP-synthetase	H^+, P	Energy retention	Membrane
Light capture	$h\nu$, e^-	Energy capture	Membrane
[Tubulin	Mechanical force	Interconnection of membranes	Membrane and nucleus]

Note Each particle has a prescribed activity and direction of flow; they are irreversible energy-consuming machines, not catalysts for the equilibration of reactants and products

table shows that very small chemical groups such as e^-, H^-, $H^+(?)$, CH_3CO^-, amino-acids, nucleotides, (H, C, N, O, P, S), can all be channelled into pathways which use defined ordered movements in synthesis or degradation within a particular particle, and there is no confusion of purpose. Most of the particles are in fact organized by the outer membrane (Figure 3). (Note that in advanced cells some of the functions leave this outer membrane to be placed in separated and more specialized, vesicle, membranes, *e.g.* the nuclear, reticulum, mitochondrial, and chloroplast membranes. We return to this specialization of different membranes later. The separated membranes will be seen to be just another way of isolating different circuits.)

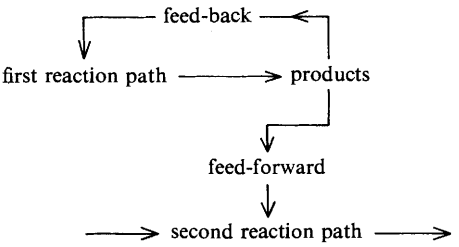
The segregation of pathways for different processing of materials could be treated by phase considerations but in my opinion this is not quite the correct model in many circumstances. I have drawn attention to microdomains as the nearest parallel on which to base ideas of biological organization.[1] A micro-

domain can be separately energized locally within an organized layer, *e.g.* a membrane. Local circuits are then permitted, involving individual domains or connecting domains. I return to this idea later. Note that diffusion is not free in the domains.

Now through each of the particles we have in effect a flow of material and/or energy, the general metabolism of the cell. This metabolism must be co-ordinated so that the cell acts as a unit. There are, I think, only two chemical ways in which a cell can be so organized: (i) by providing restricted paths for further diffusion or (ii) by allowing free diffusion of message particles but specific recognition at sites. These two mechanisms are used in very different ways. Under (i) we have already mentioned the way of handling metabolites in synthetic or degradative chains within the particles given in Table 2, and we must now try to add an outline picture of the communications network between the particles within which the main metabolic flow occurs. What materials are used in these communication channels and how do they compare with the molecules of metabolism itself that carry C, H, N, O, P, and S in different oxidation states? The nature of these carrier chemicals will be described in Section 3.

D. External Conditions.—A cell is not totally self-sufficient and clearly requires both a supply of material and energy and a way of eliminating waste products. The internal steady state of the cell is then connected to the external world and, according to the sensing devices it has, it will reflect the supplies of food and energy, the temperature, the pressure, and the various fields surrounding it. This means that the outer membrane of the cell must have a set of proteins for the uptake and rejection of chemicals, rather than for their transformation, and for sensing (Figure 1). While we have referred to the steady state of metabolism of a cell it is really the case that *steady states* of cells can vary over quite wide limits as the environment varies, and all these different steady states are related and controlled. In effect, there has to be a constant communication between the environment and the cell's internal condition. This requires the pumping of information, *i.e.* the transfer of forces or of chemicals into or out of the cell so that the inside of the cell reflects the environment. Since the single cell cannot control the environment it has to have feed-back from the interior to the membrane's external face, which interacts with this environment. In the extreme limit of adversity a cell will switch off virtually all activity and all communication with the external world and yet remain viable. It will only return to 'life' on receiving a signal that the external environment has changed favourably. By increasing cell/cell organization certain cells become more or less totally protected from these environment effects, *e.g.* in higher plants and animals, while special cells take care of them. Short-term independence from the environment also depends on internal storage devices connected to metabolism. While recognizing the vast complexity inherent in such a variability in steady state the essence of the problem rests in the communication network which maintains one and all of these states.

E. Summary of Biological Activity under Review.—At this stage of the analysis we have reduced inspection of a cell's activity to the control of metabolism, of energy, of electrical fields, and of mechanical shape. We have indicated that it is necessary to discover the nature of the small-molecule flows of chemical material, energy, charges, and mechanical stresses before we look at the control proteins which are the major units of catalysis and structure, but we have pointed out that the proteins often act in concert in larger particles or membranes. Understanding will usually rest in seeing how these larger units work. We start by looking at the controlled flow of material within the cell and then across its outer containing membrane. This is a simplified analysis of metabolism in the steady state of a cell, assuming constant environment. Such internal controls we shall call *steady-state controls.* When there is a rapid change of environment or a message is sent to a cell the cell needs to change its activity much more rapidly than steady-state controls permit. There then must be *switch controls* working from the outer membrane. All the controls must intercommunicate so that any reaction must be accompanied by some signal which is transmitted to a receiver/amplifier so as to generate an activity which either feeds back to the initial reaction or feeds on to some other series of reactions (see Scheme 3).



Scheme 3

Remember that the cell will always return to the steady state. The charts of metabolism which every biochemist has on the wall of his room show the way products and reactants from different paths are intercombined, but they give a very poor indication of the nature of feed-back and feed-forward controls. The central theme of this first paper is the control functions of small molecules and ions. The following paper describes the role of proteins in the machinery which handles the small molecules.

3 Steady-state Controls

We shall describe steady-state control under the headings:

A. Material Flow in Homogeneous Phases
B. The Energy Flow (in homogeneous phases)
C. Restricted Diffusion in Particles
D. Transport across Membranes
E. Mechanical and Electrical Steady States

F. Osmotic and/or Local Control.

Subsequently we shall consider the extra advantages gained by the division of the cell into vesicular spaces by additional membranes (Section 4) and the response of the cell to sudden changes (Section 5).

A. Material Flow in Homogeneous Phases.—We tackle first the nature of transmission of material and information between different metabolic pools in a cell in a simple single *aqueous* phase, the cytoplasm (Figure 1), which is in a slowly adjustable steady state, responding to a slow rise or fall in metabolites. The form of control can be described if we consider that transmission is by simple freely diffusing chemical entities which link different metabolic paths. In the first instance these metabolic paths are treated as if each reaction has an independent set of freely soluble single-step catalysts, enzymes, which direct the nature of its chemical transformations.

Inspection shows that the vastly over-simplified description of metabolism which we have adopted, describing it as just a transfer of the major elements, H, N, O, C, P, S (in particular oxidation states), between metabolic paths, has a core of reality in that there are small organic molecules, co-enzymes, which are designed to carry separately and in large part these six chemical elements. They are carried in the simplest chemical forms which biological systems could manage, *i.e.* A—H^- or A—H(H), A—CH_3 or A—CH_3CO(C), A—PO_4(P), A—SO_4(S), A—NH_2(N), while oxygen is carried as A—$OH(H_2O)$. Table 3 describes the individual co-enzymes, A, which both transfer metabolites and, see below, play a large role in metabolic control of their transformation.

The transport co-enzymes A are found to be present in constant large amounts in cells in virtual steady state:

$$A + X \rightleftharpoons AX \qquad (1)$$

where $T_A = [A] + [AX]$ is the total amount of A, the diffusing carrier of the input X, which is the transported group and which can be a part of the path of a given metabolic flow or of two flows (see feed-back in Scheme 3). A can be NAD, NADP, ADP, thioredoxin, or glutathionine, co-enzyme A, *etc.*, and X can then be H, phosphate, e^-, or CH_3CO^-, respectively. The control is essentially a fine control over a range of rates of flows of metabolites which are regulated by hydride-reducing potential ([NADH]/[NAD]), phosphate potential ([ATP]/[ADP][P]), redox potential ([thioredoxin]/[thioredoxin^{2-}]), or acetyl potential ([Co–A–acetyl]/[Co–A]), *etc.* The limits of such steady states are not very widely variable since all the carriers, usually co-enzymes, are present in about equal molarity with the receptor sites, all are present at around 10^{-3}M, and their binding constants are around $10^4 M^{-1}$ for both forms of the carrier. The limit of effectiveness of the control is roughly from 90% to 10% of one form, *i.e.* two decades in the ratio [A]/[AX]. In fact, even this swing is probably much greater than that which occurs in most cells.

As all the above co-enzymes are in quite high concentration, changes are clearly relatively slow and all these systems of controls are in effect well buffered.

Table 3 *Some basic element flow units of biology*

Metabolite (element)	*Reactant and control carriers (co-enzymes)*	*Protein-catalysed reactions*
Electron (e^-)	Metal ions, gluthathione	Many, *e.g.* energy conversion, oxysugar reductase
Hydride ($\vec{H}$)	Pyridine nucleotide (NAD)	Many, *e.g.* energy conversion
Methyl ($\vec{C}$)	Methionine	Methylation
Acetate ($\vec{C}$) (and other carbon fragments)	Co-enzyme A	Fatty-acid synthetase, *etc.*
Phosphate ($\vec{P}$)	Adenosine (guanosine) triphosphate (ATP)	Many, *e.g.* glycolysis and oxidative phosphorylation, DNA, and RNA synthesis
Nitrogen ($\vec{N}$)	Pyridoxal phosphate	NH_3 exchange
Amino-acid ($\vec{N}$)	Adenosine (guanosine) monophosphate (AMP)	Ribosomal synthesis of proteins
Sugars ($\vec{C}$, $\vec{H}$, $\vec{O}$)	Uridine diphosphate, dolicol phosphate	Many, *e.g.* glycoprotein formation
Nucleotides ($\vec{C}$, $\vec{N}$, $\vec{O}$)	Nucleotide phosphates	RNA/DNA synthetases in nucleus
Hydride/electron ($\vec{H}$), ($\vec{e^-}$)	Co-enzyme Q (quinone)	Energy conversion (membrane)
Sulphate ($\vec{S}$)	Adenosine phosphate–sulphate (APS)	Sulphonation
Energy ($\vec{\Delta G}$)	Triphosphates	Energy utilization
Light quanta ($h\nu$)	Chlorophyll	Energy capture (membrane)

Note Although there are carriers for H, C, P, N, S, and e^-, there are no simple carriers for O. Rather, this element is carried in carbon compounds or H_2O or O_2. Note different carriers for different oxidation states

This means that, for example, the redox control of thioredoxin (glutathione) extends from -0.15 to -0.25 volts at the most and does not interact with the region of control of NAD (NADP) at around -0.45 to -0.35 volts. Chemical recognition of the large co-enzymes gives great selectivity to even redox control which would not be present if simple cations or anions had been used in redox control. [The main disadvantage of the free redox cations (Fe^{2+}, Cu^{2+}) is that

they would introduce general non-specific one-electron redox reactions into the cell reactions. In fact, such *one*-electron steps are clearly confined to within particles and membranes; compare the immobile cytochromes, flavins, ferredoxins, and azurins with the above mobile two-electron redox reagents – see below.] All these internal controls of cells are quite general to a wide variety of cell types. This is not surprising as all cells must move H, C, N, O, P, and S in very similar reaction pathways, keep oxidation-state control, and get energy from the same metabolic paths. More details are given later.

There is also a second way in which these steady-state controls of material transfer in the aqueous phase are working. The first was just by the rate of supply of metabolite from one reaction path to another. For example, the supply of H^- depends on the rate of the production of NADH, *i.e.* ([input].[NAD]), and its removal, *i.e.* ([action].[NADH]), and on the rate of diffusion. This is just a hand-on of metabolites from path to path. The second control is that NAD and/or NADH can be used, but not metabolized, as allosteric controls in the input or action machinery, *i.e.* feed-back or feed-forward controls of enzymes (of catalysis); see Scheme 3. In this case NAD and/or NADH are not acting as hydride carriers but as information carriers telling the input or action circuit of the total [NAD] + [NADH] in circulation and the degree to which NADH is bound by hydride, *i.e.* the [NAD]:[NADH] ratio. The use of carriers of metabolites as *allosteric* controls of the catalysts has an obvious economic advantage. We shall observe later that much of this control is not only exerted on single free enzymes but on large multi-enzyme systems either in particles or in membranes. We must enquire about the workings of these organized units, and we shall see that virtually all their activity is also controlled through special additional proteins.

