ON THE NATURE OF SCIENTIFIC DISCOVERY AND PROGRESS – LESSONS FROM A CAREER IN CANCER RESEARCH

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ABSTRACT

*While there is a tendency for career scientists to focus throughout most of their careers on a quite narrow range of research topics, my experience over a period of more than 50 years has been broad, from carcinogenesis to diffusion theory. There have been times when intense work has been done on particular subjects, for example, in exploring the structure and function of primary cilia, now implicated in 40-50 medical disorders. Such experiences constantly inform one how best to approach difficult and often fundamental issues regarding the nature of life, and also how it becomes distorted in the development of cancer. The quest to know the basic difference between normal and malignant cells continues. In addressing these problems we also learn how little we know about such matters, and about the Universe, life and ourselves. I relate, in outline, many of the experiences I have had since they have taught me lessons about how to go about scientific and medical research and how to maintain an enthusiasm for the work. My aim here is to relate from a personal (highly subjective) viewpoint the issues that should help the reader to appreciate what is required to advance knowledge, and how to benefit from the little advice that I can offer regarding their future endeavours. **Biomed Rev 2017; 28:111-119.***

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INTRODUCTION

Perhaps the biggest lesson we learn too late in life is how to live life. It is like this in science; the longer you research, the more you begin to realise what it is all about and you do things more rationally so that many difficulties and problems finally seem to fall away. Your approach to research changes with experience, with less frustration as you get older. Recounting of one's experiences allows you to look back at those times when these lessons were learned. This reminds of two memoirs from great scientists whom I admired, the first by Lewis Thomas (1), and the second by Max Perutz (2), both books with the most unlikely titles. The down-to-earth approach of Jeff (Gottfried) Schatz (3) has also been appreciated as a recounting of our interactions with the world of science from

all different perspectives, not just research itself.

Reflection can be very useful; there can be lessons we have learned that ought to be passed on to the next generations, helping them to get ahead with the right attitude and appreciate how to tackle the more problems by developing appropriate strategies from the start. My own cluttered life in cancer research, strung along with interests in quite a few other fields, has had its moments. In recounting some of these, I hope this article will encourage young scientists and doctors to take heart in what they would like to achieve, otherwise their lives might well be full of frustration. This can only be countered by having the right mind-set, the catch 22 being that, unless you learn quickly from others, it is difficult to get into that correct mind-set until it is too late. (It is of relevance

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to note here that the best way to increase your chance of being a great researcher and a Nobel Prize winner is to have worked with one!)

SUBJECTIVITY RELATIVE TO OBJECTIVITY

Science is an approach to a better understanding of the extraordinary world and universe in which we live, mainly by observation, experimentation and rational thought. Methods we use try to take us beyond what our 5 senses alone can make of reality (we have thousands of machines that can sense many more things than the human brain), to move from the subjective towards the objective approach, which requires that we compare notes with our colleagues – science relies on and moves forward by consensus, the logic behind each step being strengthened by team work. The degree of altruism in this work is quite exceptional; and science has to be squeaky clean. False data (like fake news) or a hypothesis that might be better stood on its head, can all undermine our belief in a developing construct or concept about reality. [The word *belief* here is the one that often conjures up faith, i.e. a religious attitude, perhaps best encapsulated by David Horrobin's little known book *Science in God* (4)].

Steady progression in science pushes ahead technological advance, and this engenders ever wider exploration in science – a self-perpetuating process. It provides the tools to do the job of even wider exploration. Science tends to be unemotional in its activity, but there are times when ideas start to open up new vistas; these discoveries, whether small or large, keep us involved and working ever more diligently to unlock the “secrets” of nature. It is this aspect that I want to consider more deeply here, and my deliberations will be quite subjective. This is because retrospection is one of those mental activities that can tell us where we went wrong before and how we could have made better progress going another way (there's nothing like hindsight to be wise *after* the event). We learn a lot by our mistakes.

