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Implications of 'postmodern biology' for pathology: the Cell Doctrine

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Recent insights regarding stem cells, repression and de-repression of gene expression, and the application of Complexity Theory to cell and molecular biology require a re-evaluation of many long-held dogmas regarding the nature of the human body in health and disease. Greater than expected cell plasticity, trafficking of cells between organs, 'cellular uncertainty', stochasticity of cell origins and fates, and a reconsideration of Cell Doctrine itself all logically follow from these observations and conceptual approaches. In this paper, these themes will be considered and some implications for the investigative pathologist will be explored.
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Recently, independent strands of research in cell and molecular biology have woven a new pattern of concepts regarding how biological organisms are structured. These concepts are distinct from, yet are complementary to, the standard models that have dominated Western medicine and biology. Moving forward, this scientific process regarding biological structure may develop much as did the shift from Newtonian physics to the Relativity and Quantum Physics, which supplemented it. Note that the word is 'supplemented', not 'supplanted', for the Newtonian worldview remains a viable model for the physical world at every day levels of scale. However, at both the largest and smallest scales of observation, the refinements of Relativity and of Quantum Mechanics, respectively, were necessary for a more accurate depiction of the structure of our universe. As we will see, it is in part consideration of changes in scale of biological investigations that drives the need for new concepts and new guidelines for experimental design, for interpretation of data, and, perhaps, for understanding of disease.

The new findings to be highlighted herein include the recent discoveries of previously unexpected cell plasticity, reversibility of gene restrictions, and possible molecular bases for stochasticity of genetic expression and, therefore, of cell behavior (Table 1). It is hoped that a firm beginning in experimental findings will excuse some of the speculations that logically follow—even those that are frequently dismissed by traditionalists as 'theology'—and allow the reader to proceed with comfort into what we should now consider, literally, as the 'uncertain' terrain of the structure of the body, a 'post-modern' biology. Bringing to bear a Complexity Theory-based analysis on the problems of cell and molecular biology will hopefully make these concepts not only appealing but also exciting to the engaged biologist or clinician.

As with any intricate weaving of cloth, there is no simple, linear thread to be followed that will allow a direct path from 'here to there', let alone that will, in and of itself, allow visualization of the woven pattern as a whole. However, some narrative choices of course must be made, so I hope to be excused for following the path my own investigations followed, which have led to my formulations of some of these new concepts and approaches. Others might well weave a different tale; this paper will hopefully encourage such efforts.

Cell plasticity and the reversibility of gene restrictions

The standard model of hierarchical and unidirectional cell development and differentiation derived from early experiments where cells from one part of the embryo were transplanted to different regions. It was noted that, up to a certain point in

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Table 1 Themes in postmodern biology

'Genomic completeness'	Any cell with the entire genome intact (ie without deletions, translocations, duplications, mutations) can potentially become any other cell type
'Cellular uncertainty'	Any attempt to analyze a cell necessarily alters the nature of the cell
'Stochasticity of cell fate'	Descriptions of cell lineages (progenitors and progeny of any given cell) must be expressed stochastically
Cells comprise interacting agents of a complex adaptive system	Cells self-organize into larger biological structures (tissues, organs, organisms) and are therefore inherently stochastic in behavior and subject to 'mass extinction events'
Cell Doctrine is scale dependent	Cells are embedded within a hierarchy of complex systems and therefore only exist as defined entities on the microscopic level, suggesting that Cell Doctrine is only one of many possible, scale-dependent models for the organizational structure of organisms

development, the transplanted cells would adopt the differentiation states appropriate to the new location, but after a certain point they would retain their original state, resulting in inappropriate differentiation for the new site. Eventually, molecular biology provided the mechanistic underpinning of these observations by demonstrating time-dependent, irreversible repression of gene expression not required for the original differentiation pathway.