The above controls are within the aqueous phase but similar controls are possible in membranes. For example, co-enzyme Q, a quinone, is in high concentration and is freely mobile in the membranes of phosphorylating particles, and no doubt the relative concentration of H_2Q and Q controls the activities of these particles as well as acting as a hydride buffer at around $E = 0.0$ volts for membrane metabolism. A somewhat different membrane steady state is provided by the electron flow which is not limited by free diffusion of molecules in all cases but by a specified path of electron carriers in the membrane (or in a particle). This path, electron tunnelling, has controls on it of a strictly kinetic kind to which we refer later, noting here that both the redox potentials and distances between active sites can be controlled by binding of ions or molecules to the proteins concerned. The generation of electrons is usually from the reaction:

$$H^- \rightarrow 2e^- + H^+ \qquad (2)$$

[In the above there is some degree of over-simplification in stating that the co-enzymes act as the only diffusing control links. There is undoubtedly some feed-back from substrates themselves but the generality of control by co-enzymes must not be missed. Mosbach estimates that some 2000 enzymes are known and about one third of them use NAD, Co-A, or ATP co-enzymes (private communication). The second factor which has not been mentioned is that there is

often more than one co-enzyme for the handling of each of the elements H, C, P, e^-, *etc.* While NAD is common on degradative routes, NADP is more common on synthetic routes of hydride transfer. Phosphate is carried by ATP (general energetics) but by UTP (sugar incorporation), and by GTP (protein synthesis). There are at least two different electron-transfer carrier systems. The carrying of carbon fragments by Co–A is made more complicated by the way in which 1-, 2-, and 3-carbon fragment metabolism operates in different oxidation states. The use of more than one co-enzyme for a given element makes it possible for two pathways to be independent over considerable lengths while they are linked at particular points. An interesting example is provided by the flow of redox equivalents. Figure 2 shows that in the mitochondrial membrane there is electron-carrier control alternatively by e^- and H^-/H^+ carriers, *i.e.* one- and two-electron

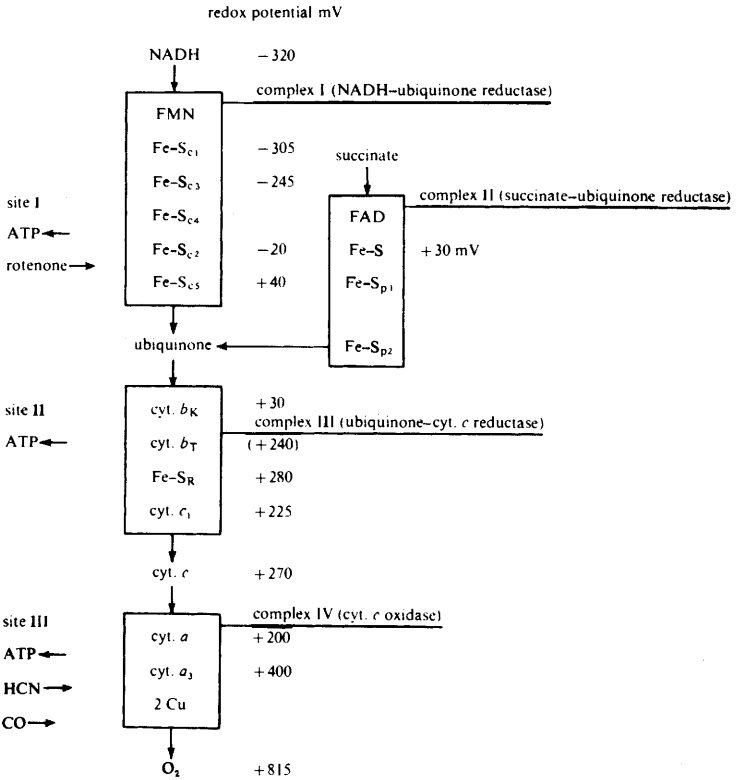


Figure 2 *The transfer of the electron* e^- *in the membrane Reducing equivalents are from* NADH, *succinate, or cytochrome* c. *Passing through four series of fixed sites (metal ions in proteins) shown in boxes, they are linked to proton movements at three sites. The compulsory movement of protons makes* P ~ P *(ATP) (see Figure* 1). *The electrons pass finally to oxygen. Note that there is rough stoicheiometry between all components except co-enzyme Q, which acts as a mobile transfer agent of electrons (as* H) *and as a buffered control.* Fe–S *represents iron–sulphur proteins; cyt = cytochromes*

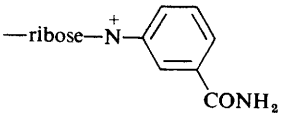
transfer agents, *e.g.* cytochromes and co-enzyme Q. Tapping points from this pathway to the aqueous cytoplasmic pathways of reducing equivalents are made at several points by different reagents, *e.g.* at very low potential the connection is *via* NAD/NADH, at higher potential of the quinones by thioredoxins, and at still higher potential by cytochrome *c*. This is not just a matter of carrying reducing equivalents to oxygen over three inputs for the synthesis of ATP. At the lowest level the whole of hydride (H) metabolism, glycolysis, *etc.*, is connected to the oxidative path. At the intermediate level the thioredoxin (glutathione) potential is connected *via* two-electron reactions to DNA synthesis *via* ribose reductases, and at the level of cytochrome *c* the chain is connected to higher-potential oxidizing agents which also produce reducing equivalents, for example peroxide reactions.]

B. The Energy Flow.—Many of the reactions of metabolism are downhill but of course the synthetic steps are uphill. It is essential to transfer not only material but also energy to them. The energy-transfer co-enzyme is a nucleotide triphosphate, usually ATP. The generation of ATP can be through the downhill metabolism of, for example, glucose (anaerobic glycolysis), but the most important sources are through oxidation of hydrocarbons (oxidative phosphorylation) and photophosphorylation. (I have written ATP as P ~ P in Figure 1 to indicate the 'high' energy of pyrophosphate.) In both the last cases the important intermediate between reducing equivalents and ATP is the proton which is generated at high energy directly either by light or by oxidation (see below) and generates ATP directly.[2,3] The ATP then aids a great variety of metabolic synthetic steps, but it also activates or controls a vast variety of other actions, *e.g.* nitrogen fixation, CO_2 fixation, contraction, and food (substrate) uptake across the outer membrane. The ATP like other co-enzymes can act so as to control enzymes in appropriate catalytic conformations or to transfer energy into a reaction path. This means that there is constant feed-back between the energy status of a cell and metabolism as well as between metabolic pathways. Metabolism and energy are very intimately linked through both hydrogen (H) and phosphate (P) movements.

C. Restricted Diffusion in Particles.—The description of the cell's chemistry so far has been in terms of simple diffusion in homogeneous phases, the aqueous cytoplasm, and the organic membrane. The transfer inside particles (restricted diffusion) must now be analysed. Inspection shows that one major mechanism uses very similar chemical devices to those in the free phases, but the 'co-enzymes' are now retained by the particles, often through covalent-bond formation. Movement by H, C, P, S, and N is then by swinging arm movements[2,4] of trapped molecular subunits of the co-enzymes NAD, ATP, ATPS, or acetyl Co–A (Table 4). The movements of the electron and the proton can be controlled in particles

[2] R. J. P. Williams, *J. Theor. Biol.*, 1961, **1**, 1.
[3] P. Mitchell, *Nature*, 1961, **191**, 144.
[4] G. Hale and R. N. Perham, *FEBS Lett.*, 1979, **105**, 263, and references therein.

Table 4 *Co-enzyme and cofactor swinging arms (group carried)*

Compound	*Swinging arms*
Co-enzyme A, pantothenic acid (acetyl)	$-O-CH_2-C(CH_3)_2-CH(OH)-(CO-NH-CH_2-CH_2)_2-SH$
Lipoic acid (hydrogen atoms)	$-CO-(CH_2)_4-CH(SH)-CH_2-CH_2-SH$
NAD (hydride)	
ATP (phosphate) (energy)	–ribose—P–O–P–O–P–OH

Note All four of the above fragments are held permanently in some enzymes. The first two are held by direct covalent attachment, while the second two are trapped as the whole co-enzyme

in a different way. Electron paths are provided by combinations of metalloproteins in long series of redox couples in space (Figure 2). There is then tunnelling between these centres. Proton movement can be controlled also in particles by H-bond networks especially at aqueous interfaces.[2] The advantages of using almost direct short paths are obvious.

Each individual particle carries out its reaction series before transferring its product to another particle. The product is often carried to a new particle by a free co-enzyme, and this co-enzyme is then recognized by a new domain particle, in which the product of the first particle is further transformed, or where it acts as a restricting allosteric control. The domains, particles, can be freely floating in the cytoplasm or attached to membranes. In the case of attachment to membrane, diffusion between domains in and along the surface of the membrane is possible for H^+ and e^- especially.

D. Transport.—The only source of food for a cell is *through* the outer membrane (Figure 1). The compounds bringing in C, H, N, P, O, and S are never in high concentration outside a cell. Thus, the outer membrane must pump food into the cell, using the energy of the internal metabolism. A gradient of concentration always exists across the membrane. The gradient is pumped by the energy carried by ATP, P ~ P in Figure 1, acting upon localized pumps in particles in the membrane (Figure 1). The pumps must not be reversible since food is more often absent than present. They 'waste' energy in irreversible controls. The pump must then be linked by feed-back from the metabolism of the cell, and the pump is just another way of handling C, H, N, O, P, or S, moving a chemical in space but not in a chemical reaction (compare Scheme 3). Now such transport can also be of charged ions when electrical potentials as well as gradients can be generated, across the membrane, see below.

E. Mechanical and Electrical Steady State.—The mechanical steady state, the shape of a cell, is maintained in many cells by an internal filament structure, linked to the membrane, and by its energy consumption. The energy status of a cell can be roughly related to the quotient [ATP]/[ADP][P]. The tension in the filament of Figure 3 is then dependent upon this quotient and upon any control

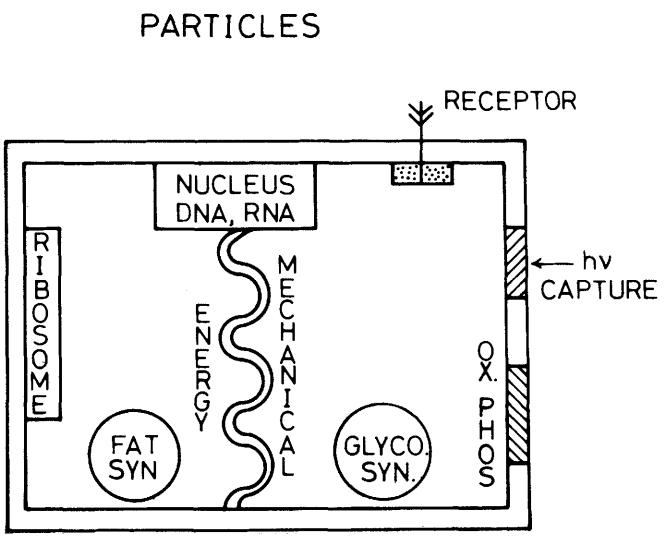


Figure 3 *Some particles in a cell. One filamentous structure is shown connecting the membrane and the nucleus. The diagram is schematic but energy,* P ~ P, *can diffuse between the particles and the filament so that the energy state of the cell has a mechanical and a chemical component*

devices which we may find (see Part II). Thus the membrane is held in tension. The tension will also depend upon external forces. Thus, external mechanical pressures can be sensed by the membrane plus the filaments and then communicated to the whole of the cell. (Note: bacterial cells do not have these filaments.)

An extension of this communication is possible if there are proteins which extend from the surface of the cell and act as sensors. The sensors can be mechanical filaments or chemical sensors. The picture of glycophorin in the red cell membrane is shown in Figure 14 of Part II. This is one way to make connections with the outside world.

[A simple extension of this picture of the cell is now possible. If we allow the energy of the cell to be transmitted through the membrane to outer proteins which are extended as flagellae (see Figure 12) then a mechanical engine is

produced so that there can be a constant drive on the cell. In this way a cell controls its position in space. Alternatively, these extended filaments can be linked together in a cellular colony, and cell–cell control through mechanical triggers becomes possible. A whole range of muscles are built up in this way. Other events of this kind are antibody triggers, surface attachments, and so on.]

