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Review

Diffusion, perfusion and the exclusion principles in the structural and functional organization of the living cell: reappraisal of the properties of the 'ground substance'

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Summary

The thesis is presented that only within very small microdomains of the cell internum might diffusion operate in the sorting of molecular affinities. Much of cell metabolism is guided and controlled in rate by the speed with which molecules that have to interact encounter one another. What is clear, however, is that the cell does not have a single 'modus operandi' but has the choice of many different strategies, each of which can contribute in different proportion to the rate of ongoing activity. It is probably our own desire to simplify things and use the most (or more) probable strategy that confines our appreciation of the overall robustness of the cell as a 'survival machine'. The main operative process at any given time (perfusion, diffusion or whatever) has always to be considered very carefully in relation to the organisational structure of the cell, which can be transient and fickle but nevertheless has been seen as involving an extensive cytomatrix, a ground substance, within an aqueous environment in which the degree of water structuring is even more fickle.

Key words: intracellular, molecular movement, transport, diffusion, perfusion, survival, cytomatrix, cytosol.

Introduction

The cell is the crucible of life. In it, molecules move or are moved. Some are moving 'randomly', driven by thermal energy; others are directed (convected). The same is true at the macro level. We have long argued that it is only in the 'dead' state that diffusion reigns supreme in the body *and* the cell. If this is so, it follows that if we stop all convection inside the cell, it will be completely reliant on diffusional activity, and the question is raised of whether any level of metabolic activity that is compatible with the 'living' state can be sustained (a resting or basal state?) under these conditions. To this we must add, what then happens when the cell is required to work 30–40 times harder during extreme exertion? Yet it is only in the past decade or so that the need for any mechanism other than diffusion at the cellular level has begun to be seriously contemplated. To understand what the problem is, we need to take another more contemplative look at what was originally called the 'ground substance' of the living cell. Only when we know more about its state will we begin to fathom out how metabolism might be regulated by the movements of ions and chemicals within the cytoplasm. Cell size has traditionally been seen as another parameter governed by diffusion path lengths, a myth that we have addressed and found wanting, being unable to account for some simple 'facts' (observations) about cells. Furthermore, like any hypothesis, this previous reliance on diffusion calls for it to be rigorously tested and demonstrated as the mechanism that can account for the rate of cellular functioning in all situations within the cell, whether it is in the nucleus or the cytoplasm. It has only been saved from this fate by an assumption being turned into 'received wisdom' almost from the start of cell physiology. The present paper falls into three categories: first, we will consider the milieu itself, and see whether diffusion is applicable as the prime cause of molecular transport/movement. Second, an alternative will be considered that the restraints might be equally important as the major determinant of, for example, cell size and metabolic range. And third, the basis for a 'nanocirculation' in the cell internum will be presented, with its relevance to metabolic regulation through encounter frequency. Here, we move into the gyrations of molecules within highly limited space, where we might still argue as to whether they are random or directed. The thesis is presented that none of these mechanisms is excluded by the cell itself. As a supreme survival machine, it embraces all, but some mechanisms are clearly more prominent at certain times than others and this will depend on the prevailing circumstance.

Diffusion, an old paradigm on which molecular interactions were based

My first task will not be to impress upon you the irrelevance of diffusion in cell biology. This has been done through many papers that can be perused at leisure in the following references **(**Wheatley, 1993a; Wheatley and Malone, 1993; Wheatley and Agutter, 1996; Agutter et al., 1995); indeed, we have been criticised for overemphasising this viewpoint (Hochachka, 1999). In this paper, some small retraction will occur – small, but necessary for a more balanced view. The most important piece of information to impart is that, whatever constructs or equations we put forward to describe conceptually or mathematically how cells work, we will never be able to dismiss the involvement of diffusion, because it cannot be avoided and will inevitably play some part within the life and workings of a cell.