Details of mechanisms for gene restriction have been reviewed in detail elsewhere.[1,2] However, in brief, there are two general mechanisms: methylation of genomic sites and formation of heterochromatin domains by interactions between chromosomes and histones (and other proteins). Clustered methylation of cytosine in CpG islands, often located at promoter sites, generally leads to restriction of gene expression (although exceptions where such methylation lead to gene activation are also known). Such methylation is mediated by tissue-specific methylases, some of which have been identified. Formation of heterochromatin, leading to stable inactivation of regions of the genome, arises from epigenetic modifications to histones, prominently including de-acetylation and methylation attributed, again, to cell-specific enzymes.

However, returning to the cell biology scale of investigation, the possibility that these early transplant experiments were only a limited window on cell potential was made evident in early work by Helen Blau and colleagues regarding heterokaryon formation, that is, the placement of a nucleus of one, 'terminally differentiated' cell into the cytoplasm of another cell type.[3] Under some circumstances, the donated nucleus changed gene expression pattern in part or entirely to that of the recipient cell. This led Blau to suggest that 'differentiation is an actively maintained state', rather than the rigidly programmed one set during embryonic and fetal development.[4]

Table 2 Demonstrations of adult cell plasticity (nuclear reprogramming)

Nuclear transplantation into adult somatic cells	Formation of 'heterokaryons' leads to reprogramming (complete or incomplete) of the transferred nucleus, conditioned by cytoplasmic factors of the recipient cell (may have physiologic roles)
Nuclear transplantation into oocytes	'Cloning'; complete transcriptional reprogramming of donor nucleus to generate all cell lineages of the embryo, fetus, and adult organism
Cell transplantation	Donor cells undergo complete or incomplete reprogramming by microenvironmental alteration cytoplasmic regulation of gene expression (may have physiologic roles)

Transplantation studies of a different type, performed in the waning years of the last millennium, suggested how true this formulation might be. In 1999, *Science* heralded three experiments published in its pages as 'Breakthroughs of the Year'[5–7] (Table 2). These papers demonstrated bone marrow cells could become skeletal muscle and liver, and neural stem cells could give rise to blood when transplanted into lethally irradiated mice. That this represented true plasticity, rather than an unexpected population of tissue specific cells lurking in other tissues, was suggested by work from my own group following single bone marrow cell transplantation into recipient mice: single-cell transplantation led to engraftment and differentiation into cells of all three embryonic lineages, mesoderm, endoderm, and ectoderm.[8] This finding remains controversial, and studies that specifically replicate the work have not been published. However, studies with a less extensive agenda have shown some degree of this plasticity, with single cells differentiating into one or two of these embryonically defined lineages, for example, *in vivo*[9] and *ex vivo*.[10]

A proof-of-principle is present in the extensive cloning studies which began, famously, with the cloning of Dolly.[11] This cloning event, at least, remains unchallenged. While cloning itself remains a difficult and fairly brutish approach to reprogramming expression of the genetic code of a fully differentiated nucleus, it indicates that there are some factors within the cytoplasm of an oocyte that, at a particular point in time, are capable of erasing the 'irreversible' gene restrictions to allow that adult nucleus to program cells of every type of the body. The difficulty in accomplishing this consistently under experimental conditions is most likely an indication that these experimental attempts remain rudimentary, rather than an indication that such outcomes are necessarily exceptional.

More recently, some of the mechanisms underlying how cells can move from one organ to another, engraft there, and take on differentiative aspects of the new home, have come to light. For example, the cytokine *stromal-derived factor-1* (SDF-1) has been implicated: its production by cells of the target (sometimes injured) tissue and its release into the circulation can create a gradient for CXCR4-positive cells (the receptor) to home to the site.[12] Understanding such signaling may lead directly to therapeutic benefits if their production can be upregulated or perhaps by giving them exogenously.[13]

There must of course be molecular processes underlying this reversing of gene restrictions, implied by the transplantation and cloning events demonstrating adult differentiative transformation. Interestingly, such mechanisms of reversibility at the molecular level were being reported during the same time period as the cloning experiments, and continue to be elucidated. Review of these rapidly accumulating developments is beyond the scope of this paper, although we have recently summarized many of them.[1]

Finally, more recent experiments have demonstrated that such plasticity events can occur via not one, but two general mechanisms: direct differentiation following exposure to new microenvironmental cues and cell–cell fusion wherein the nucleus of one cell is exposed to cytoplasmic factors of another cell, that is, the physiologic equivalent of Blau's heterokaryon work. Which of these mechanisms will dominate in an *in vivo* setting and to what extent is likely to depend on whether there is tissue pathology and on the nature and severity of the pathology.