The above represents one contact between external and internal solutions across a membrane. It is mechanical in nature. The second communication is both chemical and electrical. Obviously, an isolated cell cannot maintain a steady state of external metabolites or of external energy since it would then work against an infinite sink. However, it can use a differential of non-metabolizable inorganic ions since these are present in all waters, *e.g.* Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, *etc.* It is observed that all cells link their internal metabolism with ion gradients pumping out Na^+, Ca^{2+}, and Cl^-. By making the exchange of internal and external ions on an inequivalent basis in terms of charge, this pumping of ions gives a chemical-concentration gradient and an electrical potential across the membrane (Figure 4). We return to these devices later but it is now clear that the

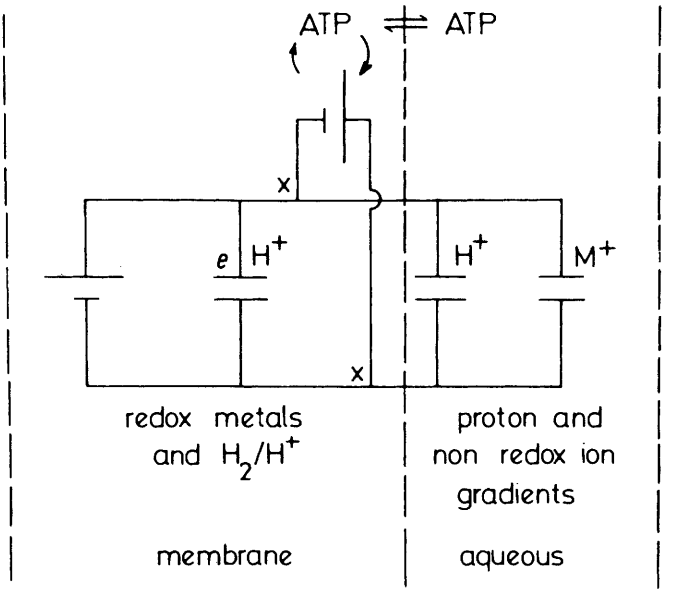


Figure 4 *A circuit diagram linking the movements of* e^- *and* H^+ *in the membrane and its surfaces to movements of ATP and ions in aqueous phases either inside or outside the cell. By siting the proteins, each gradient can be localized (see Figure* 3)

internal metabolic steady state can sense mechanical, chemical, and electrical field gradients external to itself. Figure 1 illustrates the controls of the simple cell confined by one membrane, but the general ideas are applicable to organizations of many cells when the environment includes messages, *e.g.* hormones, from other cells.

While noting the electrical component of the gradient we must not ignore the strong concentration gradient. As far as the steady state is concerned, a major function of the ion gradient is linked to osmotic control. By raising the internal solute concentration above the external, an osmotic pressure is exerted on a membrane which produces mechanical pressure on it and so adjusts membrane protein states. The production of a strong external cell wall, in bacteria and plants, allows the membrane to be forced to the shape of the wall. Here shape is not necessarily controlled by the internal filaments.

Finally, the tension, electrical or mechanical, exerted on the membrane can feed back and cause assembly changes amongst the proteins of the membrane. This means that the chemical activity in catalysis or in transport of proteins in the membrane is under steady-state control. There is then a connection to the internal metabolism. We begin to see that a single cell is not a dissectable apparatus but can only be understood at the level of the whole.

F. Osmotic and/or Local Control.[5]—We now have the following situation. Inside the cell there is a vast variety of continuous chemical transformations that involve movements of H, C, N, O, P, and S *via* co-enzymes and controlled by co-enzymes and inorganic ions (largely) and that produce hundreds of compounds. Simultaneously, energy is being moved from this metabolism *via* pyrophosphate (ATP, another co-enzyme) into a variety of syntheses, into the maintenance of physical stresses required for the structural organization of the cell and into the pumping of food and ions into and out of the cell across the outer membrane. The cell's osmotic and electric potential balances are maintained and simultaneously the cell's control apparatuses are linked to the environment through ion pumps and chemo-mechanical receptors. While most pumped ions, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, are linked to controls only through mechanical handling, one cation, the proton, occupies a special position in that it is directly related as H to the whole metabolism (Figure 1).

The cell now has two very different possibilities which are based on the organization of particles in the membrane and which are predetermined by genetic control. It can be planned so that the cell volume as a whole is isotropic when every volume element of the internal aqueous phase is at one fixed chemical potential of each chemical. (This is the basis of chemi-osmosis). A more subtle development allows a cell to position the input and output of ions, food, *etc.*, in such a way that the cell has forced, fixed directions of flow in the steady state. The cell cytoplasm is anisotropic and uses energy to maintain this anisotropy. At the extreme, input and output could be placed at diagonally opposite poles of a cell when currents constantly flow through it. At another, inputs and outputs are put very close together so that local circuits are achieved. The multitude of devices which can be based on local circuits but not on chemi-osmosis has led the author to argue that chemi-osmosis is not a major device *in vivo*. (See Figure 5.)

When considering the pumping and gating of ions and molecules through a

[5] R. J. P. Williams, *FEBS Lett.*, 1979, **102**, 1.

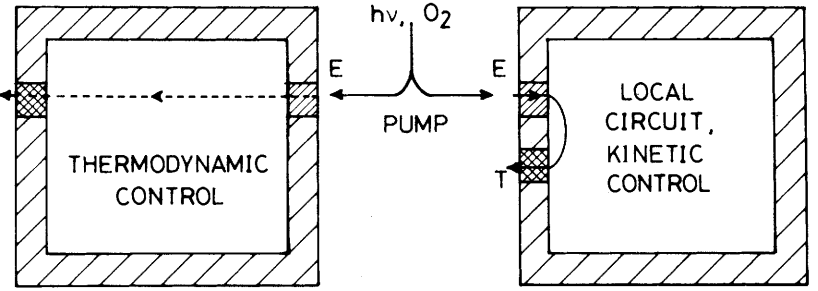


Figure 5 *A schematic diagram illustrating the distinction between a slow fine general control based on an equilibrated steady state of large capacity (left side) across a membrane and a localized membrane control. The latter circuit can be used for very fast response (see Section* 4)

membrane, a quite new type of steady state can arise which is not really possible using free fast diffusion inside a cell. Since the pumps and gates can be localized in different regions of a membrane, control of diffusion will allow steady-state gradients to exist from one region of the cell to another. The cell then reflects the outside environment in the sense that a compass needle reflects magnetic fields. Its internal metabolic transformations are related to the external conditions through a predetermined set of axes within the cell based on cellular organization of particles. Both the metabolic activity and the shape of the cell can be affected so that running through a cell will be differential stress lines and differential flows. The steady state is definable in terms of energy and concentration plus energy and concentration gradients. Such local differences around a cell are of great advantage in sensing and responding to outside conditions.

Finally, note again the role of the proteins in all these controls. We return to this role in a second article. In essence, the flow of the non-metals, H, C, N, O, S, P, and of energy P ~ P in the cytoplasm is controlled by enzymic catalysis, *i.e.* kinetic control over bond-breaking as is general in organic chemistry, and by allosteric control of the catalysts using co-enzymes in two ways. The flow of inorganic ions including the proton is controlled by physical barriers to thermodynamic equilibration, membranes, and the degree of offset from equilibrium is due to energy input at the membrane using protein transport devices. The flow of the electron uses protein organization in the membrane or particles. In all these activities the structure and dynamics of the proteins must be examined if we are to obtain an understanding of the activities (see Part II).

4 Steady-state Control Using Internal Vesicles

While in some cells the control is all at the level of the outer membrane, in others a vesicle is used internal to the cell. In such cases where a cell is divided internally into compartments by a second membrane and one type of metabolism is separated from another by the membranes, then we can increase control devices

from one aqueous phase and/or one membrane phase by adding controls in two aqueous phases across an internal membrane as well as differentially in separate membranes. Some control must now link across the internal membrane, reflecting the two aqueous steady states. Of course, the steady-state controls must remain very general, in high concentration (well buffered), but must pass through the membranes. Figure 6 and Table 5 give examples.

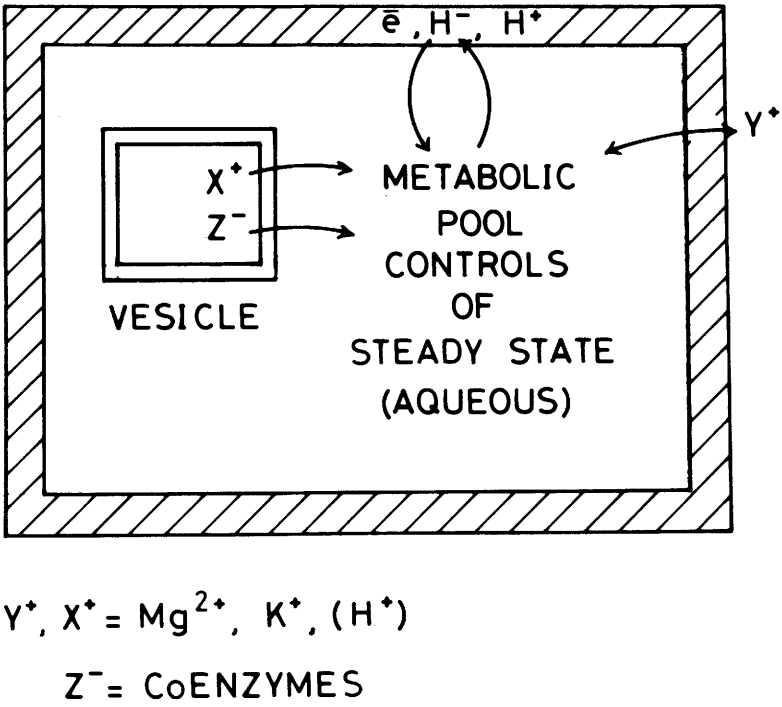


Figure 6 *A vesicle inside a cell is shown as containing both anions,* Z^- *(ATP), and cations* X^+ *(*Mg^{2+}*), which can be liberated to the cytoplasm on reception of a signal at the vesicle membrane* (e.g. *light in the case of the chloroplast*). Z^- *and* X^+ *then affect the cytoplasmic metabolism which is linked to other membrane or external processes* via Y^+. *The diagram applies to calcium triggers of muscle and to the bioenergetics of mitochondria and chloroplasts where* X^+ *flow could be proton movement*

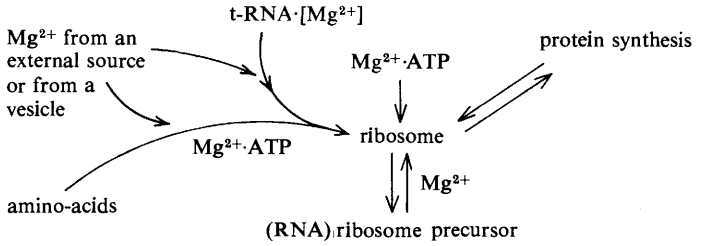
Table 5 *Some 'steady-state' vesicles*

Vesicle	*Control chemicals*	*Function*
Mitochondria	H^+, Ca^{2+}, ATP	Energy from oxidation
Chloroplast	H^+, Mg^{2+}	Energy from light
Plant vacuoles	Organic anions	Store, waste collection
Endocytotic/exocytotic (amoeba)	Membrane controls(?), Ca^{2+}	Ingestion and waste

A. Material and Ion Transport.—The total content of a control chemical can be divided between the cytoplasm and the vesicle solutions, and the steady-state level of an internal chemical can be adjusted not by chemical transformation but by differential pumping between two internal compartments. Consider, for example, the magnesium content of plant cells containing vescicular chloroplasts, chl (see Figure 6). The total magnesium in the cell is fixed, T_{Mg}(cell), but it is in two compartments: $[Mg^{2+}]_{cyt}$ and $[Mg^{2+}]_{chl}$, where cyt is the cytoplasm. The division into these two pools is known to be related[6] to the light falling on the cell, which causes an increase in $[Mg^{2+}]_{cyt}$ from $[Mg^{2+}]_{chl}$. The vesicle membrane contains the light-capture apparatus. The change in $[Mg^{2+}]$ levels can then change enzymic activity in the cytoplasm. [This type of control can well be an osmotic control or a local control (see later) since the surfaces of chloroplasts are illuminated irregularly and unevenly by light, and Mg^{2+} free diffusion is not very fast.] Now this example is particularly important since Mg^{2+} is involved in a vast range of enzyme activities and in RNA and DNA synthesis. The control could then extend to the rate of growth of the plant cells. A similar change of $[Mg^{2+}]$ has been discussed in the growth of *E. coli* cells in the absence and presence of food;[7] see below. Notice control now depends on diffusion control, limited by pumping and gating the Mg^{2+} flow across a membrane and not by chemical transformation in one solution in a variable metabolic steady state, *e.g.* see control by NAD/NADH above. The parallel between Mg^{2+} control and that by co-enzymes inside cells is close in that (i) they both could be directly related to metabolism, (ii) both use high concentration of the messenger material, 10^{-3}M, and (iii) both act on many metabolic paths, Mg^{2+} through control of phosphate metabolism and NAD through control of hydride metabolism. Neither of them would seem to need amplification and they are both slow fine controls working over relatively narrow but high concentration ranges. We shall find that calcium ions and cyclic AMP behave in a quite different way. It may well be that potassium ions act like magnesium ions, but sodium ions do not (Figure 6 and Figure 10).