ON THE NATURE OF DISCOVERIES

A discovery should be a sudden revelation. The word “reveal” occurs in almost every paper published these days; check for yourself, it is more dramatic than “found” or “shown”. Usually a discovery implies or connotes that it was an unexpected event. That is not strictly accurate because, although it can be the case, there have been many conceptual “discoveries”, e.g. the Higgs' boson, that were postulated decades ago but in this case only found recently following the

development of the CERN accelerator. This raises the question of which kind of event can be credited with the title of a discovery. My main concern is that the literature today has become overloaded with discoveries – researchers showing that they have found things “for the first time”, a claim to fame, implying a *significant* step forward. Primary research papers need to be about original work; they should be rejected at submission if they have nothing new to report, unless it is to corroborate some previous finding. I would like to suggest here that every little step forward *might* be called a discovery, but that simply trivializes what I believe is worthy of the name. Indeed, what most “first timers” claim is that they have been researching a phenomenon that needs an explanation. Evidence is collected that amounts often to a small piece of data that helps support a hypothesis; the collation of lots of pieces of evidence through team work will then decide whether there is sufficient support for it. But this flies in the face of a different philosophy espoused by Karl Popper (5). Today experimentation on hypotheses is carried out *to prove** them; this attitude is laudable up to a point, but a more pragmatic approach is that we should be testing our hypotheses to destruction. Popper maintained if they can resist this assault, they might bear some truth. Of the several ways he put this point, perhaps the one that tells us that there is never an absolute truth and that all hypotheses remain “suspect”, is: “*The game of science is, in principle, without end. He who decides one day that scientific statements do not call for any further test, and that they can be regarded as finally verified, retires from the game.*” Darwin's hypothesis has stood the test of time and has been duly called The Theory of Evolution, but assaults have been made on several aspects of it such that we now have NeoDarwinism. The simple lesson is summed up in a short cliché – “nothing is written in stone”.

(*In truth, this word *prove* originally meant to test something; whisky is proved/proofed – tested to ascertain its level of alcohol on the Gay-Lussac scale (e.g. 40% proof). Today “prove” usually means to establish, verify, or confirm validity.)

CARCINOGENESIS – THE RELEVANCE OF ACTIVATION

One of my first positions as a post-doc was to use the new electron microscope that the department in which I held my first post had acquired. The EM at that time had not long been introduced and having learned to use it during my postgraduate days at the Institute of Cancer Research in London, I was determined to continue with it. In Aberdeen, it was helpful in following adrenal damage (adrenal apoplexy) caused by

dimethylbenzanthracene (DMBA), a powerful carcinogen that rapidly induces mammary cancers in rats, but had this unusual side effect. The tumour aspect was my main project, in which I had found that depressing liver function unexpectedly reduced its carcinogenic activity whereas logic would have suggested that, in slowing it from being rid from the body, its carcinogenic potential should presumably be enhanced, knowing that the liver is important in metabolizing and destroying most foreign compounds. The result was completely the converse of what was postulated; far fewer tumours were induced. The lesson here is that we often learn much more from experiments that go in an *unexpected* manner; it calls for a rethinking of the whole problem. It soon became clear that hydroxylases were at work in this metabolic pathway of usually eliminating toxic compounds, but when they were strongly suppressed in this case, DMBA seemed to be inactive. The postulate had then to be changed that some metabolic action was necessary to activate it, and with colleagues in London we soon showed that it was the 7-hydroxylated derivative that was the carcinogen (6). This quite small “discovery” was very a memorable occasion because Charles Huggins, a close colleague of my chief in Aberdeen (Alistair Currie – who coined the word “apoptosis”) was visiting the day we got proof that this derivative (metabolite) of DMBA was the active substance. (Huggins several months later won the Nobel Prize, but not basically for his work on this model of carcinogenesis.) After this finding, the metabolism of potential cytotoxic substances became a focus of research, especially with the action of aryl hydroxylases on aromatic carcinogens.

ADRENAL DAMAGE AND THE FINDING OF AN EXTRAORDINARY ORGANELLE

This prevention of the activation of DMBA also meant that the adrenal was protected (7). This takes us to the next step in this story. The histology was clear cut that the derivative was the active damaging agent in this small organ, but even today we do not know why it in particular is damaged rather than any other organ in the body. EM exploration of adrenal damage confirmed that almost complete obliteration of the two inner layers, the *zona fasciculata* and the *zona reticularis*, had occurred, whereas the *zona glomerulosa* (seemingly spared according to the histopathology) was only mildly affected. However, its cells were less well organised into “glomeruli”, and they had unusually large and deep vacuoles in their Golgi regions, seldom seen in the controls. In