We have advocated for 'new principles' of cell plasticity, principles that logically follow on these varied experimental results.[14–16] The first principle is referred to as the principle of 'genomic completeness'. With cloning as proof-of-principle, supported by an ever-expanding demonstration of the reversibility of gene restrictions described above, one may state that 'any cell which has the entire genome intact can become any other cell type'. This is not to say that such transformations are necessarily physiologically possible, but once experimental or therapeutic manipulations are brought into play, plasticity of cellular differentiation is limited only by the ingenuity and extent of effort by researchers, not by molecular impossibilities.

Uncertainty and stochasticity

The second and third principles follow on combining the above genomic plasticity with a still valid truism regarding the nature of cells: 'the internal and the external co-determine the cell.'[17] To wit, if the microenvironment around a cell (or if the cytoplasm around a nucleus) is altered, then the nature of the cell, namely its pattern of gene expression, is likely to change as well.

A key corollary that comes into play is the argument that any attempt to observe a cell, let alone manipulate it, will, by definition, alter the cell. The similarity of this idea to Heisenberg's Uncertainty Principle from quantum mechanics led to our designating this second principle as 'cellular uncertainty'.[14–16] However, approximately a decade before we published on this concept, it had been proposed by Chris Potten and Markus Loeffler, arrived at through their collaborative work in mathematical and computer modeling of stem cell processes of the small intestine.[18] Quantum 'uncertainty' was first thought, even by Heisenberg himself, as perhaps merely a limitation of technological innovation for examination of subatomic particles, but eventually was revealed to be an irreducible feature of universal structure. This cellular application of the term was initially intended more as metaphor rather than as true and exact analogy. We will return to this topic later.

For many years, there has been serious debate as to whether cellular processes, particularly those involving differentiation, were determined or stochastic.[1] Experimental results for many years could be used to argue both possibilities. However, considering *genomic completeness* and *cellular uncertainty* in light of each other, one is logically required to then state that cell lineages, to the best that we can observe them, are stochastic processes and that to describe the progenitors or progeny of any given cell under observation, one must speak in terms of probabilities.[14–16] Moreover, in describing a population of cells, there must be recognition of inherent variation, no matter how well characterized the population. In other words, cellular heterogeneity is irreducible, and descriptions of the cell populations must be expressed in terms of probabilities.[15,16] The third principle is accordingly designated the 'stochasticity of cell fate'.

Increasingly, data accumulates and, along with it, a growing (although not yet complete) consensus that cell differentiation is, indeed, stochastic.[19,20] This is not to say that it is completely random and/or chaotic, but rather that there is a constrained randomness in cell development. Again, we will return to the notion of constraint later, as well, and find that it represents a key point in understanding the organization of living structures.

There are possible molecular bases for such stochasticity. While there are the possibilities of an eruption of quantum effects into molecular behaviors,[21,22] a more intuitively direct example is found in the work of the Cremer laboratory of the Ludwig-Maximilians-University, in which they fluorescently labeled euchromatic and heterochromatic chromosomal domains in living cells and then observed the movement of the label in real time.[23,24] As expected, the heterochromatic regions were essentially stable and unmoving, particularly those that

were localized to the nuclear membrane. However, euchromatic regions were not stable, but, rather, moved very freely within the nucleoplasm, sometimes outside of the chromatic domain, sometimes inside. The movement was best described mathematically as a 'random walk'.

Given that access to transcription factors that regulate gene expression depends on exposure to those molecules afloat in the nucleoplasm, the randomly changing exposure as euchromatic genes move from the interior to the exterior of the chromosomal domain, and back again, means that there is inherent stochasticity to gene expression. Again, in all likelihood, it is not to say that it is completely random, but that there is a constrained randomness (probably constrained in part by structural/architectural aspects of adjacent chromatin regions).