Signals can also be sent directly to particles rather than to enzymes. For example, ribosomes (Figure 3) are stabilized by Mg^{2+} ions so that the very existence of ribosomes is related to $[Mg^{2+}]$. The critical concentration range is around 1.0mM. For animal cells this concentration is maintained by the blood stream, but for unicellular organisms in fresh water the external concentration of magnesium is $< 10^{-5}$M. (Note by way of contrast the high magnesium concentration in the sea.) Unicellular organisms in fresh water must pump magnesium into cells across the outer membrane. Pumping requires energy. Internal $[Mg^{2+}]$ is then again related to convertible energy levels. Protein synthesis *via* ribosomes is connected therefore to $[Mg^{2+}]$ in a very complex way, using Mg^{2+} pumping across many membranes (see Scheme 4).

[6] J. Barber, Ciba Foundation Symposium 'Chlorophyll Organization and Energy Transfer in Photosynthesis', 1979, **61**, 283.
[7] J. E. Lusk, R. J. P. Williams, and E. P. Kennedy, *J. Biol. Chem.*, **243**, 2618.



Scheme 4

Returning to the vesicle-containing cells, *e.g.* chloroplasts, we can express the $[Mg^{2+}]$ steady-state control once it is set up under, say, a steady flux of light as

$$\Delta G(\text{light flux}) - \Delta RT\ln[Mg^{2+}]_{chl} = \Delta RT\ln[Mg^{2+}]_{cyt} \qquad (3)$$

where an efficiency factor should be included for the conversion of light into usable work for magnesium pumping. The magnesium-concentration changes are probably similar to potassium-ion changes in plant cells and both may be related to proton-concentration changes in some way (see Figure 6). The above equation should be compared with that for the ADP/ATP couple

$$\Delta G(\text{metabolism}) + \Delta RT\ln[ADP]_{cyt} = \Delta RT\ln[ATP]_{cyt} \qquad (4)$$

where internal cell energy is switched about using covalent-bond synthesis (no membranes) rather than using concentration gradients of cations.

A vesicle in a cell can also be used to divide metabolic activities, not just concentrations of inert transmitters such as Mg^{2+}, so that one flow of *metabolism* is inside the vesicle, *e.g.* mitochondria carry on Krebs cycle reactions, while the other major metabolic pools are in the cell cytoplasm (Figure 6). In this case the control levels of the usual steady-state control chemicals, *e.g.* NAD and ATP, Z^- in Figure 6, can be differently poised in the two aqueous phases, and there is not equilibrium *across* the mitochondrial membrane. Control is now by the pumping and gating of the steady-state transformable co-enzymes, particularly ATP/ADP, as well as by the pumping and gating of the diffusion of non-transformable ions such as Ca^{2+}. (Mitochondrial activity is known to affect calcium-ion levels in the cytoplasm.)[8] Once again, mitochondria are not evenly distributed in cells, moreover, like chloroplasts, they can have a very large weaving recticulum which inserts into particular structural features of the cell (Figure 7). Control can be local and indeed it is often likely to be local since a diffusion gradient will be established from the chloroplast or mitochondrial surface to the extremes of the cell cytoplasm (see Section 7, 'The Shapes of Cells and Vesicles').

The pumping of calcium by mitochondria[8] and of magnesium by chloroplasts means that in a plant cell two different types of vescicle are using two different

[8] A. L. Lehninger, E. Carafoli, and C. S. Rossi, *Adv. Enzymology*, 1967, **29**, 259.

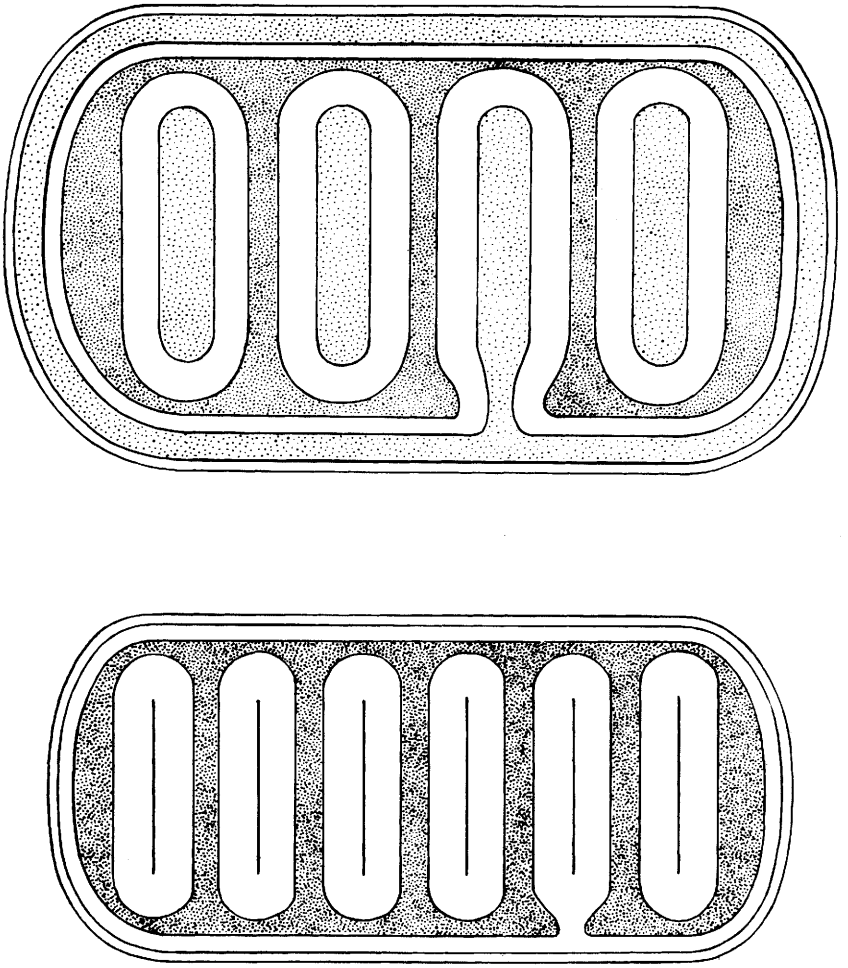


Figure 7 *A frequently observed design of a vesicle membrane is shown. The outer membrane is a simple volume-limiting shape, but the vesicle membrane is highly convoluted and its space is controlled by mechanical changes based on the energy status of the vesicle; above de-energized, below energized. In the diagram a section of the vesicle membrane is seen and the four inner compartments are continuous. The diagram applies to mitochondria, chloroplast, and sarcoplasmic reticulum membranes*
(Reproduced by permission from *J. Ultrastructure*, 1979, **547**, 417)

cations to regulate metabolism so that there is no confusion of purpose.[9] In muscle cells there is another vesicle, the sarcoplasmic reticulum, which pumps calcium, but it is specially disposed around the fibres of muscle. Release of this calcium triggers contraction and several enzymes, but this action is not part of

[9] R. J. P. Williams, *Biochem. Soc. Trans.*, 1979, **7**, 481.

the steady state – see below. All three vesicles described here have in common a weaving invaginated membrane to allow local differentials in function.[9]

The steady state is now very complicated (Table 6) since there are two pools of

Table 6 *Some steady-state controls*

Input	*Transmission device*	*Receptor*
X = H^-, P, *etc.* from metabolism	Mobile co-enzymes (and substrates)	Second metabolic path of allosteric protein
e^--donor	(*a*) Direction of tunnelling in membrane (*b*) Mobile co-enzyme	e^--Acceptor protein
ATP	Tension in filament	Connective fibre membrane protein DNA (membrane-linked)
Mg^{2+}	Opening of channel of membrane	Metabolic path or allosteric protein
Mitochondrial ATP	Transport	Cytoplasm, ATP-binding protein
Na^+ field	Opening of channel of membrane	Field-affected gate

[A] + [AX] to consider [see equation (1)] and an energy input between them. In this case it is not just the ratio [A]:[AX] which adjusts control but also total [A] + [AX] in the pool of each phase. Thus the control influence of vesicles, *e.g.* mitochondria, on the cell cytoplasm works through (pumped) diffusion of [ADP] + [ATP] in the same sense as the chloroplast works through total [Mg^{2+}] while simultaneously it works through the ratio [ATP]:[ADP] in the (plant) cell cytoplasm. (Notice that there is no ATP pumped by some membranes, *e.g.* the thylakoid membrane.) In so far as other steady states, *e.g.* of hydride and acetyl, are linked across the membrane so that different conditions exist in the mitochondrial inner space and in the cytoplasm, the same control elements are exerted by NAD/NADH and by a Co-A/Co-A–acetyl, *i.e.* both total concentrations and ratios are important.

In essence, we are distinguishing now four types of steady-state control: (i) that based on the total concentration level of a carried component of metabolism belonging to two paths, *e.g.* H^-, (ii) that based on the total concentration of the carrier of a component of any one path, *e.g.* NAD + NADH, (iii) that based on the organization (particles) and use of enzymes in the cell, (iv) that based on a physical constraint on the diffusion of the carrier or a required component for the use of a carrier, *e.g.* Mg^{2+}, by a compartment barrier. In all cases control can use chemical synthesis and degradation and/or physical restriction as message-controlling devices. An organic compound can be used in all four ways, *e.g.*

ATP (see below), but an inorganic ionic device, *e.g.* Mg^{2+}, can only be used in control by confining it physically. This distribution will also apply to switch controls. Finally, note that in so far as there are many different membrane-enclosed compartments there will be many different possible exchanges of information and that all can be controlled differently (see below) since each membrane acts as a differential energy input.

B. Electrical and Mechanical Control of Vesicles.—Many cells constantly change shape, and some cell outer membranes regularly break into internal vesicles which cross the cell only for their membranes to recombine with the outer membrane. This type of endocytosis/exocytosis activity enables food to be digested as the vesicles are transferred across the cell. This activity is an extension of the mechanical dynamics of the cell membrane under the influence of some fibrillar internal 'muscular' activity. Other vesicles appear to be moved around within the cell. More noticeable are the changes in membrane structures of vesicles themselves in different cell steady states, indicating that their membranes are in adjustable tension locally, much as is the outer membrane (Figure 3). The parallel with the outer membrane is again clear in that electrical potentials, due largely to the transport of inorganic ions, are generated across the vesicle membrane surface, and once again they are undoubtedly variable at different points along the membrane. The vesicle generates ion gradients within its volume as well as across its membrane. (In passing, note that some cells use undulating or breathing motions of their outer membrane to circulate the cytoplasmic fluids and vesicles.)

C. Position of Vesicles and Particles within Cells.—Given the complexity of cells which have many vesicle systems it is obviously advisable to look at the organization of them relatively to one another. Similarly, we can ask if all the large particles which act in different metabolic paths are organized or not. This point has not yet been tackled by biologists in any depth and I can only give a general impression from my own reading. My conclusion is that there is organization which can be adjusted. For example, mitochondria and chloroplasts are placed where they are most likely to be needed, *e.g.* around muscle fibres and near drive points for locomotion. The organization is seen at all levels of the positioning of vesicles and particles in cells. Questions then arise as to the mode of organization which prevents free diffusion of particles and vesicles but allows their ordered transference. It would seem to be likely that the particles and vesicles move along particular chemical-potential gradients to points where they can assist function. The movements can be through the cytoplasm or along the membrane. Internal cell organization is dynamic and related to external cell movements along gradients and/or to cell–cell organization.

D. Summary of Value of Vesicles.—Vesicles allow an extra dimension to the partitioning of cell material and energy based upon diffusion control. They allow a greater degree of separation of function while allowing ion currents to

control *internal* activities of the cell cytoplasm in a way which was only possible across the outer membrane in a cell without vesicles. Ions such as calcium and magnesium are then made into major internal steady-state control elements in addition to co-enzymes. While particles already allowed the movement of organic groups to be regulated by the swinging of covalently attached units (Table 4) this is not a possible mode of control of movement of ions. Instead, energy is used to give potential and concentration gradients across vesicle membranes. The vesicle membranes are usually highly convoluted so that local domains control function. The vesicles can also restrict movement of free organic molecules. The vesicles sense chemical-potential gradients in cells and respond to them. Chemical-potential gradients include here chemical concentration and all types of field. The gradients in cells are now exceedingly complex even in the steady state.