several cells there appeared to be whiskers or strands in these vacuoles, until I found one that was truly remarkable (Fig. 1). It was an almost perfect longitudinal section of a primary cilium (8-10). The diplosome base could not have been more perfectly sectioned and all the attendant processes were present, the EM’s magnification being sufficient in those early days to show even the finest detail. Having had good training in biological sciences, I was perplexed because this cilium was solitary, deeply invaginated in these vacuoles, and not of the abundant 9 + 2 variety – the motile cilia on epithelial cells that we all learn about undergraduate courses. I was quite astonished at the image itself, and also bewildered by what on earth it was and what it was doing there; we had never heard of primary cilia throughout all our education in the biological sciences. The literature soon showed that this was a known structure that had been seen in 1898 by Zimmerman (11) – quite remarkable for that period considering the resolving power of microscopes that were available. But his finding hardly attracted any attention before the late 1950’s. Even then no more than 2 or 3 researchers took any much interest in it. For me it was such an unusual finding that it had to be of importance; I immediately became hooked on it and it has remained an obsession right through my life. Hypotheses began to be constructed about this organelle (or more accurately a complex of organelles), one being related to the teaching of Dan Mazia (12), who had posed a simple question – how is it that a typical cell divides into two and not any other number? The notion was that the centriole was responsible for ensuring that there were two poles at division controlled by its own division into two that then migrated apart, becoming the poles of the spindle. How could this be the case in cells where the centriole pair (diplosome) was trapped in the base of a primary cilium complex? Did this prevent the cell from further divisions? In the final analysis it was shown that the centrosome – into which our complex organelle was embedded – was responsible for the bipolarity of division. By implication this means that the primary cilium probably was not directly involved. The conclusion is that we hang on to hypotheses sometimes because they look so convincing, until some discovery call for a rethinking. Clearly the role of the centriole was not what we had thought, which led me to an onerous task of writing a monograph on it – *The Centriole: a Central Enigma in Cell Biology* (8) – in which Chapter 6 is devoted to this unusual cilium protruding from the diplosome.

THE PRIMARY CILIUM

The obvious question is - what is its function? The small number of reports on primary cilia after the end of the 1960's meant that it still remained of little interest to cell biologists, but I have always contended that "nothing a cell does is without significance", so what is the significance of the primary cilium, other than it might block division if it also affects the centrosome? It does not because rapidly dividing cells can have primary cilia, which are quickly resorbed by the time the cell has reached preprophase. The one idea that did arise was that the primary cilium can suppress attempts by the cell to mobilise its centrosome for division, i.e. it inhibited progression into mitosis, effectively suppressing rapid proliferation. However, this notion proved inconsistent with the data.

Because many cells do not develop cilia as well as the one shown in Figure 1, the general opinion throughout the world of cell biology remained that they were vestigial and rudimentary structures, something left over from antiquity in the early evolution of eukaryotic cells. Indeed, many are quite poorly developed cilia in some cell types, especially those proliferating very rapidly. But I was not satisfied, and that is why I continued to research this cilium throughout my life. If nothing is insignificant in what a cell does, the lesson is that by sticking with the problem and having lots of determination, you can succeed. You need to ask the right sort of questions, and have the tenacity and motivation to keep researching it. At the risk of becoming tedious on this subject, let me simply add that the sensory function of the primary cilium was established in the 1980-1990's, but not fully appreciated until the beginning of the 21st century.

If the cells of the body do not form primary cilia, pathological conditions would surely arise, as I clearly espoused in 1995 (13). When this became evident in conditions such as polycystic kidney disease (14), research became intense and soon after there were more than 600 papers being published a year on the primary cilium. We can confidently say that at least 40 disorders can be attributed to defective primary cilia, and today that figure may well have risen to 50 (15). It is also remarkable that many are neurological problems that affect a whole range of behavioural/psychological patterns. I would never have imagined how such a turnaround at the fundamental level of the cell could take place over a short time relative to this organelle that had been discovered more than a century earlier. The lesson is that you cannot brush things aside; there are phenomena, they have their existence, and they all have their explanations. Looking at this whole episode, my

concern is that we ought to consider, when the discovery of the true nature and significance of this complex organelle became obvious, what a rapid change in thinking occurred over 10-15 years, and how much the whole episode resonates now throughout medicine.