A complexity primer for the experimental biologist

Stephen Hawking has said that 'the 21st century will be the century of complexity.'[25] Indeed, Complexity Theory, with its notion of *emergent self-organization*, has become an important lens through which to view topics germane to many of the hard and soft sciences.[26,27] However, cell and molecular biologists have been slow to adopt its analytic approaches. This is surprising, as its basic principles are (surprisingly) simple and may have profound benefit for biologists, physicians, and definitely for pathologists.

For example, a persistent issue for critics and researchers of adult plasticity findings is the events are usually infrequent, no more than single digit percentiles or even as low as one in 100 000 cells of an examined tissue.[28] Occasionally higher, robust engraftments have been noted (eg in radiation pneumonitis in mice[8] or in fibrosing cholestatic recurrent hepatitis C after liver transplantation[29]), but usually it is a low-level phenomenon, a fact often pointed to by critics of the field.[28]

One approach to considering this seemingly trivial level of engraftment lies in considering whether it is another form of constrained randomness that we have referred to previously in this essay.[30–32] Such constrained randomness or *quenched disorder* is central to the functioning and characteristics of *complex adaptive systems*. Complex adaptive systems are any group of interacting individuals that fulfill certain behavioral criteria and will therefore self-organize into large-scale structures that can adapt to environmental alterations.

A common and readily understandable example of a complex system would be ants and the colonies they give rise to. There is an exceedingly precise social and physical order to ant colonies that develop without any central designer (the Queen Ant's function is reproductive, not organizational). If one programs 'virtual' ants—single units that interact with each other and the local environment the way real ants do—then they will self-organize into a virtual ant colony, not coded by the programmer.[26] They do so because, in common with most other complex adaptive systems, they fulfill four criteria:

1. *There are large numbers of individuals*: Number is important for the nature of emergent structures arising from their interactions. Fewer 'ants' will generate one type of colony; as the population increases, the structure will change in response. In human terms, a village is different from a city, which is different from a megalopolis.
2. The individuals respond only to local cues, without monitoring the large-scale emergent structures of the system as a whole.
3. Some of the interactions between individuals must create homeostatic, negative feedback loops. If there are only positive feedback loops, or if such positive feedbacks predominate, there may still be self-organization, but these are energy expending, nonadaptive systems (eg tornados, hurricanes).
4. There is constrained randomness, quenched disorder in the system. Too much randomness and the system is likely to be completely disordered or chaotic. Too little and it will be rigidly determined and unable to make adaptive changes.

In ant colonies, the few ants that are not, for example, following the food line are the ones that are instrumental in exploring new pathways if your foot interrupts the food line or if the current food source runs out. So the limited randomness can be said to allow for exploration of new states that will allow the system as a whole to adapt.

Likewise, dispersed cells of slime molds assemble into an adaptive multicellular organism in response to temperature changes. Real ants emergently assemble into colonies. People emergently assemble into neighborhoods, economic markets, and political structures. The collective flora and fauna of the Earth generate the self-regulating Gaia system described by Lovelock.[33]

Slime molds are a particularly appropriate example to raise in this context as they hint at a way of looking at all multicellular organisms: their cells fulfill all of the four criteria and therefore one may consider the organization of tissues, organs, and bodies (plant or animal or other) to be the emergent properties of interacting populations of cells. So, for humans, one may consider that embryonic and fetal development and postnatal tissue maintenance and repair may all represent the emergent self-organization of cells.[30–32]

The mathematical corollaries of complex systems have import for our understanding of the behavior of such biological systems. For example, even with

precisely the same starting conditions, the emergent properties of similar systems will be different and unpredictable, even if all the rules governing interactions between individuals are understood. The constrained randomness in the system makes precise predictions impossible. The *fact* of emergence can be foreseen, but its precise character remains unknown until the system is allowed to actually 'run'.