E. Storage Vesicles.—There is a class of vesicle which cannot be included easily in the description of the steady state as used here since it involves other activities of the cell, particularly triggering rapid responses. If during metabolism a cell deposits chemicals, some of which are products of metabolism, in a vesicle then, although these chemicals are removed from the steady state, they are not waste, rejected, products but are stored. Storage vesicles of many chemicals are listed in Table 6. The storage is of both salts and organic materials (including proteins). The re-entry of these stores into metabolism can only be achieved by breaking of the vesicle which is clearly to be included under triggering control not steady-state control since it will only come into play on a change in demand from the environment. In particular, we note the storage of calcium ions, transmitters, hormones, and digestive enzymes (see Figure 10).

F. Cell–Cell Organization.—An alternative way in which biological systems extend organization and separation of function is through the use of organized patterns of cells. In an advanced organism the outermost cells can be likened to the outer membrane of a single cell and the internal organs can be likened to vesicles. Cell–cell communication and control is through chemical, electrical, and mechanical methods, much as in the single cell, and despite the increase in complexity both in chemical messengers, *e.g.* hormones, and in the number of different physical barriers to diffusion, *e.g.* the different cells are organized to form membranes from cells, the nature of the communication network is not altered in a fundamental way. In this article, therefore, we shall continue to analyse the properties of single cells, assuming that multi-cellular systems are not really different in kind.

5 Loss of Steady-state Conditions

We have treated all cellular alterations as slow buffered changes from one metabolic condition to another. In this case the response of the whole cell was involved since the rate of change of conditions was slower than the rate at which internal cellular systems could be altered. The time periods involved could be of the order of seconds to allow internal equilibration in a large cell, well buffered

by various controls. Living cells also respond very rapidly to the first hint of a change in the environment. We must suppose that there are local effects at particular points on, say, the outer membrane which respond by sending very rapid messages to the cell. The first photons captured by an algae must start it off toward the light source and it must not wait for an internal steady state. This means that we must examine how a rapid switch can be made – a redirection of the steady state – so as to take advantage of knowledge of external gradients. The new knowledge of the environment needs to be amplified for in itself it will not have sufficient strength to adjust the whole cell metabolism. Only when the cell finds itself in a new constant environment will a steady state be re-attained. At all other times gradients of all chemicals within the cell will be changing in given directions. The cell will respond to these gradients by adjusting locally shape, electrical potential, and chemical pumping. These asymmetries set up currents of ions, energy, and chemicals within the cell volume which are used to activate the cell's response further through amplifiers.

6 Switch Controls: External Stimuli

A. Chemical Switches.—This second type of control is usually based upon sudden or pulsed release (creation) of chemicals from a store and is represented by the fast control sequences

$$\text{pulsed chemical synthesis} \rightarrow \text{action} \rightarrow \text{degradation} \tag{5}$$

$$\text{pulsed entry} \rightarrow \text{action} \rightarrow \text{exit} \tag{6}$$

The first needs a synthetic device for initiation *inside* the cell, which must be switched on from outside the cell, followed by a degradative device for removal of its products. It also requires a store of a precursor in high concentration. The second control is mechanical as much as it is chemical; its description is in terms of gates to entry and pumps for exit. It requires a store of the transmitter or messenger itself *outside* the cytoplasm of the cell. The *input* to these controls is in no way necessarily connected with cellular metabolic paths; contrast co-enzymes. The relationship to the steady state is that either the steady state provides the store of precursor in the synthetic switch, usually ATP goes to *c*-AMP (cyclic adenosine monophosphate), or it produces a very high concentration gradient of ions across the membrane of the cell. It is the stores of external ions and of organic co-enzymes which are switched to give a new signal due to a sudden change in external environment.

We take the first case (equation 5) to distinguish switch from steady-state controls. Consider two types of control based on ATP:

$$\text{ATP} \rightleftharpoons \text{ADP} + \text{P (steady-state control)} \tag{7}$$

$$\text{ATP} \rightarrow c\text{-AMP} + \text{P}_2 \text{ (synthesis) (switch control)} \tag{8}$$

The first is the control discussed above which is readily balanced, buffered, and at high concentration, 10^{-3}M. ADP is held by the energy of the cell in a relationship with ATP written in the general language:

$$\Delta G_{\text{met}} + RT\ln K = \Delta G_{\text{steady state}} \quad (9)$$

where ΔG_{met} is the continuous free-energy input from metabolism which elevates the steady-state $\Delta G_{\text{steady state}}$ of reaction (7), the phosphate potential, away from equilibrium K.

By way of contrast, metabolism does not hold *c*-AMP at a measurable level, $< 10^{-7}$M, and [*c*-AMP] is always raised as a rapidly synthesized short-lived pulse which reflects a more or less transient phenomenum impinging upon the outer surrounds of the cell. The *c*-AMP is produced from ATP stored inside the cell when the outside of the cell receives a message, from a hormone, say. It is a completely unbuffered transient. We can draw a picture of concentration changes of the two types of control as in Figure 8. Note the concentration axes. The time

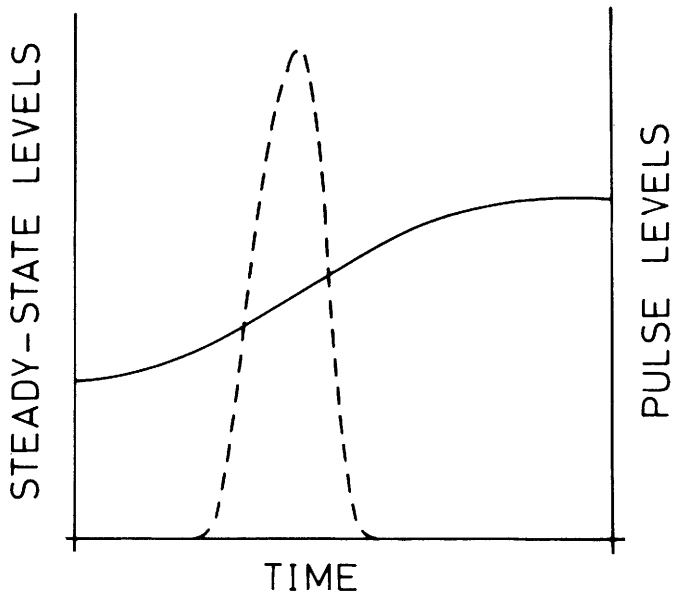


Figure 8 *Slow changes in levels of steady-state concentrations in the range* $10^{-3\cdot5}$—$10^{-2\cdot5}$M (*continuous line*) *compared with pulse-control changes from 'zero' to* 10^{-5}M (*broken line*)

scale of action is also quite different. The *c*-AMP is quickly destroyed by degradative enzymes, giving non-cyclic AMP, which does not work as a trigger.

The requirement for this type of switch control is therefore a protein in the outer membrane which is only an enzyme (for ATP → *c*-AMP) when the cell experiences a sudden change of environment. The protein that undergoes the initial switch from inert to enzyme must then have an external activator, a receptor site, and must respond to this activator by changing its shape (structure) to that of the enzyme form. In a multi-cellular organism the external agent can be a hormone, which may have been released by external changes of light inten-

sity, temperature, humidity, pressure, field, or chemicals, from a stable storage device (vesicle) in a distant cell. (There is no steady state of *c*-AMP during the switch reaction.)

The second way of introducing a transient chemical is to leak suddenly into the cell a chemical carrying the message. Typically, calcium is used in this regard.[9] The normal in-cell level of calcium is 10^{-8}—10^{-9}M. Injection of calcium, the opening of a channel to the external store of $> 10^{-3}$M through an external event, raises the calcium level to 10^{-5}M in about 10^{-4} s (locally much faster). Removal is by pumping the calcium out, and it falls back to 10^{-7}M in some 10^{-3} s. There is no effective steady state. A point which is often overlooked is that the pulse-control energy must be put into the control while the cell is recovering so that low metabolic activity of a cell (rest) is a high storage-energy state and the free energy of the store runs down when action is generated. (In-cell controls parallel in their energy status the metabolic activity of the cell and are highest at rest. Thus, the resting state is high in ATP and low in calcium while action drops the level of ATP or creatine phosphate as the concentration of calcium rises.)

It pays a cell to have very many metabolic flows connected to the same switch-control information, just as we noted the interlinking of the steady-state controls. It follows that all sorts of outside effects will be translated through the same chemical messengers, *e.g.* *c*-AMP, and receptors into the same internal changes of metabolism. In other words, although there are many different cells doing many different things in response to a variety of outside influences, they could use the same type of transmitters and amplifiers, and their final response in large part must be to switch-on energy, which then causes a set of events predetermined by the differentiated character of the cell and its position in the organism.[9] Obviously, 10^{-5}M-*c*-AMP or -Ca^{2+} can do little directly to change the state of a cell which has a concentration of enzymes in a metabolic pool of greater than 10^{-3}M. These messengers must and do act through cascade amplifiers, proteins, which act on enzymes (Figure 9).

A further problem with such a switch in a low concentration of a chemical, *c*-AMP or calcium, is that it must link to the whole cell buffered activity through the steady-state systems. Therefore the amplifiers to which the message is connected must have longer time constants than the half-life of the pulse, for only in this way can they affect the flow of substrates. A typical example is given by the flow of calcium in the triggering of nerve synapses or of muscles. We write this in the following way. The pulse is:

$$\text{high calcium outside cell} \rightarrow \text{influx of calcium into cell} \rightarrow \text{output of calcium by pumping} \rightarrow \text{high calcium outside cell} \qquad (10)$$

Internal calcium cycles here from a low ΔG to a higher ΔG to a low ΔG, *i.e.* rest → action → rest. There is an energy dissipation in this control circuit which is very small but it allows the switch of a considerable energy flux inside a cell since the rise of calcium concentration in the cell is connected to several amplifier devices which themselves are connected into other circuits [equation (11) and Figure 9].

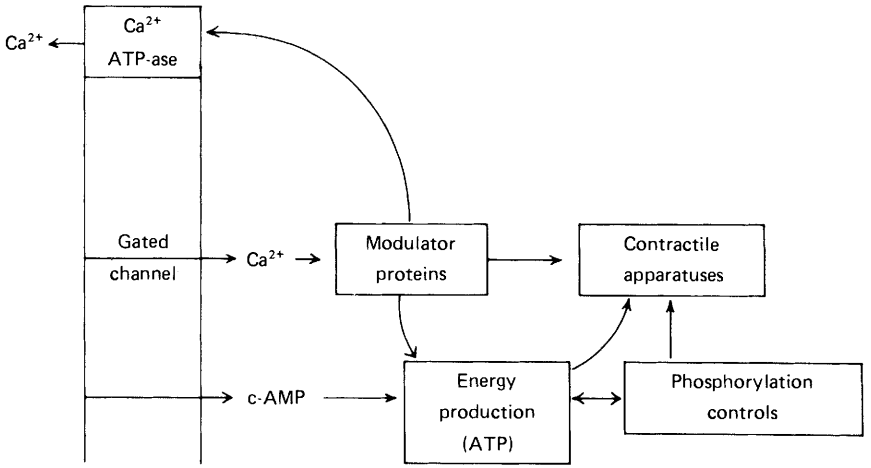


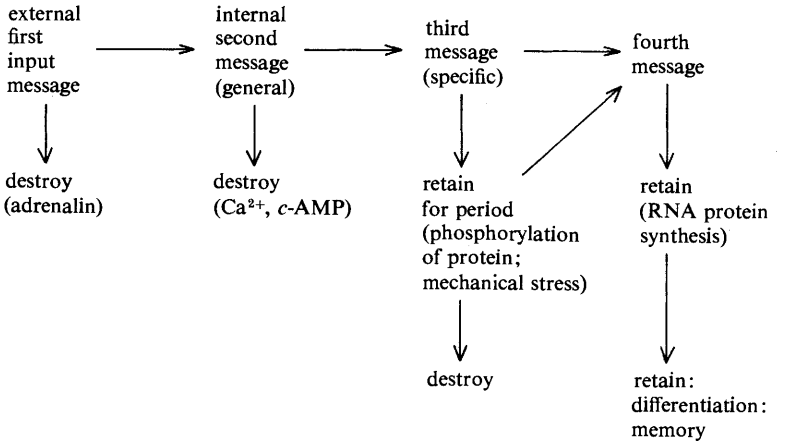
Figure 9 *A schematic representation of the multitude of targets of triggered changes of calcium and c-AMP. It is essential for pulse action but also for restoring the cell back to its steady state*

$$\text{Ca influx} \rightarrow \text{amplifier} \rightarrow \begin{matrix} \text{glycolytic flux} \\ \uparrow \\ \text{phosphorylation } (\rightarrow \text{differentiation}) \\ \downarrow \\ \text{dehydrogenation (oxidative metabolism)} \end{matrix} \qquad (11)$$

The amplifier is the transformation of an inactive to an active state of an enzyme by phosphorylation *via* a protein kinase. The protein kinase is itself an enzyme which switches on many other enzyme molecules by this phosphorylation. The amplified message then sets in motion flow through a metabolic path, *e.g.* glycolysis. Removal of calcium by a feed-back mechanism (Ca^{2+} ATP-ase in Figure 9) which switches on the calcium pump restores the calcium-concentration gradient and prevents further synthesis of the protein kinase, but phosphorylation remains. The action of phosphatases in the cell finally removes the effect of the protein kinase by removing bound phosphate, deactivating the enzymes, and the cell is restored to its initial (rest) condition. There is then a time sequence of rise in Ca^{2+}, rise in protein kinase, rise in enzyme activity, flux of chemicals, and then a fall in the same sequence. Many activities of the cell are then partially controlled by the level of calcium (see Scheme 5). Ca^{2+} and *c*-AMP are often called second messengers.