Complex systems such as a living cell cannot be reduced to simple equations

We may all have been looking for 'simplifying' constructs because many believe that science is an attempt to simplify our understanding. But we must bear in mind that complex systems that respond to innumerable environmental factors may not easily be reduced to simple concepts that would apply under ideal conditions, as do, for example, the gas laws in physics. Schrödinger (1945) pointed out that if physicists had started with living systems as the focus of study, we would have a very different set of laws in front of us today. Similarly, Einstein knew that living systems were less amenable to analysis using only physical laws if for no other reason than their complexity. Indeed, he went so far as to state that the scientific method itself might have shortcomings, although this overstates the case because, given infinite time, we could conceivably work out every factor's involvement:

'To be sure, when the number of factors coming into play in a phenomenological complex is too large, scientific method in most cases fails us…Occurrences in this domain are beyond the reach of exact prediction because of the variety of factors in operation, not because of any lack of order in nature.'

While it would be nice to think that equations for cellular functioning based on the physical laws of diffusion adopted and adapted by Fick (1855) from Fourier's analysis of heat transference in metal bars (Fourier, 1828; see Freeman, 1878 for a translation) would apply, and although they might seem largely capable of representing the situation in a living system, the evidence has piled up to show that there is no way in which diffusion alone can make a major contribution to much of cellular physiology (reviewed in Agutter et al., 2000), and it does not provide the 'connection of profound generality' required of a concept that Einstein mentioned in the continuation of the above statement:

'We have penetrated far less deeply into the regularities obtaining within the realm of living things…What is still lacking here is a grasp of connections of profound generality, but not a knowledge of order in itself.'

However, despite all that we have written in defence of the notion that diffusion is indeed largely irrelevant in living systems, we cannot avoid the fact that it nevertheless occurs and will naturally (and without apparent additional energy expenditure) contribute to such activities as nutrient uptake by cells. An important issue concerns these 'energy' requirements, and three points that rarely get aired ought to be considered.

First, in living systems, most molecules do not generally move, but are moved, when we consider what would happen if everything depended upon Brownian motion and the law of mass action (Wheatley, 1993b). The most pertinent comment in this regard was made by Johnson (1983), who recognised a grey area at the molecular level when considering the movement of molecules within living cells:

'This is the region of scale where flow and diffusion are not clearly separated; where the concepts of temperature and molecular movement overlap; where it is not clear whether molecules move or are moved; where the ideas of active and passive lose their meaning'.

They are moved by other molecules or indirectly by energy liberated from their interactions with other molecules [the seminal work of Einstein (1905) and von Smoluchowski (1908) dealt with 'Brownian motion', which is not diffusion *per se*].

This leads to the second point: diffusion ought to connote its full (and precise) scientific meaning and not the vernacular used in the quotation by Darnell et al. (1986; see below). It has to imply that there is a tendency of molecules, through their thermal agitation and jostling with others (Brownian movement), to move down their physical gradients, from regions of higher concentration to regions of lower concentration. 'Diffusion' of molecules, meaning the random jostling of Brownian movement, is not a correct use of the word in a scientific context, and such a connotation has to be strongly resisted. It is this problem that almost certainly led in the early days of (cell) biology to diffusion being seen as the obvious way in which molecules 'sorted themselves out'. The assumption is so ingrained that almost everyone falls into the trap until he or she has repeatedly been shown its inadequacy and their attention has been drawn to rational alternatives. But the real issue is that if diffusion does assist in such activities as the uptake of nutrients or release of waste substances then gradients do need to exist. Without them, i.e. without 'sinks' to effectively help create them, diffusion is of little or no relevance (use). The discontinuous gradients that exist at cell membranes are not created without considerable energy input. Therefore, the energy that needs to be expended to make diffusion of any value to a cell, e.g. in nutrient uptake, is mostly spent in manufacturing and sustaining gradients that give the system the unlevel playing field or step on which diffusion can effectively

provide the molecules to move in a particular direction. It is this vectorial aspect that becomes the truly important issue in cell biology, but diffusion is not always (often?) an efficient means of vectorially transferring molecules.