Mathematically, if one creates a phase diagram of order and disorder, complex systems occupy a zone that lies on the border of completely determined ordered systems and chaotic (ie fractal) systems. However, as the randomness in the system keeps it in a state of dynamic exploration of new and adaptive states within that border zone, it is likely, with sufficient time, to wander off into mathematical chaos leading to what is often referred to as a 'mass extinction event'. This has been invoked to explain diverse phenomena including, for example, the collapse of stock markets, of civilizations, the extinction of species, and the decline of inner cities.

Cell Doctrine, revisited

A key aspect of complex systems is that they can exist in hierarchies. As I have discussed previously, this feature leads us to the perhaps strangest aspect of 'postmodern biology', namely the need to reconsider Cell Doctrine, the foundational paradigm for Western medicine and biology.[34,35]

We return to the ant colony. From a distance, an ant colony might have the appearance of a dark, shifting, though solid shape on the ground in and on which it organizes. From up close, however, it is clear that it is not a solid thing at all, but rather organized behaviors of the individual ants. At this level of scale, the ants appear to be single entities, as the colony appeared from a much greater distance. However, as we move on to the microscopic scale, we can now understand that the ant bodies (or any bodies, including our own) are likewise not discreet entities, but are the self-organization of still smaller structures. Depending on the scale of observation, the emergent self-organization of these interacting entities can either have the appearance of a definite *thing* or dissolve, on lower scales of observation, into a multitude of interacting smaller things. 'Thingness', one might say (with apologies, perhaps, to Theodore Geisal, aka 'Dr Seuss'), is dependent on scale.

Moving up the hierarchy, we also see how bodies of diverse kinds self-organize into larger-scale entities such as cities, cultures, and ecosystems. Indeed, Lovelock's Gaia hypothesis is a reflection of precisely this: at the global scale of observation, Earth and all it contains does, in fact, constitute a single discreet, adaptive entity. It is simply difficult to see it when one is part of it.

Think about the city in which you, the reader, live. You probably consider it to be 'your city' as do most of your neighbors. However, within a century or so, no one alive today will be living in that city, and yet the city, as an entity, will still exist (barring a mass extinction event). The life of a city (bee hive, ant colony, rain forest) transcends the individual lives of the beings that inhabit it and constitute it. Thus, also, bodies temporally transcend the individual cells that comprise it at any given moment.

But what of these cells? One may also argue that these, in turn, represent the self-organization of lower-level, interacting entities, namely biomolecules. Biomolecules certainly fulfill the first three criteria to form a complex system: they are numerous, interact with homeostatic feedback loops, and only interact on the local level, with other molecules, but without monitoring the larger system as a whole. At the purest level, their interactions one-with-another are governed only by the biophysical properties of their component atoms, and by the likelihood that any two biomolecules will encounter each other. Do biomolecules, however, display quenched disorder? Is their likelihood of mutual encounter nonrandom?

Once again, recent, parallel studies in another field shed light on this question in an unexpected manner.[36,37] Interactions of biomolecules are energy dependent. The energy required is often provided by breakdown of energy bearing molecules such as ATP. It has long been presumed that movements of biomolecular motors, for example, are driven by such energy. Recent observations of single molecule interactions reveal a surprisingly different dynamic.[38]

Take, for example, work from Toshio Yanagida's laboratory looking at relationships of actin and myosin.[39] Real-time observations of the movements of a single myosin filament along a single actin filament reveal that the hydrolysis of ATP is insufficient to power the bending of the myosin hinge and, thus, its sliding movement along the actin filament. Rather, movement of the myosin filament is random, in response to the Brownian motion of the water in which it is suspended. The energy derived from ATP does not move the filament, but rather constrains the random, Brownian-driven movements into the physiologically required direction.

This molecular version of quenched disorder is now recognized in a host of interactions, including ligand/receptor binding, RNA/DNA interactions, and dynein and kinesin movements.[35,37] Thus, biomolecules do, indeed, display the quenched disorder necessary to form a complex adaptive system and therefore to self-organize into cellular organelles and cells, themselves.[36] And, as such, the nature of the cell needs to be reassessed.