Now let us consider a second path of metabolism by cells. Just as glycolysis produces ATP so does the oxidative metabolism of the cell. The two must not act independently. In fact, on admitting oxygen to a cell the primary action of mitochondria (cell) is to lower $[Ca^{2+}]$ so that glycolysis is reduced. It is also true that high ATP feeds back and shuts off both glycolysis and oxidative phosphorylation.

[In passing, it is interesting to observe the above differences between the use of *covalent synthetic organic chemistry inside the cell cytoplasm*, employing membrane



Scheme 5

surfaces to make *c*-AMP, and that of *ionic inorganic chemistry employing the diffusion barrier across membranes*, of calcium ions. Dual organic/inorganic control already seen in the steady state is also exerted in these switches of steady states. The use of ATP, a co-enzyme, to make *c*-AMP in the switch synthesis is not unique, and a GTP (giving *c*-GMP) switch reaction is also known. No similar switches are known that use the other co-enzymes. The only other ionic switch message carriers known to the author are the proton and the sodium ion (see below). It is also important to note that after amplification and while the switch control is removed there remains a longer time interval while a covalent chemical modification of enzymes, phosphorylation, acetylation, or even methylation maintains new activity.]

B. Mechanical Switch Control.—In previous sections the chemical messengers of the *steady state* were connected to mechanical stresses through the ATP levels. By making some part of the utilization of ATP dependent upon calcium ions (or *c*-AMP), mechanical stresses can be made a part of switch controls. Thus the filament in Figure 3 could be controlled by an external stimulation (Figure 9). This is the basis of muscle action, and tension can obviously be suddenly developed and followed by rapid relaxation or slowly developed and sustained according to the nature of the impulse received from the environment and the enzyme apparatus linked to the fibres (see the following article). There would appear to be a continuum of possible mechanical responses from steady state to switch changes. It is important to observe that the switch tension develops locally

since calcium diffusion is very slow. The cell responds in an anisotropic fashion according to the locality of the impulse, and this is more closely directed in space by the directions of the fibres. The cell is highly organized by these mechanical links.

C. Electrochemical Switches.—Charge-carrying across membranes introduces a new switch possibility in that if the charge is not compensated it sets up a new electric field, ψ, which can be used as a message device. For example, virtually all biological membranes have a field across them due to the pumping out of sodium ions and their passive but unequal replacement in the cell by potassium ions. The out-of-balance of charge is very small in concentration terms compared with the ion concentrations which are in excess of 0.1M. However, the potential difference is appreciable. If diffusion rates of Na^+ across the membrane, so far carefully controlled, are altered suddenly, then a very small amount of ion diffusion, a change internally (from rest) of some 10^{-5}M—Na^+ in a local region of the membrane, causes an inversion of the membrane potential since it is an uncompensated charge movement. The local change quickly develops an eddy current of influx in neighbour regions so that the whole membrane region changes polarity *without initially affecting the chemical activity of the cell.* A message runs down the membrane with little chemical consequence. Converting sodium movement to a membrane potential switch means that although Na^+ ions are very concentrated they act in the same trigger sense as a calcium flux and not in the steady-state manner of magnesium or potassium fluxes. Now, sodium ions are transmission devices without effect on the metabolism. However, at *certain local* areas of the membrane this Na^+ current opens a calcium channel (Figure 10). Calcium influx locally now has a large *chemical* effect since its influx is connected to the amplifying devices described above, and it can cause discharge of storage vesicles (Figure 10 and Table 7). The exact position of the gates for Na^+ and Ca^{2+}, together with the restrictions on diffusion provided by the shape of the cells, decides the way in which control messages are propagated. The action which follows depends on the connection or lack of it to an amplifier (vesicle store) and thence to metabolism. In this type of device, change of outside conditions at one end of a cell gives local change in metabolism at a quite distant part of the same cell. The cell structure is here the limitation, and it imposes at the time of switch activity considerable internal gradients in cell cytoplasmic chemical potentials, which in turn start currents of all kinds in the cell as well as across the membrane.

Just as we drew attention to the invaginated structures of vesicles which allowed localized activation[5] and different activities to be put in different parts of a membrane, so the exterior membrane of the cell can be involved in long protusions, *e.g.* nerve cells, and very different activities are found in the different parts of the cell surface.[9] It is essential for a full appreciation of the sophistication of biological machines to understand the different chemical gradients which are set up both across membranes and within cells (and their surrounding fluids in the case of organized cell systems) so that most states can only be described in terms of flow and not in terms of concentration or chemical potential averaged over relatively large volumes.

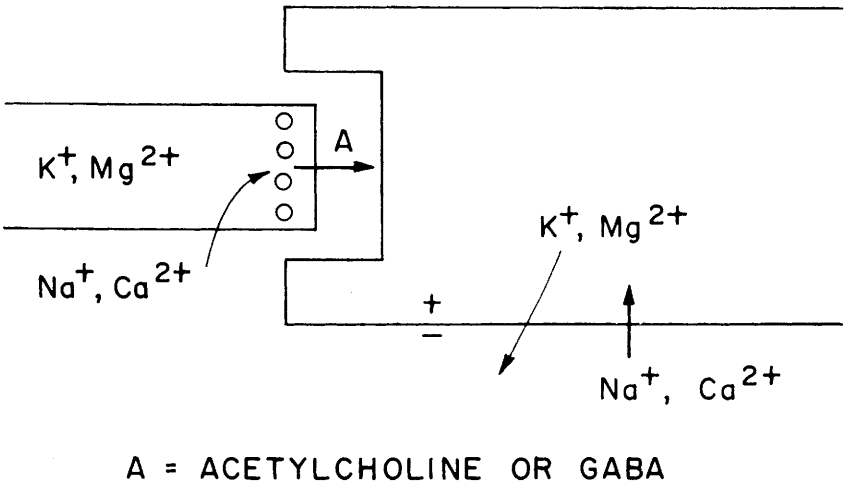


Figure 10 *The left-hand cell has a* Na^+/K^+ *gradient. Influx of* Na^+, *a nerve message, causes eddy currents to flow from left to right until a* Ca^{2+} *channel is opened near* A. *The calcium releases the contents of the vesicles which now trigger the nerve message in the right-hand cell. There follows flow of* Na^+ *and* K^+ *across the membrane of the second cell*

Table 7 *Some internal vesicles for triggering*

Vesicle	*Control chemical*	*Function*
Synaptic vesicles	Acetylcholine, *etc.*	Nerve transmission
Hormone vesicles	Adrenalin, *etc.*	Readjustment of cell steady states
Lysozomes	Enzymes	Digestion
Sarcoplasmic reticulum	Ca^{2+}	Muscle contraction
Mitochondrion	Ca^{2+}, H^+	Energy production
Chloroplast	Mg^{2+}, H^+	Energy production

Note Many of these stores are triggered by Ca^{2+} influx

D. The Proton Trigger.—The proton is a unique chemical entity and is used as such in biology. It can be synthesized, by redox reaction, when the membrane receives a message, O_2 or light (*hν*), and in this sense it resembles *c*-AMP. Its store is just organic reducing equivalents. On the other hand, its production must be coincident with the generation of a negative charge, either an electron in the membrane or an hydroxyl ion in a part of space remote from the site of H^+ production, so that it simultaneously gives a field and a pH gradient. In this sense it resembles a Na^+ or Ca^{2+} ion pulse. Finally, it is an energy-carrying substrate and as such acts on the membrane to produce ATP or other chemical events including transport. A very complex diagram for proton action is shown

in Figure 11. Protons are also selectively recognized like Ca^{2+} and, unlike Na^{+}, are chemical triggers.

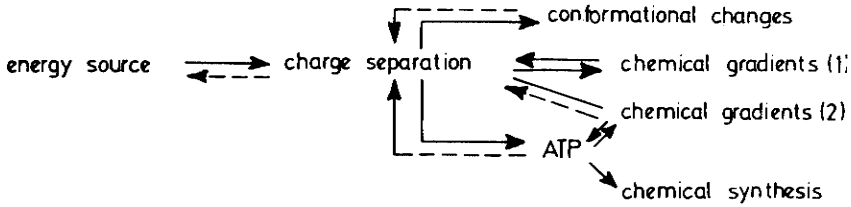


Figure 11 *The initial act of energization of membranes is often just charge separation which generates protons and electrons. The proton causes gradients of ions* (1), *molecules* (2), *and especially ATP, and it affects mechanical and electrical membrane states. Initially the actions are local but prolonged energization will lead to proton gradient storage in vesicles*

The proton migration is only in some ways like that of the sodium ion, and there is one very important difference. Firstly, let us stress the similarity. The proton generates a field and in so doing alters the chemical potential of all positive charges including all protons. Like the sodium ion but unlike the calcium ion the total proton concentration is high. However, unlike the sodium ion (or initially the calcium ion) it is *bound* protons that are overwhelmingly present in the forms of $-NH_3^+$, $-COOH$, $-OPO_3H^-$, and so on. Thus, even at pH = 7 the bound proton concentration is 55M (water), all of which is available for local transport mechanisms. After an external signal all these protons immediately set up general local proton eddy currents. However, there will also be faster specified proton pathways and sites of proton recognition since there is selective binding (pK_a values) by the contributors to the highly buffered proton concentration, *e.g.* the *membrane* proteins and lipids which contribute to a proton pool. The free energy change due to the field is then largely that of *bound* protons in the region where the field has developed. *Immediately* on generating a field there is a force acting on protons bound near the point of generation of the field, and general proton flow can commence in special paths. (Note that this does not just refer to the free aqueous protons.) Overwhelmingly, this flow will be in the membrane and it will occur in any nearby membrane channels which permit downhill proton flow (compare the opening of sodium channels). In one case the channels are of the ATP synthetase (Figure 4). Energy capture is triggered.

Now let us consider a pH change as compared with a change in $\log[Ca^{2+}]$. $\log[Ca^{2+}]$ was affected locally initially and slowly diffused in the highly charged medium, being held up, buffered by the protein negative charges, local binding. It did not generate a prolonged steady state but was a rapid fluctuation (Figure 8). The effect is usually very localized. But note that migration is of the exact species which enter the cell. Contrast a pH change. There are protons everywhere due to the water and the membrane surface. Within 10^{-6} s the pH change has spread through Grothus mechanisms some distance from the site of entry, but the fastest

migration is within the membrane phase which has acid/base groups that catalyse proton migration. Before bulk pH changes can occur, sites, *e.g.* the ATP-ase channels that are sited *very near* the site of proton generation, must undergo a drop in local pH. This drop will only be *reduced* as general equilibration occurs. Pulsed or triggered ATP production will then short circuit the setting up of steady-state gradients. The very construction of mitochondria and chloroplasts, which are the sites of ATP production, with long tubular sections (compare nerve and muscle cells) is of a design which assists this domination of local overbulk effects (Figure 7). Not only is the physical construction correct but the regular placing and very high concentration of ATP-ase units near sites of proton production are exactly correct for such a mechanism. Subsequent steady-state changes as in chemiosmosis are then a useful storage device and a useful steady-state device, but protons can be used both in this sense and in triggering.