These considerations also bring home the third point that seldom gets voiced: diffusive activity within cells, far from being set up to assist metabolic functions as the norm, will more usually be acting counterproductively, i.e. as a destructive force that constantly dissipates gradients in which much energy had been expended in establishing them for one species or other of a particular molecule. This explains in many cases why cells have to be unceasingly active ('unresting', in the words of Gerard, 1940). The difficulty here is for us to quantify diffusion in terms of its global activity in a cell and then to apportion this to constructive and destructive actions. If we only see or measure the former then we ignore the latter, which may be *n* times more prevalent. Exactly what their relative contributions are would be difficult to estimate, but one other factor cannot be ignored at this time: the extent to which the cell is an organized and 'stable' structure; i.e. it is not a pool in which chaotic behaviour rules.

Looking down the microscope, it is hard not to accept that cells are relatively stable structures. However, once the level of resolution is increased enormously, one starts to appreciate how fast a cell has to run just to stay on the same spot. To give a more descriptive analogy, it is like looking down on traffic from an aeroplane at 35 000 feet. It hardly seems to be moving. Then, as you come into land, you pass over roads at about 100 feet and the traffic is zooming along, seemingly as fast as you are. This can be seen in cells along various transient tracks, but it takes exceptionally good instrumentation to follow it in small cells (Wheatley et al., 1991) and the resolution of equipment needed to quantify it has to improve by 1–2 orders of magnitude. But the intact living cell is not jostling like one sees Brownian movement in a cytoplasmic bleb caused by injury. So, while the cell internum *is* very active, thermal motion of most of its molecules is greatly restrained and Brownian movement becomes barely perceptible. This alone should be a convincing argument that the cell is well organized and, especially, that the milieu is not necessarily of a simple aqueous consistency, and calculations ought not to automatically assume that this is so (Mastro and Keith, 1984). The structure we see in cells is indeed a physical representation of its metabolic processes; in brief, the cell is the functioning entity in a holistic sense and should *not* be seen as a structure *within which* function occurs. So, if we have largely ruled out the jostling expected from Brownian movement in a fluid phase, we need to know more about the cell in terms of its organization and especially about the milieu in which everything takes place. The milieu is aqueous without a doubt, but the question concerns its state and how it interacts with other molecules to create living substance.

Superb organization: the essence of the living state

The problem was picked up early in cell biology. Looking at cell metabolism in relation to the intricate structure of a cell (of which precious little was known at the time), one eminent scientist, Sir Rudolph Peters (1930), stated that:

> *'Extreme order has to be reconciled with a fluid anatomy…This cannot be done adequately without borrowing concepts from physiology, and especially from neurology. The cell must be considered as a reflex entity, structurally organized so far as even its chemistry is concerned, with chains of chemical substances acting as it were as reflex arcs…Our (sensitive) mosaic may radiate its effects throughout the cell. It is perfectly possible to appreciate how a co-ordinated structure may be maintained in a medium which is apparently liquid. This theory is all that is needed to enable us to understand how substances can reach a special site in the cell. Between the chains of molecules, fixed by their radiating webs, there will exist paths from the external to the internal surface, the* ***capillaries*** *of the cell' [My emphasis].*

In other words, since as many as 4000 reactions may be occurring simultaneously in a quiet cell, without the little crucible boiling away as vapour by the heat dissipated, each and every one has to be harmoniously controlled. There is no factory on earth that comes anywhere near this complexity and, at the same time, gives the fidelity or replicative performances while remaining flexible and adaptable to its environment. But then life has spent 3–5 billion years perfecting this act. Such a contention is totally against the notion that everything in a cell happens by the sorting out of molecules from one another based on random movements (Berg, 1993; see Agutter and Wheatley, 2000), i.e. by a process that has wrongly been called diffusion. It has taken nearly 70 years to move away from the diffusion paradigm, from the fatally flawed statements in some of the first editions of our current chosen textbooks on cell biology (although new editions have made amends to some extent). Darnell et al. (1986) in their first edition wrote:

> *'Diffusion is the entropy-driven process by which molecules distribute themselves in whatever volume is available to them…Because a cell coordinates its metabolic activities by diffusion alone, the rate at which molecules diffuse throughout the cell limits the typical cell's size to between 30 and 50 μm in diameter. More specifically, in most cells, whatever their shape, no metabolically active interior region is more than 15 to 25 μm from the cell surface.'*

Choice of the main scenarios

If we are to dwell on diffusion, then we must go back to the quotation above and see that Peters (1930) used the words 'apparently liquid'. It is relevant that life seems to involve water, and water is usually a liquid, but in association with other molecules – especially proteins – it can assume properties quite unlike normal water. Nevertheless, if our paradigm changes and 'normal' liquid water is not entirely representative

of the internal milieu, then alternatives have to be considered and we need to know how these would impact on physiology. One of a small handful of authors who looked seriously into these matters was Peter Hochachka. At the expense of reiterating many of our own findings and deliberations, these have been summarised in a very adequate way in his seminal paper, in which he discussed the relative merits and contributions of two rather extreme schemes of work on which metabolism might be based (Hochachka, 1999).

The first scheme referred to the prevailing scenario for many years in the 20th century, that the cell was a bag of enzymes in relatively simple solution. They can work like this *in vitro*, so why should they not work like this *in vivo*? The other model that was proposed dealt more with a gel-like or structured system through which molecules are directed with great precision so that there exist thousands of microdomains in which careful pathways are followed by the appropriate molecules, leading to the desired products and responses in the right place at the right time. Acceptance of this state of affairs is long overdue, but it is probable that today those adhering to the first scenario are probably a small minority to whom few are likely to pay attention in the future. But science demands that we test hypotheses and gain consensus only when adequate experimental evidence is available for us to make proper judgement. Just as we have called on those adhering to the notion that 'diffusion suffices' to prove their case by experimental demonstration of evidence for their claim (and not just the extrapolations; Krogh, 1941; but see Hoofd, 1992), those adhering now to the second scenario are in just the same position. It has to be shown that the dynamics (kinetics) involved are either by directed movement or, at the least, that they could *not* have been accounted for by diffusion, i.e. random molecular walks, alone. [In this discussion, all mention to processes such as facilitated diffusion and other similarly sounding processes are excluded, because *assisted* diffusion cannot technically be diffusion at all, where that is meant to imply only these random walks of molecules within gradients.]

Back to basics: reappraisal of the 'ground substance'

Being intent on their experimentation, few students in science, and perhaps more so in biology, tend to spend time reading its history; that of experimental biology has recently been covered in fine style by Lutz (2002). When it comes to the nature of the living substance, only the occasional book enters into the provenance of the many ideas within 'cell theory', but one more heterodox volume has a commendable amount of information on the early history that I wish to cover here. Indeed, even if in no other way, Ling (1962) has done great service here by bringing out the relevance of its history and how ideas have come and gone with fashion and new evidence.

Having agreed that the cell contains definite organelles, from the obvious ones, such as the nucleus, mitochondria and lysosomes, to the many small ones, such as synaptic vesicles, Golgi cisterna and cell membrane, that actually exist and are not artefacts of preparation for examination, it is not too much for the structuralist to now include some large macromolecular complexes, such as ribosomes, microtubules and proteasomes, as miniature organelles, even if they are not bounded by membranes. Accepting that the nucleus is tethered in a cell (which can be proved by a variety of techniques and follows from the common sense observation that if it was not then it would lie hard against the membrane at the 'bottom' of each cell after centrifugation), it follows that many other organelles have a non-random distribution.

The changes in distribution of organelles with, for example, the division cycle is a subject of considerable topical interest; for example, if we watch the fate of the nuclear envelope and its components (Salina et al., 2001). I only wish to extend this one stage further here by suggesting that the mini-organelles mentioned above, and perhaps even free macromolecules and macromolecular complexes, are probably not randomly distributed in the cell. This argument simply reduces to what I said another way round above; that the cell is superbly organised and there is no reason to suppose that it is not similarly organized at all hierarchical levels. Having covered everything from the nucleus down to large receptor molecules being rather precisely located, e.g. usually on the cell membrane in the case of the latter, the question is what is there left to discuss? And here the history must be read, for it comes down to the nature of the milieu in which all these structures exist and all the functions of the cell occur; and what happens to it and within it that makes for 'the living state'. We know that Szent-György (1971) referred to this water-base as the mother liquor without which life would be impossible (Harold, 2001). This being the case, then water is the essence of life – the molecules '*dancing to the tune of the solids*', according to Szent-György.