Just as ant colonies and cities are defined entities on one level of scale, but lower down are clearly not entities at all but are rather the self-organization of

smaller things, so too are cells only defined entities on one level of scale. Higher up, they are invisible (and thus remained only one hypothetical possibility for explaining the substructure of the body until the invention of the microscope). Lower down, they likewise cease to exist as definable entities, but instead merely a product of the dynamic, organizational interplay of biomolecules. Thus, from this perspective, a cell, as a 'building block' of the body, has no inherent existence independent of the mode of observation. 'Now you see it, now you don't.'[34]

Returning to the question of cellular uncertainty, we must now consider that, in the absence of such inherent, scale-independent existence, cellular uncertainty is inherent, rather than simply a result of current technological limitations.[34,35] For example, from Greek times, clearly prior to the availability of the microscope, there was active debate about the substructure of the body.[40] One school of thought was that the body was made up of ultimately indivisible subunits ('atoms'—the borrowing of terms once went from biology to physics, rather than the other way around). The other school of thought was that the body was comprised of an endlessly divisible fluid. With the advent of the microscope in the 1500s and subsequent observations of cell membranes and cell walls, the argument was decided, as the empty box of the cell (like the cell of a monk or of a prisoner, hence the term) could not be subdivided. When the 'furniture' of the empty cell was visualized in the ensuing decades (20 years before nuclei could be stained and confirmed), Cell Doctrine took its place as the champion of the debate.

However, now we see that on a level of scale down, the body is, indeed, an endlessly divisible fluid. For biomolecules, intra- and extracellular are meaningless terms. What if, in fact, the earliest technology for microscopic observation had been different? What if nuclei were the first structures to be clearly recognized? Then, the fluid continuum would have become the dominant paradigm, with these odd little balls floating in the fluid more curious than contradictory. If 20 years passed and then cell membranes were discovered, then biologists might well have clung to their 'Fluid Doctrine', simply modifying it to include semipermeable partitioning of the fluid compartment by the presence of phospholipid bilayers—the 'plasma membranes'. How many different models for the body might there be? How much do we miss, by excluding these other perspectives from consideration?

Some implications for clinical and investigative pathology

Cell Trafficking and Multiorgan Engraftment

If injured tissues can produce signaling molecules which recruit cells from the circulation, might tumors that preserve this function or upregulate it *de novo* also do the same? For example, some tumors produce one such molecule, SDF-1.[41] If so, might this explain inconsistent findings regarding the monoclonality (or lack thereof) in benign neoplasms such as parathyroid and thyroid adenomas?

One might also consider that the ability of progenitor cells of various kinds to enter the circulation and traffic between organs may be the normal, physiological variant of what becomes metastasis when it is tumor (stem?) cells that are involved. Indeed, might the pathology of tumor metastasis give important hints for the understanding of these more recently recognized plasticity events?

Cellular Uncertainty

Given the exquisite sensitivity of cells to their microenvironment, one must consider the possibility that any attempt to isolate cells, let alone characterize them, in advance of some sort of 'conditioning' procedure, is, in fact, already part of the conditioning process. Consider, for example, perhaps the simplest isolation step, when one extracts blood cells, already in a fluid suspension, by venupuncture. The cells are exposed to marked alterations in flow dynamics and exposed to a nontemperature-regulated metal surface as they are sucked up into a syringe. Expelling them from the syringe entails more of the same. These mechanical interventions have been demonstrated to alter cell behavior.[15] How much more so will mechanical and/or enzymatic tissue disaggregation, violently breaking cell:cell and cell:matrix linkages, alter the behavior of a cell? It is disingenuous at best to assume that these are simply 'neutral' isolation steps.

Let us then say that the cells are immunolabeled for FACS sorting. Aside from additional turbulence, temperature, and material contact influences, the binding of antibodies to surface proteins must also be considered a conditioning process. For example, depending on which antibody clones are used, CD45 activity may be unchanged (although blocked from additional binding to factors in the culture media), upregulated or downregulated.[42] Again, clearly the isolation step is also a conditioning step. At least, however, in this example, some predictability about the impact may be made.

However, consider sorting for CD34, a molecule central to many hematopoietic stem cell isolation/enrichment procedures, but one for which no precise functionality has yet been defined. We have essentially no idea of the impact of binding antibodies to isolate cells expressing this molecule; indeed, we may not even be certain if the binding of detecting antibodies is in fact pushing the cells in the direction of stem cell functioning.