AMINO ACIDS AND THEIR UNUSUAL BEHAVIOUR

In this section I want to address another discovery that is totally unrelated to the story just related. I had been working on protein synthesis and turnover for some years. Out of this came two particular findings that left me again wondering why we have been misled by the "received wisdom", hypotheses (on few "facts") that in many cases had been held for many years. Received wisdom needs to be rigorously challenged from time to time to ensure it retains any vestige of truth. The first of my two main findings deals with a question that came out of experiments in which we investigated the kinetics of amino acids being incorporated into proteins. The pathway is well known through tRNA acylation and mRNA, but the kinetics worried me. The observation that led to very many experiments over the following years was simple, but its explanation was not. By adding a radioactive amino acid to a culture, cells form an intracellular pool and proteins become labelled. To measure the pool and the labelled protein, cells are spun out of the labelling medium and the pellet resuspended in phosphate buffered saline (PBS, pH 7.4) before being recentrifuged to wash them. This would be repeated twice or perhaps 3 times to ensure that all free labelled amino acid in the medium has been removed. On final resuspension in PBS, trichloroacetic acid is added and the proteins precipitated. The supernatant will contain the contents of the *acid-soluble* pool. But if the kinetics of labelling are followed using very short exposures times (i.e. far less than the 30 or 60 min that most researchers used), we found that over the first 5 min proteins were being labelled with *linear* kinetics despite an acid-soluble pool of unlabelled amino acid still being present. The exposure times eventually went down to 5 and 10 *seconds*. The obvious explanation was that the free amino acids given to the cell must have bypassed the acid-soluble pool, contrary to the received wisdom (Figure 2). In fact, it usually took at least 5 min (sometimes as long as 20 min) for the label to equilibrate with the acid-soluble pool, indicating quite a slow turnover, again confirming that free amino acids were being preferentially incorporated. Two points arise: (i) the labelling times used by almost all researchers were far too long (from 200 to 400 times too long!) for the events just de-

scribed to have been detected – the initial kinetics are totally obscured; and (ii) the assumption held probably by all other experts was that the amino acids would be transported into the cells through special channels into the acid-soluble pool, from where they would be assimilated into protein – this would mean that incorporation has to be exponential for at least the first 5 to 10 minutes (Fig. 2). Both assumptions started to crumble because of our simple demonstration; the ploy was to label cells very briefly and remove them for analysis as immediately as possible, and in particular spin them down through a solution that did not allow any of the labelling medium to come down with the cells, effectively isolating them from the medium, and also allowing no exchange across their membranes during this process. This was achieved with AR-200 (a low-density oil from Pharma, Heidelberg, Germany). The cells were washed in PBS once and the supernatant collected, which now removed free amino acid that had accumulated within the cells during the very brief exposure. The pellet contained the proteins and the acid-soluble pool, the obtained by traditional trichloroacetic acid treatment. The results were compelling in two regards; (i) there was a *free* pool on amino acids within the cell that was measured in the first wash supernatant, distinct from the acid-soluble pool. Its level of radioactivity was much the same as in the initial labelling medium when the volume was measured and the activity normalised; and (ii) the acid-soluble pool was only poorly labelled. Amino acids get in and out of cells *extremely rapidly*; equilibration across the membrane has to be virtually instantaneous, challenging the ability and capacity of any transporter system to be involved as the kinetics of formation for the free pool, although they may be involved in forming the acid-soluble pool in time. This simply does not match up to requirements for labelling the proteins, which otherwise would follow an exponential for 5-15 min if they came from the acid-soluble pool (16; reviewed in 17). Despite the evidence reviewed in the last cited reference and a considerable number of follow-up papers that supported our finding, the received wisdom has remained the same to today.

The lesson is that, unless your discovery is acknowledged, understood and championed thereafter by the experts in the field of the now defunct received wisdom, it can remain in limbo for a lifetime or even longer. After many discussions with a guru on the subject of intracellular pools, Murdoch Mitchison, we were able to conclude that the cell does harbour three pools, a freely exchangeable amino acid pool in rapid equilibrium with the external medium, an acid-soluble pool that contains amino acids that somehow become trapped

in the cell and only relatively slowly equilibrates with amino acids in the medium, and a degradation pool of amino acids (or more correctly very small peptides) that are released by degradation of proteins. Proteasomes release amino acid chains of 5-7 residues that cannot exchange quickly with the external medium. (Unfortunately our deliberations were never fully published as Murdoch passed away.) The acid-soluble pool still needs to be defined (redefined) and its kinetics more clearly understood. Incidentally, without going into details here, the degradation pool has to be reduced to single amino acid residue before they can be recycled and re-enter proteins, which happens only when there is little or no external pool. Their chance of doing so in regular culture conditions is very unlikely unless the cells are starved of amino acids externally and the acid-soluble pool has already been assimilated and exhausted. Many lessons can be learned from these studies, but the one I would emphasise yet again is that sometimes we fail to appreciate how fast things happen at the molecular level in the cell. Techniques (or more accurately the way they are applied) can often be inadequate to get information at this high level of resolution, in this case simply on a time axis.