Latterly, more and more investigators are concerned with the nature and state of the water inside cells and, especially, the properties it assumes when it meets any kind of surface or conditions within cells (e.g. vicinal water; Drost-Hansen and Singleton, 1992) or special kinds of molecular surfaces (primarily proteins) such that it becomes more gel-like (Pollack, 2001). There is a world of difference between these two extremes, and this is perhaps the most crucial message that has to be imparted in this article – that we need to know exactly how the state of water inside the living cell differs from that in a beaker of tap water, as depicted by Wheatley (1993b).

Redefining the 'ground substance'

So what was originally meant by the 'ground substance' of a cell by early cytologists? If it was not water (in which case why would they have not called it ground solution or liquid instead of substance, which implies a more solid nature), then what was it originally considered to be? The answer comes from Wilson (1904), which is the second edition of his treatise that stands as the cell biologist's bible. Having agreed that protoplasm includes everything, then the cell partitions into the nucleoplasm (all that is the nucleus) and the cytoplasm (the

rest). The 'fabric' the cytoplasm, with all the solid bits and pieces it contains, could be taken as all part of a meshwork (granules of various kinds wrapped up in a honeycomb-like reticulum, referred to as the spongioplasm by Leydig as early as 1885; see Wilson, 1904) and clearly deserves the name 'cytomatrix'. What was left in the intervening spaces, '*the cell-sap, enchylema, hyaloplasm, paramitone, interfilar substance, etc*.', to quote directly, was the ground substance. Almost everyone (i.e. those that did *not* confuse it with the cytomatrix) later assumed that the ground substance was water, with salts and some other substances dissolved in it. Its viscosity would be close to that of ordinary water. The question we should be asking today is, if the latter day cytomatrix exists as a dynamic structure with the properties ascribed to it by Porter (1984) and Clegg (1984) under the term 'microtrabecular lattice', what is the nature of the material surrounding it, which must largely be comprised of water? If we knew this, then the ways in which micro- and macro- molecules travel through it might be better understood. The very fact that molecules such as gelsolin have been found illustrates the dynamic way in which the state may also vary from instant to instant, from one condition to another, because not all the protein in the cell is wrapped up in the cytomatrix and organelles. Thus, the interstices contain a thinner protein solution. Is this material that can transform quickly from gel to sol, or does it have to involve the making and breaking of the microtrabecular lattice, alone or together? If we knew the answers, or at least how to find the answers, our understanding of cell function would take a quantum leap.

Water, ions and molecules, big and small

The water content of the cell is approximately 40–44 mol l^{-1}, a meaningless value unless we are thinking of water dissolved is some other phase. We have mentioned vicinal water, but the point about this is that its properties are changed because it becomes more organised and structured (in a sense, more ice-like in its self-association through hydrogen bonding). The issue that has dogged most people in recent years is the extent to which something like this probably occurs within the cell. Within a crystal (a highly regular molecular surface), one or two layers may be associated, although at the crystal surface this may be more. At the surface of proteins, several layers may form and, in exceptional circumstances, where polarization greatly enhances this effect, we might get 1–3 layers that constitute a phase with altered properties to those of free water and that certainly shows differences in its selective solubility (the reason for K^+ being preferred to Na^+ within cells, as argued by a number of proponents of this idea, e.g. Negendank, 1982). Although the number of layers could be greater, there is no real evidence to prove this, and it is certainly stretching the imagination to conceive of ≥4 such multilayers. If this was the case, water structuring inside cells would start to approximate to short proton relaxation times in NMR spectroscopy as it moved towards an ice-like form (Wiggins, 2001). Cellular NMR has been achieved, but only of huge egg cells. Its resolution may never be good enough to analyse water in lacunae of some 20 nm in diameter within the microtrabecular lattice. If small protein molecules can link up such areas, then the whole system might move close to being gel-like, but it would be very sensitive and undergo phase transitions very quickly from gel to sol and back, as suggested by Pollack (2001). We cannot eliminate such behaviour of water and its associated ions, as well as big and small molecules within cells. Any theory that embraces metabolic regulation has to take into account the nature of the ground substance and the fact that it can change dramatically from one moment to the next. But what is lost in stability can be a real gain in flexibility, and it is perhaps the greatest attribute of living organisms that they have exploited exactly those conditions that make themselves constantly more adaptable to their circumstances. If they cannot adapt, they are going to be eliminated sooner rather than later; and we only ever see the successes (Wheatley, 1997), the survivors, those that have been selected out and exist today in an unbroken lineage of existence since the first dawn of life.