Stochasticity of Cell Origin and Cell Fate

As already noted above, when dealing with a single cell or population of cells, one cannot be certain of the lineage pathway through which it or they descended from a progenitor. Likewise, their progeny can only be stochastically predicted.

Additionally, any population of cells must always be considered heterogeneous. When researchers ascribe homogeneity to an experimental population, they always are speaking in terms of an exceedingly limited set of markers. Differences in location during the cell cycle alone dramatically alter gene expression and differentiative potential of the cells under investigation.[43] Even if one controls for this by synchronizing all cells in a population via cytokine/chemokine manipulation, how many other factors remain uncontrolled? Examples of other implicit sources of heterogeneity: different locations within tissues or inside laboratory vessels resulting in differences within a population in terms of cell:cell contact, crowding, nutritional supply, etc.; circadian variations dependent on timing of cell isolation and light/dark controls during culture or *in vivo* experimentation.

Both of these concepts force a recognition that the conclusions about histogenesis of tumors, based on phenotypic similarities, are also suspect. While, indeed, many tumors retain aspects of the original cell mutationally altered to lead to the tumor, it is never certain for any particular patient's tumor, even if a general class of tumors is likely to be so derived. The need to say 'likely' in this context further highlights the necessity of recognizing that these processes are stochastic.

Exciting recent developments regarding epithelia–mesenchymal transitions also point in this direction.[44] Even the assumption of separating the stromal responses to tumors from the tumor cells themselves, that is, the hyperplastic from the neoplastic, becomes fuzzy. Benign stromal reactions may sometimes reflect a more controlled growth of cellular progeny of the tumor-originating mutated cell itself. In this case, assumptions of histogenesis based on phenotype have missed a greater (and astonishing) complexity to the genesis of neoplasia and its effects on surrounding tissues.

The Complex Nature of Cell Biology

The emergent self-organization of any complex system is defined, in part, by the nature of the interactions between the individuals as well as by the size of the population comprising the system. Thus, embryonic and fetal development, while sharing many features of postnatal tissues, are also quite different, although it is all one extended unfolding of self-organization of the participating cells.

Similarly, tumors, from a complexity perspective, are also not substantively different from normal tissues, they are simply different forms of self-organization as the rules governing cellular interactions change, perhaps in response to injury (eg keloid formation, osseous metaplasia in torn muscle) or to mutations. Change the cellular signaling between parathyroid cells and one gets a parathyroid tumor, similar to normal parathyroid gland, but different, too. Change the signaling sufficiently, so that positive feedback loops come to predominate, either by genetic shifts that upregulate these or that lead to loss of homeostatic feedbacks, and one gets an energy consuming, nonadaptive self-organization: cancer.

One may also consider issues of tissue engineering, which is typically thought of as, just that, an *engineering* problem: create an architectural framework and stack the cellular bricks inside it to make a structure. However, this is not how tissue organization actually takes place. An alternate approach might be to simply place the appropriate cell types in a setting and, by shifting the setting, influence how they organize themselves into larger, tissue-like structures. An example of this is in the exposure of hepatocytes within experimental artificial livers to serum from patients in acute liver failure: the cells begin to form bile duct-like structures. Cells *will* self-organize, we simply need to create the starting conditions that promote a likelihood that the self-organization will be structurally similar to normal tissue and, therefore, be of use for grafting and repair.

Mass Extinction Events Involving Cells and Tissues

The statistical inevitability of the so-called 'mass extinction events' of complex systems applies to cell behavior.[31–32] This means that some organ failures may not be strictly a response to an external insult. Some organ system collapses that are currently classed as idiopathic (eg aplastic anemia) might represent the statistically likely mass extinction of that tissue. While mechanisms may be eventually found to explain some cases, detailed population studies might reveal a mathematical probability similar to that already described in mass extinctions of other complex systems.