ON LONG-LIVE AND SHORT-LIVED PROTEINS

The degradation pool has still not been properly defined; at that time we were experimenting on turnover kinetics, and proteasomes had not been discovered. However, we followed the formation of the pool using labelling procedures, which inevitably led my team to looking at the kinetics of protein degradation. This resulted in a visit to the laboratory of Joe Amenta in Pittsburgh, who had been studying short-lived and long-lived proteins, seen as two quite distinct classes (18), i.e. some gene products are proteins that are relatively stable and others that have much shorter half-lives. This notion is not wrong; indeed it would be difficult to believe otherwise. But there was a catch, and one that was closely linked to what has just been discussed on the basis of the intracellular pools. If proteins are labelled again for a very short time (no longer than few minutes), a much clearer distinction of short-lived from long-lived proteins should have been much more obvious. But once again, to the contrary, our data showed something quite remarkable and unexpected regarding the two classes of proteins. Proteins were labelled very briefly and their degradation kinetics followed quickly and accurately within minutes throughout the first hour at intervals of between 1-5 min initially and every 5 min thereafter their degradation was much faster than expected. But, even more intrigu-

ingly, protein profiles on electrophoretic separation showed that *all* proteins were undergoing rapid degradation and only after ~60 min did any clear distinction begin to be detected between what could now be called short-lived and long-lived proteins (19). The schema in this last reference to explain this finding remains theoretical (20), but was bolstered by the earlier discovery by Laskey et al. (21) of chaperones that suggest that nascent proteins are chaperoned into their rightful place in the cell matrix.

In brief, if new proteins do not become integrated quickly (we need to remember than 5-15 min is a significantly long time in biochemical terms), they are vulnerable to protease activity in the (now discovered) proteasomes. About 30% (yes, one third!) of all proteins that have been synthesised are destroyed within 20-30 min. Why had previous experiments by other researcher failed to uncover the kinetics of this gross level of early turnover? It is simply because they used labelling times usually of an hour or more, totally obscuring what happens within the first half hour from the very start of labelling – i.e. the same criticism as above, that we do not look soon enough at the events and their kinetics occurring in the metabolism of cells at the molecular level.

These findings came under considerable criticism on the grounds that the synthesis of proteins is an expensive activity in energetic terms. Such a huge turnover in the first half hour seems “profligate” and surely evolution would have made synthesis much more efficient. This is a matter on which much deliberation was required, until an explanation began to emerge. This was reached by taking some analogies. First, nature is often very profligate, as in the spawning of fish, many more eggs being released than necessary, but this allows some to survive and a few embryos to reach maturity. The second related to house building. If you build a house without having a surplus of bricks and then run out of them, the work has to come to a halt until a new supply is sourced; but if you order a surplus to requirements, there will be no hiatus. In life the requirement for proteins both for maintenance and growth demands that a surplus has to be available; the need for the essential ones must be met at all times. But not all those synthesised will find their place, so continued synthesis with turnover gives a much greater the chance of maintaining both structure and function. If there was no turnover, the cell would quickly clog up with the surplus to immediate needs. Another criticism is that these findings must be due to some “fault” in the technique or the analysis of the data. The data are objective; nature never lies. The problem with any argument for the status quo is that it is not that our simple

experiments were technically flawed; indeed, the exactly the opposite is the case, as explained above, i.e. *previous work* had been technically flawed, or at least inadequately designed, to get to the root of the problem because of their long labelling times. We often learn to late about the need for very careful design of experimental protocols; few postgraduates and post-doctoral scientists get formal training in design, as also in the analysis the data. Statistical packages are used to determine whether a significant difference has been found between experimental and control groups. Only a handful of tests are now being used, and few people understand why they are applying a particular test, and what the statistical analysis actually measures. I was fortunate in having a course over several weeks in my early postgraduate days. The advice therefore is to seek training and expertise advice when delving into masses of data needing to be analysed.