Exclusion principles, adaptation and survival

Following on from the above considerations, it is clear that cells have to operate despite the fact that small, or even big, domains within the protoplasm may be constantly changing their state. The question that many raise is probably a facile one: what state can be described as the one supportive of or characteristic of life. My answer is that we should recognise a principle that ought to be considered by anyone who champions one hypothesis to the exclusion and often the detriment of another. Since the cell itself does not operate in this way, but uses whatever mechanisms are at its disposal at any time, scientists in this field (or any other, for that matter) ought to accept that no single theory is going to prevail over any other that has any degree of plausibility in it. The cell will use any mechanism that renders its new state compatible with continued operation. The exclusion principle is one that is incompatible with good science. To make a simple illustration of this point, it would be foolhardy of a physicist to expect the same outcome from a particular disturbance to a glass of tapwater and a block of ice, yet both refer to pure H_2O. If the cell, for particular reasons in relation to environmental stimuli, switches its outer cytoplasm from being a gel to a sol or *vice versa*, no single set of equations or law of diffusion can be applied, just at it would be wrong to average them in trying to find a physical description.

Some other microdomains to consider

Because water has different and rapidly interchangeable states, we have to consider the circumstances in which it is found. Thus, within a microtubule of 25 nm diameter, the 14–15 nm core is presumably filled with an aqueous solution. If three layers of water are vicinally associated with the wall, then about 1.1–1.2 nm need be subtracted, leaving a free core that might be 3–4 μm long and <13–14 nm in diameter. That

is enough for approximately 40–60 molecules of water to stretch across. Movement within this core could be followed to see how quickly it occurs. Odde (1998) has attempted this with different agents that might or might not be associated with epitopes on the inside of microtubules (an idea that his work has largely demolished). If no association occurs with wall materials, it would take a long time for small molecules to find their way up into these microtubules. It would be difficult to imagine that they had any form of assistance, and therefore it should be possible to work out the diffusion coefficients and check whether these were of the same magnitude as the diffusion coefficients of the same molecules in tap water. It would appear that this was not far from being a reasonable match (Odde, 1998).

So, diffusive processes are clearly operative in these microdomains, although it is extraordinarily difficult to verify this claim, just as it is only surmise that acetylcholine liberated at the presynaptic membrane *diffuses* across that short gap to the post-synaptic membrane at nerve junctions (Wheatley, 1998). This does not exclude, therefore, the possibility that molecules go down their concentration gradients by other means, such as by flow of the milieu, the pumping action of other microstructures or some expulsive or quick capture process. What it says is that molecules can still move relatively freely in these domains if and when squeezing and pumping do not operate. We need to know how a substrate in a tightly coupled pathway leaves the first enzyme as its product and becomes the substrate of the next enzyme to act upon it. Does it diffuse within a nanodomain such that it has a small interval of 'freedom', or is it passed on without ever losing its 'tethering' in much the way that Miles et al. (1999) have indicated from their work on tryptophan synthesis?