E. Internal Vesicle Switches – Relays and Mechanical Switches.—The difficulty with a switch which requires a source of, say, calcium is that the external environment is not very high in calcium, say 10^{-3}M; moreover, this store is general to the whole cell. A better device is made from a relay of the initial switch activity from outside to a local very high concentration of calcium. This requires vesicle storage of calcium to be released by calcium and is a very simple amplifier. Just as the chloroplast is used as a vesicular source from which magnesium is released on exposure to light (see Section 4A) so is calcium released from a vesicular source by a pulsed extra-cellular trigger signal in many cells. Striking examples are in the fertilization of eggs or in the activation of contraction in muscles. In the last case the switch is no longer chemical or electrical but is mechanical transmission (Figure 12). The calcium trigger is then effectively localized. Organic transmitters such as acetyl choline may also be released in this way.

F. Storage Vesicle and Triggering.—While a vesicle can act as an independent metabolic pool and a trigger amplifier, it can also act as a more permanent store or a depository for waste. The materials to be stored could be substrates, but on the whole a vesicle is an unnecessary complication for this purpose since H, C, O, N, and P can be stored in polymers, *e.g.* glycogen, protein, polyphosphate. More useful vesicle storage is of catalysts or poisons not required in the steady state but obviously required irregularly, *e.g.* they are switch enzymes or inhibitors. This is very frequently found in that poisons, enzymes for digestion, hormones, and enzymes for protection are stored in vesicles well removed from the steady state, to be released only when danger or food is present in the environment by fusion of the vesicle and the outer cell membrane (Figure 13). The storage of waste is interesting here. Two examples stem from the inevitable intake of calcium and silicon. Both are relatively easily rejected through cell membranes but can also be rejected to an inner vesicle. Once concentrated there they can be turned into solids and thence on 'rejection' from the cell into shells and bones. The steady states of calcium and silicon in animal and plant life help to form the structure and the marvel of shape and form in biology.

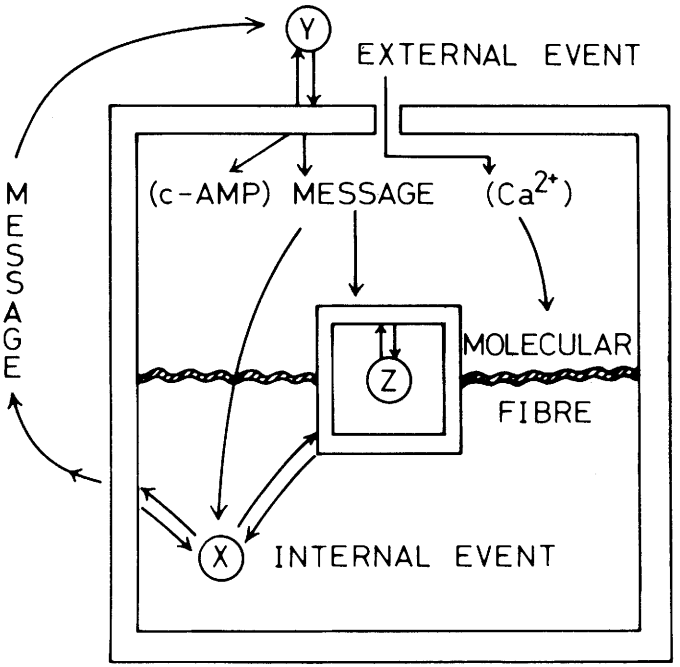


Figure 12 *An external event triggers the influx of calcium ions which can act directly,* e.g. *on a fibre or on a vesicle, to cause release of* Z *(calcium or other ions internally) which then acts at* X *to cause a message externally and which could back-react through* Y. *This feed-back is schematic, and several cells could be involved when the message could be that of a hormone*

7 The Cell Anisotropy: The Shapes of Cells and Vesicles

A running assumption in many discussions of cell chemistry is that we can legitimately use concentration terms. In steady states of isolated cells this is likely to be correct but it is not likely to be correct when a cell is undergoing its activity in an environment which has no symmetry. For many cells their place in an organism makes them extremely asymmetric, and their functions include making a connection between fluids of different kinds. Similarly, the photoactive surfaces of chloroplasts can only face the light along certain edges. This asymmetry will generate asymmetry in concentration terms, and in many cases the positioning of enzymes in the membranes has evolved to meet this anisotropy. There are two ways in which this can be done. One is to repeat the organization regularly all over the cell but to activate it locally, *e.g.* mitochondria and chloroplasts are probably organized in this way (Figure 7). The second method is to locate particular enzymes in particular areas of the cell. Clearly, epithelial cells are designed in this way. It would now appear to be necessary to develop a theoretical framework of local concentrations and diffusion control. This will not be attempted here although it, and not theory based on generalized steady-

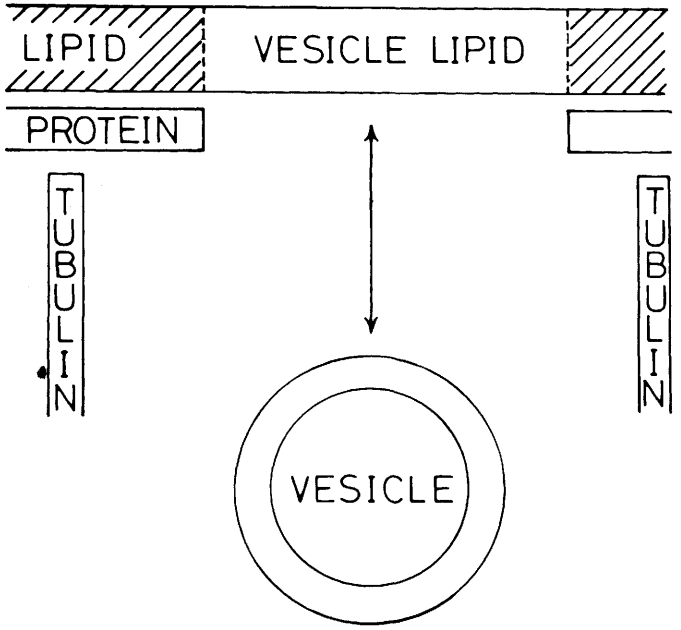


Figure 13 *Triggered loss and recovery of a vesicle where the contents of the vesicle are rejected outside the cell,* e.g. *neurotransmitter, neurotoxins, digestive enzymes,* etc.

state concentrations in cells, is certainly correct. Thus, anisotropy of cells allows the development of continuous flow from one site to another. The flow can be of charge carriers when a current flows in the cell continuously. Of course, the flow could be in the non-aqueous as well as in the aqueous phases. We consider the flow of the electron in the non-aqueous membrane phase first.

Before entering upon this description we must stress that it is not just the placing of individual enzymes in cell organization which gives the anisotropy. The construction of the whole cell (or organism) has developed in response to evolutionary pressure so that each cell has a shape based on the structural proteins in the membranes. Enzymes are then placed not just on the left- or right-hand side of a cell but in very special localities. The placing of vesicles is equally specified.

8 Electronic and Electrolytic Components of Biological Cells

The electronic circuits of biology are hop-conductors, metal ions, or, unusually, organic aromatic molecules, in protein matrices of particles or membranes. There appears to be a general rule that distances from 15 Å to at most 30 Å could be crossed, assuming a dielectric constant of 5. The rates of transfer of electrons remain fast with unimolecular rate constants of $\geqslant 10^3\ s^{-1}$ and the transfers are not then rate-limiting. Detailed analysis has been given at recent symposia. The

electron paths may be as long as four or five centres before the electron circuit meets a chemical reactant, *e.g.* O_2 (Figure 2). Again, the circuit may deliberately change from inorganic metal electron carriers to organic carriers so as to give rise to proton-linked motions of the electron. It is unusual for inorganic electron transfers, $Fe^{II} \rightarrow Fe^{III}$ or $Cu^{I} \rightarrow Cu^{II}$, to be pH-dependent, but the redox reactions of non-metal (organic) systems involve the ionization of protons, *e.g.* $O_2 + 2e^- + 2H^+ \rightleftarrows H_2O_2$, quinone $+ 2e^- + 2H^+ \rightleftarrows$ hydroquinone, and $NAD^+ + 2e^- + H^+ \rightleftarrows NADH$. Thus, this change from inorganic to organic electron carriers allows the migration of the electron to create local proton gradients as it passes down conducting systems of the kind inorganic → organic → inorganic → organic. The electron potential-energy drop in the circuit can then be used to generate proton gradients which in turn and in a separate circuit that is electrolytic (protonic) can be used to connect to other electrolyte circuits Na^+, K^+, and to the production of ATP (Figure 2). Thus, electron flow is linked to different chemistries and gradients. The suggestion that such a scheme held was put forward by myself and Mitchell in 1960[2] and was first demonstrated by Mitchell.[3]

In man's electronic circuits there are other devices than wires and power sources. We have switches, condensers, valves (gates), and so on. Careful inspection of the proteins which carry electrons suggests that metal centres in them can be switched on and off by local binding to the proteins of, for example, protons. Again some metal ions are clustered around active enzyme sites to make a condenser. We do not understand the sophistication of these devices yet, but it is becoming apparent that the electronic circuits are highly regulated through the properties of the protein matrices which have controlled dynamics.

In man-made electronic circuits devices using one current carrier, the electron, are linked by connection through wires (conductors of special spatial construction) and then by restriction of flow by limiting the numbers of current carriers in a circuit (resistors), by circuit breakers (switches), by current regulators, valves, and transistors which are triggered above voltage thresholds, or by condensers which store electrons up to capacity limits. The circuits of electron-conducting components are surrounded by electron insulators, air or plastic. This scheme, although it holds in the above organic phase of membranes, is little used in biology. The more usual circuits of biology start not from generalized insulating space with inserted threads of conducting materials, wires, in designed organization but from generalized conducting space, water, with constructed thread-like *insulators* in designed patterns, fats in membranes (Figure 14). Moreover, this biological device has multiple current carriers, H^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and organic anions and cations. Circuit design for any one carrier now depends on the controlled breakage of the insulation threads made by aqueous paths across and along the insulator thread. Since the insulator thread can have a detailed spatial construction with chemically specified paths for the different current carriers it can make a multitude of different connections from within one large conducting aqueous domain, the cell cytoplasm (Figure 14). However, it is also possible to have within the insulation thread differential power sources, *i.e.* very different chemical potentials of the current carriers are generated in the

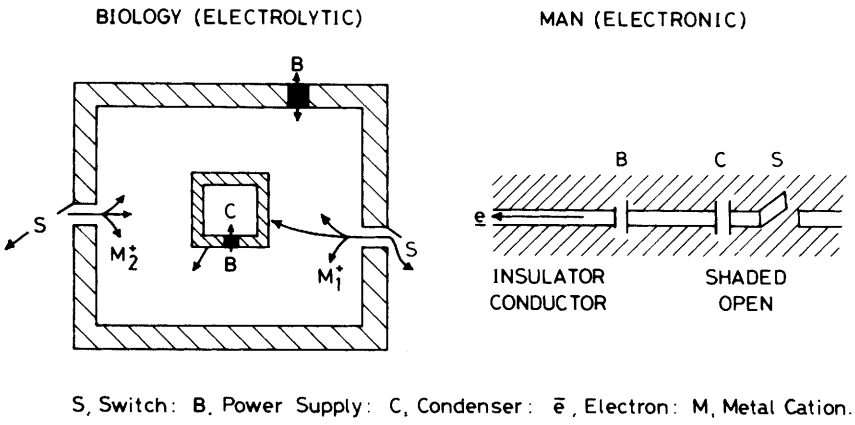


Figure 14 *The construction of electrical or chemical circuits. On the right is shown a typical electronic circuit both of man's devices and of cell membranes. On the left is an electrolytic device which can also be a chemical device, used in biology (see text)*

general conducting domain through controlled use of energy in the generation of different concentration gradients. Additionally, there can now be localized points of generation of power to the individual ions (localized pumps) and localized gates. The design permits specified current flows of different ions. An overall circuit design is now as shown in Figure 14. Steady small monitor currents flow continuously in the cell in different directions, but they can be triggered to give much larger pulses by opening the channels specifically. The channels are variable resistors since they often occur in clumps.