Our “discovery” of the rapid turnover of *all nascent* proteins began to be appreciated when Yewdell’s group at NHI (Bethesda, MD, USA) had been investigating defective ribosomal products (DRiPS) for some time (22). In a follow-up paper, Yewdell acknowledged that their discovery was indeed a rediscovery of my findings some 20 years earlier (23), a second eureka moment as Yewdell himself called it. What is remarkable is that their acknowledgement of this finding has not yet met with “general approval”, i.e. we have not moved on since those days. The lesson is that there are complex issues regarding many phenomena and they need to be untangled if we are to gain a clearer insight. However, we have to consider whether the findings, while adding to the general pool of knowledge, has since had any influence on pursuits in the field of protein turnover. It also reinforces the fact that things happen very fast at the molecular level in cells, and techniques must be used that take rapid and early kinetics into consideration. There is probably a whole world of new findings to be uncovered if a very short time axis is taken into consideration and appreciated in experimental work on cellular metabolism.

ANALOG AMINO ACIDS

The new findings on protein turnover kinetics led us to using amino acid analogs. Some are sufficiently like their homologs to become incorporated into protein, provided there competition for incorporation is minimised (i.e. the natural amino acid is removed from the labelling medium). The analogs mostly explored were ethionine, para-fluorophenylalanine (pFPhe) and canavanine (an analog of arginine). We had been working with analog amino acids for years, and then realised

their relevance more fully in following proteins that cannot properly integrate in the cytomatrix, particularly those incorporating pFPhe (24).

In brief, proteins that incorporated some labelled analog (usually about 5-6% as efficiently as their homologs in the absence of the natural amino acid) were very rapidly turned over, as anticipated; only a very small percentage of these proteins became integrated into the cytomatrix. But even a few “analog proteins” can markedly disturb cell metabolism and behaviour. This led us to seeing the response of tumour and normal cells to analog amino acid incorporation, where the former were far more actively growing and therefore synthesising proteins considerably faster. Cell death was particularly noticeable in tumour cell populations. This work continues to this day with colleagues in Ukraine, because it has opened up the possibility again of the use of analogs in cancer therapy (25). Analogs had been explored for many decades; they have their place in combination therapy of tumours, as in vivo experiments have shown (26). This story goes further because cells that have synthesised and integrated analog proteins become considerably more sensitive to other agents, including cytotoxic drugs, irradiation and elevated temperatures (42-44°C) (27). The lesson here is that fundamental work at the cellular level must be taken seriously in their translation to medical science because they can offer rational explanation as to how the agents can best work in combination. There will be optimal concentrations and timings of application to achieve the best results, for improving treatment protocols and regimes in the disorders such as cancer. Translation from bench to bedside is now much more relevant than even a decade ago. Customised (personalised) medicine takes cognizance of this fact.

The reduction of arginine in the blood to just 3-5 % of its normal concentration can stop many tumour cells growing and actually kills many. Amino acid analogs can then enhance the destruction of remaining cancer cells, but exactly how to optimise the application of two agents remains a challenge, demanding rational thinking as to how each acts under particular circumstances and then in collaboration. The problems of making combination therapy highly effective are not trivial, and trial and error methods are laborious and unproductive.

DIFFUSION AND ITS LIMITATIONS IN CELL METABOLISM

Our findings on free and rapid exchanges of amino acids across the cell membrane indicate that, over the dimensions

of mammalian cells (on which most of our investigations took place), diffusion is fast enough to explain the kinetics we observed in formation of an intracellular pool and rapid incorporation into proteins. Almost all biomedical scientists learn that diffusion is the main process by which molecules in their random walks move from areas of higher concentrations to those of lower concentration (Fick's Law). The size of molecules is important in diffusion, but it is too slow to account, for example, for the integration of proteins in the more distant parts of the cell. From a plentiful supply of oxygen molecules in the lungs, it would take about 8 years for them to start reaching the big toe by diffusion alone if the body was a static structure mainly of water. Macromolecules would take almost an eternity to move this distance, which is overcome by the body having a rapid circulatory system that can deliver them in seconds. Not surprisingly therefore, distribution of macromolecules at the cellular level by diffusion is limiting, which is seldom taken into account because people consider the dimension (distances) molecules have to travel seem to be very small. Therefore, the prevailing opinion (assumption) is that diffusion is adequate. But this is not the case, and it has to be overcome by cells also needing an intracellular circulation. To follow flow at this level, one has to be prepared to use highly specialised equipment, in this case the acoustic (laser-Doppler) microscope (28). This can clearly show that there is *directed* mass movement within the cell, an intracellular circulation. Necessity is “the mother of invention,” and newer techniques are often required to move on.