Molecular complexes may be designed either way. If the proteasome takes in a faulty protein, it will be acted upon by a sequence of enzymes, but it may have to roll around inside the barrel of the proteasome between each enzyme reaction – which is why it would seem plausible, in teleological terms (Agutter and Wheatley, 1997, 1999), to have such a structure. Oster (2002) recently put forward the notion that myosin might be tethered by one foot to actin but that it requires diffusion-like freedom to pivot and wobble until it strikes the point where its other head can meet the next actin molecule. What is much less likely is that a molecule rotationally showing maximum motion within a small domain might also be translationally moved within the compass of reactive sites (assuming that domain is approximately 20 nm) very quickly, but its chances of getting right across the cell would be infinitely smaller if it had to negotiate unassisted thousands of curtains of cytomatrix, the received wisdom of the 'crowded cytoplasm'. Again, we return to the scientific definition of diffusion, and rotational motion through thermal agitation is *not* diffusion. But within the microtrabecular lattice, we probably have domains of approximately 20 nm in diameter in which molecules spin around making many contacts with the vast surface of the matrix on which most metabolism takes place (also a hypothesis with strong supportive evidence that is now so generally accepted). The freedom required here is for small molecules that can be highly mobile (≥500 Da) and need only cover a few nanometres to be of use, but even bigger protein molecules would gyrate with sufficient speed to enter many interactions.

Bringing molecules to these sites and maintaining the gradient through their utilisation at these sites probably involves more than simple diffusion, and here we must think not of the resting cell, in which action can be quite leisurely, but when the organismal demands on a cell are at a maximum and there is a need to deliver supplies fast. Nevertheless, one can still ask the pertinent question of whether life can be sustained on diffusion alone, however 'leisurely' or resting we try to make the circumstances. This is where Coulson (1986) has made the greatest contribution, although indirectly, to this debate. For at the level of general physiology, his principle simple states that delivery is usually the rate-limiting factor determining how fast a tissue or organ can work. Inside the cell, the same principle should apply, and we have considered that delivery by perfusing enzyme beds can be used to speed up reactions as well as effectively regulate the metabolic rate (Clegg and Wheatley, 1991; Wheatley and Clegg, 1994). [For more information on this aspect, refer to Wheatley (1999).] For much of the metabolic activity that takes place within a cell, there is now a groundswell of opinion that little is left to chance (Brownian motion) and that perfusion of enzyme-studded surfaces is the order of the day. While this is not **exclusively** the case – one of the major points that has been emphasised throughout this article – it is clear from the work and carefully considered arguments of Hochachka (1999) that he had arrived at this same conclusion, one which ought to be heeded for its explicit handling of the confusing data in this field. The 'capillaries of the cell' reported by Peters (1930) refer to a nanocirculation (if the term 'microcirculation' is reserved for the capillary beds in tissues first seen by Malpighi). Within the cell, the nanocirculation would indeed exist mostly as transient channels suffusing the matrix, where catalytic activity and most functional activity is concentrated (e.g. Getzenberg, 1997).

Concluding remarks

The cell uses many different mechanisms to achieve the movement of substrates, products, waste, etc., and it operates them at the same time. Diffusion will be involved, although in general we think that, in active metabolism, it offers little means of gearing up in the way that perfusion can and that it more often operates in a negative way to dissipate gradients developed for other purposes. No simple equations can be applied, unless the system (the living cell) is in a particular set of circumstances where one might become predominant. Unlike scientists who want a simple answer with a manageable equation into which to plug their data, the cell does not like this exclusion principle. It embraces any device that supports its metabolism and survival. At the various hierarchical levels involved, the importance of these different mechanisms might

change, and in the smallest domains of the cell (20 nm), the degree to which freedom of (random) movement plays a part in sustaining reactions (especially in pathways) remains an interesting problem that, with technology that will need to be developed in the future, may bring some of the much-needed answers.

I am saddened that Peter Hochachka will not himself be able to read these words, but they have been written in his memory – a man I never met yet seem to know so well. We have walked a long and difficult intellectual path 'together', and his strength and direction in previous years has given me the tenacity and encouragement to continue in this fundamental work on the most fascinating subject on earth, that of how a cell works.

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