Likewise, for example, the occurrence of the rare organ collapse of individual patients within a population affected by a usually self-limited disease (eg hepatitis A) may relate to statistical aspects of organs as emergent phenomena, rather than something specific about the person so affected. Epidemiologic studies of a scale and type not yet performed may be necessary to evaluate these possibilities.

This principle also means that for organisms to be truly, adaptively alive, they must also inevitably die. There is no such thing as a fountain of youth for

physicians to identify; life and death or as inseparable as two sides of a coin.

Limitations of Cell Doctrine

This essay on the need to invoke alternate models of the body—these 'post-modern' concepts—to explain phenomena, may border on what some researchers may dismiss as 'theology', 'philosophy', or downright 'nihilism'. The ramifications are powerful, and can be experimentally tested.[1,34,35] For example, there are testable and reproducible phenomena of the body that cannot be explained in simple anatomic terms. Perhaps the most well documented of these would be acupuncture. The 'problem' that acupuncture poses for Western medicine is that its meridians—conceptual constructs central to the successful practice of acupuncture for therapeutic benefit—do not correspond to any known anatomic structure.[45] Dissect down along the meridians and there are no nerves, vessels, muscles, or lymphatics in evidence. If the effects of acupuncture cannot be described in some measure through anatomy, then it cannot be fully explained by the building blocks of that anatomy: cells. As long as Cell Doctrine remains the only model for the body, then some aspects of the body, like the acupuncture meridians and their effects, will remain poorly understood or even unrecognized.

Understanding of tissue physiology and how that physiology is disrupted in disease may, therefore, sometimes, be best promoted by leaving Cell Doctrine behind. Consider electrical conduction through tissues: fluid dynamics may better describe (and yield experimental approaches) than cellular dynamics. Conduction through muscle is obviously well established. Less well known, perhaps, is recent work, for example, showing that calcium waves propagate through the liver.[46] How might such physiologically demonstrable effects relate to disease in the liver? We know that bone repair can be facilitated or directed by manipulation of electrical current, so even this least fluid tissue in the body can still be described as a fluid continuum.[47] Are there aspects of disease that reflect such a model?

It behooves clinical and experimental pathologists, as the true *tissue biologists* in medicine—at higher, integrative levels of scale above those studied by cell and molecular biologists—to perhaps consider new and creative approaches to modeling tissues and the pathologic processes that can effect them. Only two models have been discussed here; how many might there be if we consider different scales and different modes of observation?

Summary

We see that parallel revolutions in wide ranging studies of biological systems are leading to surprising conclusions about the nature of the body and, therefore, the nature of pathologic conditions affecting the body. While reductive approaches have been and remain extraordinarily powerful for the study of disease, regeneration and repair, we are increasingly in an age where ever finer experimental techniques and the explosion in detail and data which they provide, now require a systems approach for analysis with brings these diverse elements together in newer, more powerful understandings.

While uncertainty, literal and figurative, may overwhelm or confuse investigators in such a postmodern era of biology, it also provides the door to astonishing potential, both for what the body is capable of and for our ability to understand it, in health and in disease. We should embrace such change with energetic creativity, balanced of course by rigorous experimental testing and careful assessments of reproducibility. Established paradigms should not be surrendered lightly, nor, however, should they be rigidly held when accumulating evidence highlights their limitations. Even as they have limits, they will, of course, remain useful, within the limited experimental frameworks, which gave rise to them (as Newtonian mechanics is still relevant for sending people to Mars, even though quantum and relativistic mechanics has also been delineated).

To conclude, I would quote two nonpathologists whose lessons, nonetheless, are central for continued progress in our field. Barbara McClintock, Nobel Prize winning geneticist, once said, 'There's no such thing as a central dogma into which everything will fit…any mechanism you can think of, you will find—even if it is the most bizarre kind of thinking. Anything. So if the material tells you, 'It may be this,' allow that. Don't turn it aside and call it an exception, an aberration, a contaminant. So many good clues have been lost in that manner.'[48]

And Goethe summarized the essential first lesson for the training pathologist, but which even the most senior pathologist, whether clinician or investigator, should remember and affirm: 'Thinking is better than knowing, but looking is even better.'[49]

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