Refinement of the circuit, which is really a set of positional power sources plus resistors, is to include capacitors. The main biological capacitor is the vesicle which is made by a 'spherical' insulation thread, vesicle membrane, within the conducting cell matrix (cytoplasm). The condenser is discharged by the linking of the two insulation threads, vesicle and outer cell, or by activation of gates in the vesicle thread insulator. Complex circuits can be built by giving the vesicle insulation thread its own power source, its own ion selectivities, pumps, and its own gates. By suitable location of such complex threads of vesicles within the encircling insulator thread of the outer membrane an array of local circuits is made so that local messenger chemistry can be performed within a cell, Figure 12. It must be remembered that since the current carriers are specific, often ions, they can carry out specific *chemical* changes on reaching their target.

Further intricacies in the circuiting follow from the alignment of cells, since in this way specified ion currents link new power sources together. All the circuit connections are localized by the positioning of gates, pumps, and vesicles within

each cell. Thus, a huge communication net is established within an organism. Full advantage is then taken of organization to link electrolytic currents and chemical activity in special localized zones.

Because man can use electronic circuits he can employ magnetic fields to connect an electronic message to a mechanical device, *e.g.* in a doorbell. The biological equivalent in the circuit of Figure 10 is to connect at some point a Na^+/K^+ message, for example, to a calcium transmission which then acts as a chemo-mechanical converter, *e.g.* in muscle. Whether in man's or biology's circuits, once the energy of the initial message is changed to a magnetic or a calcium second message locally so as to produce a mechanical change then the mechanical change can be put to many other uses than just tension development. It can be used to open gates, releasing new chemicals from behind diffusion barriers. This type of feeding of chemicals is widely used by man and in nature. However, while the magnetic second messenger acts as a switch, the calcium, which can also act just like a switch, can in addition act as a microscopic device for activating catalysts. It is as if a magnetic field activated the enzymes of the cell directly. Of course, both electrical currents (voltages) and Na^+/K^+ currents can also act *via* voltage switches (gates). The interchanging of electrical, mechanical, and chemical devices is at the heart of man's machines, but it is also at the heart of biology where the machinery is molecular.

The very important conclusion is that the understanding of biological systems lies not just in a knowledge of chemical potentials in given volumes of solution but of the energized flow of chemicals in fields. This change from a conventional thermodynamic approach to an inspection of dynamics is true not only for the small molecules but for all the structures of the cell. In fact, there are no structures in the conventional sense, as we shall see when we tackle the nature of proteins in biological systems in the second paper. To use an analogy, a map shows a river as a structure and as such is a poor representation of a river. Short-term changes in the river are changes in the rate of flow, yet these are also the origin of the development of the river which lead to changes in the map. We started this article with a map of biological concentrations but have concluded it by describing the rate of flow of chemicals. It is the changes in rate of flow which are so significant in signals and so on. Moreover, it is these changes which lead to the change of cell structure called development and differentiation,[10] topics which require a brief reference to the time dependence of the cell.

9 Time Dependence: Development

In order to describe the nature of a cell's activity it has been necessary so far to reduce control to an apparent stationary state of flow of small molecules and ions. While this is often a good and very useful approximation it is clearly not an adequate description of cellular activity since each cell develops, most divide, and all are finally destroyed. There must be a steady and controlled drift in the steady state which is related to aspects of cell metabolism that we have not described.

[10] R. J. P. Williams, *Bioenergetics*, 1970, **1**, 215.

This drift is due to the continuously adjusting progression of synthesis of large molecules (see Figure 1) which we have omitted since we have described all products as rejected waste. This part of metabolism is distinct from the turnover of substrates in the maintenance of the communication net between the pools of metabolic intermediates, of energy, and of signal-generating circuits and their responses to environmental effects. The conventional description is that during the 'steady state' in these small molecule and ion turnovers there is a slow accumulation of new polymers. The analogy with the steady state of man's factories fails since the cell factory has the second activity of reproducing the whole factory while it generates other products. When these cell chemicals and proteins reach a critical mass the cell's activity undergoes a dramatic switch followed by division.

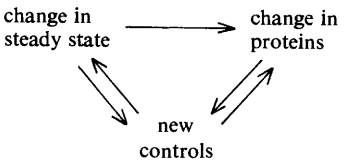
The slow drift of the steady state, developmental growth, is now to be coupled to the previous description. There is, of course, synthesis of proteins, fats, and polysaccharides, but the synthesis of fats and polysaccharides is a secondary event based on protein catalysis, and the production of protein is therefore the basic synthetic activity. What are the controls on it? In fact, we do not have to alter our model of the cell's activity to see where the controls lie. To incorporate steady-state protein growth we need only include feed-forward control from the intermediates to the synthetic growth reactions in ribosomes. In fact, peculiar as it may sound at first hearing, all the cell steady states we have described include already growth in this sense since the polymers are the products which accumulate. A cell is a trap for its own products. The feed-forward controls on protein growth are then the energy (ATP, GTP, *etc.*) and the supply from metabolism of amino-acid units of C, H, N, O, and S. The effect of ions on the machinery, ribosomes, has been described already. It is well within this description that the rates of accumulation of different products will change with time since the steady drift of the 'steady state' is now under the influence of the slowly changing polymer product levels (protein levels) in the cell. The inevitable continuous (irreversible) production of polymers in a cell predetermines the whole cell cycle. All cells change behaviour continuously unless protein (polymer) synthesis stops. Although it is not conventional to use differentiation as a description of this process the cell does differentiate continuously. In a constant environment cell growth is differentiation of necessity. But since we have linked the internal steady state of small molecules to the environment (see above) change of the environment must also be linked to the pattern of cell differentiation. Not only the rate but also the kind of change is controlled, *i.e.* development and triggered (true) differentiation. Differentiation is usually restricted to this second alteration in polymer content, although frequently it is just a product of time.

Of course, the concentration of proteins, catalysts, acts back on the 'steady state' of substrates and we again enter a description of complicated feed-back controls. These feed-back loops extend forward to the synthesis of RNA and DNA and so to the cell cycle. The idea of a steady state of a cell has now been abandoned in favour of a steady progression of steady states, just as we had to abandon the idea of a steady state in one environment in favour of a progression

of steady states as the cell faced changes in environment. In both cases the progression is internally controlled in that the cell can modify protein production based on controls and can change its environment by self-propelled movement. Organization of cells only adds sophistication to this pattern for one cell is the environment for another.

10 Maintained Change in Environment

It is obviously a stress on a cell designed for one type of environment if it is placed in a very different one, *e.g.* from out of the light to in the light, or for an organism, *e.g.* a baby going from the womb to independent life. The steady state must change. One change is that of the controls which we have analysed and which occurs immediately, but an additional and more drastic alteration is the alteration of the protein machinery at least in part. This is called differentiation or the production of new catalytic and structural elements, or even of present ones in quite new amounts. The whole cell (organism) can then take on a new appearance, and new functions, activities, will appear. How is this controlled? Since it must be related to the change of environment it is clearly convenient to make it work through a newly synthesized or released chemical much as a trigger control works. It must act also through the original set of controls. We now need a relay of the kind given in Scheme 6. One clear possibility is that a dramatic



Scheme 6

switch to a new permanent environment makes a dramatic and *sustained* change in the conventional trigger controls. For example, a sustained change in calcium-ion internal levels will alter a cell. Such alterations can be irretrievable switches. Alternatively, in an organized system of cells the production and storage of a control chemical (hormone) in one cell can be newly activated so that a new chemical-potential gradient is developed throughout the organism, *e.g.* steroid hormones. These hormones act at the DNA/RNA level, passing through the outer and nuclear membrane and bypassing *all* the relays. Such control is exerted by steroid hormones. As the expressions of DNA and RNA are regulated by proteins, so changes in these proteins either by chemical modification, *e.g.* phosphorylation, initiated by long sustained calcium pulses or by the direct binding of a steroid hormone will alter this expression.

Elsewhere I have elaborated on these functions and tried to relate particular cell changes to known external effects. One example is a change in the type of nerve message impinging on muscle cells. Slow muscle cells can be forced to

differentiate into fast muscle cells. Another speculative example is the difference between short- and long-term memory. Short-term memory is an electrical store which degenerates before it is reflected in growth. Long-term memory arises when repeated electrical stimuli cause growth and new neuronal connections.[10]

11 Summary of Transmission

If the above picture is correct then a cell needs few chemicals to affect its internal metabolism (Table 8). We can list nine or ten major freely mobile organic trans-

Table 8 *Types of mobility*

Class	*Example*
Free diffusion, specific acceptor	Small-molecule co-enzymes, H, C, P, S, (N), (O); energy
Swinging arms	Cofactors in particles, H, CH_3CO, P?; energy
Hopping diffusion	Electrons in redox chains, protons on surfaces
Gated diffusion	Membrane confinement, inorganic ions H^+, Na^+, K^+, Mg^{2+}, Ca^{2+} (co-enzymes sometimes: H, P)

Note All control rests in proteins

mission agents permanently in the cell cytoplasm for steady-state control, NAD (NADP), ADP (ATP), Co–A (various forms), glutathione (thioredoxin), and possibly *t*-RNA. To these we add the content of K^+, (H^+), and Mg^{2+} ions which can be used in steady-state control if they partition between two aqueous environments under some energy input. The switch control represents an invasion of the cell by three or four transmission agents of a different type, Ca^{2+}, (H^+), Na^+, *c*-AMP, (*c*-GMP), and perhaps one or two others. (We are deliberately omitting agents for gene control such as sterols.) The second set of transmitters acts on the first through amplifiers (see Figure 9). The second set of transmitters can be triggered by a great variety of messages from outside the cell. These messages include a list of hormones as well as the passing of electrical pulses (Na^+), and a given cell has its switch transmission and amplifier sensitive to a limited number (perhaps one) of these external agents. Thus, we have equation (12).

diverse set of messages diffused in, say, blood stream → specialized cell receptors → Ca^{2+} and *c*-AMP response → protein amplifier →

metabolic path changes, *etc.* (see below) → switch-on steady-state and feed-back control (12)

The events which follow receipt of the message and amplification always include destruction of the message (but not its response through the amplifier) and the generation of energy by activating glycolysis. However, each cell has a particular response as well as this general one, and the cellular expression depends

upon the type of differentiated cell which receives the signal. Even here, however, there are some gross similarities. A cell in general responds mechanically to a calcium input, although this mechanical response can be of several kinds. In muscle cells it is merely a contraction of a fibre while in cells containing vesicles it is contraction of a fibre so as to release the contents of the vesicles. The last act starts a new train of differential chemical messages, hormones, transmitters, *etc.*, or even just a new nerve pulse.

Finally, we stress again that it is the dynamics that are so impressive about biological control of small molecules and their flow. The apparent permanence of biological steady states and structure often leads us to miss the constant input of energy which is essential to its maintenance. The structure of a plant is not the same as the structure of a house but is more akin to the structure of a river. Stop the input of energy and the river dies, increase it and it changes at first as a rate of flow, *i.e.* its level, and hardly perceptibly in shape, but slowly it changes in shape, too. The whole structure is energized and can be energized at different levels. We wish to show that this is true of the large molecules which make up the 'structures' as well as of the flows of small molecules. This means that biological macromolecules do not have a 'structure' in the sense that a house has a structure, but they have a variety of energized states dependent upon the energy which is flowing through them (see Part II).

Appendix: The Use of Inorganic Elements in Control

The inorganic chemist will have noticed that in this account I have treated the transfer of a large number of elements. In the first row of the Periodic Table I have described the movement of H, C, N, and O while monitoring general metabolism and its control by organic carrier co-enzymes. Somewhat parallel chemistry was found for P and S, but particularly in the case of P it is energy and not material transfer and control that is involved. Very different roles have been found for Na, Mg, K, Ca, and Cl *ions* in that they are not involved in the metabolism of organic material but in information transfer through the switching of electric-field and chemical-concentration gradients. The proton often links information, energy transfer, and metabolism together. Apart from these elements the other major inorganic elements in biological systems have catalytic roles – Mn, Fe, Co, Ni, Cu, Zn, Mo, Se. The inorganic chemistry which has evolved within biology is an almost incredible matching of each element to functional value. Biology then provides an operating synthesized example of much of inorganic chemistry, not treated in Groups and Periods, but in which many diverse elements act together to give a unity.

I trust that it has been noticed that in this article I have written but one or two organic reactions. It is my purpose here to show the movements of the chemical elements in metabolism and control. Thus, in keeping with the spirit of the Liversidge Lecture it is always the inorganic and physical features of biological systems that are stressed.

Finally, note that I have given only a few references in this article and most are to reviews that I have written. In fact, most of the ideas I have outlined can be

found in textbooks of biochemistry and the only originality in this article is the manner of assembly and the stress. I trust that nobody will be offended by the absence of extensive references.