WIDER IMPLICATIONS

The implication of the findings is that diffusion in general plays only a small role in the distribution of molecules throughout the cell as well as the body, as I have set clearly out the arguments that are contrary to the received wisdom [29]. The more aqueous laguna in the cytomatrix (among the trabecular lattice) of cells measure ~20 nm in diameter, enclosing a volume where diffusion will be fast enough to maintain metabolic activity. But the lattice runs throughout the cytoplasm; its structural elements will be inhibiting exponentially diffusion over distances greater than a few microns much more than if the cellular internum was completely aqueous (30). The lesson here is that general “theories” propounded for years may have much less relevance than we are led to believe, and can (ought to) be challenged on many grounds from time to time. Their limitations need to be recognised and understood. Once again, we need to appreciate that new insight is likely to raise

many wider issues; for example Clegg and myself discuss the implications of these findings on the control of rate of metabolism in cells and animals, which can be intimately related to the rate of delivery of essential nutrients to them (31), which can be a remarkably simple explanation, if only partially correct as other activity might be involved. This is another case where the (young) research scientist must always be aware of the “bigger picture” and see their data within its wider context; even a small advance can change the overall concept far more than one might expect. It also reinforces the fact that everything in nature is connected.

CONCLUDING REMARKS

Scientific research requires careful observation, investigation of the phenomena to which it draws attention, the formulation of rational hypotheses, and the training necessary to test them (to destruction on the Popperian basis). Linus Pauling remarked that scientists ought not consider only one explanation for a phenomenon; they need to postulate several hypotheses and have a means of selection. This means that you approach the most likely hypotheses first as these will probably lead sooner to an explanation of a phenomenon. For some unknown reason, many investigations start from a single hypothesis and try to prove it at the expense of considering that there can be several other explanations; indeed biological systems are inherently complex and several hypotheses might need to be intertwined to reach proper insight. Untangling the individual elements requires a very open-minded approach, logical thought progression, and the ability to eliminate untenable hypotheses by appropriate experimental analysis, which is as important as trying to show (establish) that your hypothesis is “correct”. Discoveries can be small, but sometimes big ones can come from a completely new approach to a phenomenon. Lateral thinking in effect is the making connections in the brain of ideas that did not previously seem to be related, but then subconsciously these associations can pop out literally in a Eureka moment. My experience through a considerable range of different observations and experiments has taught me to apply scientific method to testing many hypotheses about them. It has taught me to be sceptical of received wisdom in the sense that, while it might contain some elements of truth, one should not necessarily rely on widely held propositions. In modern parlance, you do need to think *outside the box*; to paraphrase Schopenhauer, *to see what everyone sees, but think what no one else thinks*.

The fascination of scientific research is not just in formulat-

ing hypotheses, but in executing very exacting experimentation to test rigorously their invalidity or validity. It is not the end result, however gratifying this might be when making a major finding (a breakthrough), but the pleasure in doing the work itself. It also results in a self-perpetuating interest because each little finding opens the doors to many, often unexpected, new avenues to be investigated; indeed, research is a never ending game, as espoused again by Schopenhauer, who intimated that if you think you have completely resolved some issue, you are in fact opting out of the “game” (as quoted above). There are times when frustration becomes quite intense, and leaving a problem for some months has led to a sudden moment when some perhaps trivial event or thought suddenly resolves a predicament that was the cause of the frustration (again those little Eureka moments that make life worth living in the field of research). My career has been rewarding in this way and I have that same inquisitive nature now as I had at the start, which was about 60 years ago when I first began as an undergraduate studying cell physiology with James Danielli of cell membrane fame in London. While their lipid bilayer nature seems to be a long accepted “theory”, it remains a hypothesis and has been challenged on many fronts (there are holes in it!).

Life is one long lesson in understanding life in all its dimensions, this world and our universe. Nothing is totally sacrosanct; *the only thing constant is change*. And finally one word of caution; it has also taught me that we are only just beginning to have insight into all the workings of the cell and thus life itself. We should be under no illusion that we have got deep into the problems. We are still only scratching the surface. If there is one question as a biomedical scientist that remains central for me, it is that of Schrödinger – *what is life?*

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