Ray Peat's Newsletter

Tissue Firmness and Elasticity, and Rothen's Resonance: Forgotten Physics and Aging

The most interesting problems exist exactly where the professionals assert that there is no problem at all.

The space between cells is of interest to many specialists, but it is relegated to the background in mainline medical thinking. In reality, the stuff between cells is the biggest part of a complex organism, and it contains many of the answers to questions of individuality, immunity, growth, aging, healing, sickness, and regeneration.

Respiration is essential for the existence of higher organisms, making it possible to maintain complex and adaptive structures, containing appropriately differentiated cells. I want to introduce some ideas about how respiration is involved in the creation of structures both inside cells and in the spaces between cells.

The idea of "containment in the cell" has directed attention away from the ways in which a cell knows what it is and where it is, and where it should be, what it should do.

The material in between cells has been dismissed as little more than glue, but it is where some of our most important things happen.

But first, I want to outline some of the mental habits and fixations that have been retarding biology and medicine for a long time, keeping people from thinking about these organismic issues.

THE BIAS AGAINST SENSITIVITY

In dead vegetable matter, "cells" were the empty spaces in the fibrous framework. Plants could be grafted or propagated from cuttings, making it clear that one type of plant tissue could generate the other types. The "essence" of plants was considered to be little more than growth, and their seeds had the disadvantage of introducing individual variation. Grafting and cloning animals didn't seem to work, and the idea grew up that the animal's essence was contained in the germ cell, while the plant's essence was in its vegetation.

"Contained" in the "cell," our "essence" had only to reveal itself. A man's semen was his essence, which introduced the soul into the egg, animating it. Replacing "preformationism" with "epigenesis" simply introduced the idea of individualism, by saying that a new individual came into existence by fertilization, but it didn't

Carbonated water is such a common thing, chemists are embarrassed to talk about it.

All the water in respiring organisms contains a considerable amount of carbon dioxide.

Carbon dioxide binds to proteins and to other amine-containing polymers, and dissolves in water, reducing the pH, so that the interactions of polymers and water are strongly affected by the concentration of CO2.

Carbon dioxide modifies biological materials and structures in and around our cells.

end the belief that our identity was "contained" "genetically" *within the cell.*

The neat little compartments seen in plant tissues became a sort of mental armature that no one questioned. Almost all of biology has been reduced to the idea that "the necessary information is *contained within* the cell, in its genes." The "Central Dogma" of molecular biology was stated as "information flows from DNA to RNA and from RNA to protein, and not the other way." To speak of cellular sensitivity (as Lamarck did) is to assert that information flows *into* cells; the fanatical opposition to Lamarck that has characterized biology in the 20th century has blended into a general hatred of "vitalism," the idea that life could be explained in terms of its special, living qualities. **The dogmatists insist that life must be explained in terms of physics as they understand it, and it happens that their "physics" is nothing but a doctrinal relic kept in place to justify their conceptions of biology.**

The idea that cells are governed by their responses to whatever surrounds them has taken a long time to develop, and the dogmatists still feel that there is something impermissibly "vitalistic" in the idea that cells are sensitive or responsive. To be sensitive is to receive information from "outside," and so the anti-Lamarckian ideas of evolution and adaptation were extended into the fields of development, immunity, aging, even learning. "Clonal selection" has been proposed to explain everything that happens in the organism, from development to cancer, because this view is compatible with the doctrine that information flows only from the genes to the cell; the cells simply die if they don't contain the necessary information. "Programmed cell death," in which a genetic code--a program, like a computer program--specifies the time at which a cell will die, is a characteristic part of this doctrine about the nature of a complex organism.

I think it is necessary to look at these established ideas, which are absurd and unfounded, because we are forced to wallow in them every day, until people have come to treat them as if they were intelligent, intellectually responsible, descriptions of reality; if we realize that silly ideas and connotations have been built into our language, then we can begin to give serious attention to the "anomalies" of science.

"Anomalies" are facts that don't obey the law, and so they are of special importance for anyone who cares about understanding things. If you have some parts left over after you have assembled a puzzle, maybe the puzzle wasn't correctly assembled. So-called anomalies are often the most productive places to look for solutions.

CELLULAR JUDGMENTS

That cells may be small or large or giant hasn't seemed problematic to most biologists. The assumption seems to have been that they "are big enough to contain their essential genes."

Once or twice, I have got biologists to talk about the question of how a cell knows that it is big enough to divide. I think the essence of their answer is something like "they count the molecules," which is to embarrassingly compound the puzzle, like the farmer who explained his marvelous ability to rapidly count the cows in a field by saying that he "simply counted the teats and divided by four."

The same kind of question can be applied to the thickness of layers of extracellular material, such as the basal lamina of capillaries. For example, granted that a cell should rest on a structure of a certain uniform thickness, how does a cell know when to stop secreting the material? When it is modifying the chemical composition of its extracellular matrix, how does it determine that the right composition has been achieved, when the substance is outside the cell?

Cells have to, in some sense, perceive themselves and their environment, because their environment is an essential part of their existence, governing what they are and what they can become. To understand the nature of cellular perception, we have to look beyond existing descriptions of what cells are.

In plants, the fibrous, cellulose-based walls between cells don't prevent all interactions between cells, but they do keep the cells from wandering, and they act as stable regulators of the cells' functions. Animal cells also have a carbohydrate-rich layer surrounding them, which

powerfully regulates their functions, but this layer is much more adjustable than the cellulose framework of plants.

The chemical and structural plasticity of this intercellular material is an essential part of the greater sensitivity and complexity of animals, and while it is now conventional to describe this material as part of a signalling and regulatory system, I think it might also be appropriate to think of it as **part of the cell's sensory system.** It forms a buffer zone around a cell that is strongly conditioned by the cell's own activity, and when this zone is altered by the presence of another cell, or when its composition changes because of injury or infection, the influence from that buffer zone has to change processes in the cell. **For example, if we compare these layers to a film of soap around a drop of oil, the surface tension--and therefore the shape--can change radically under the influence of physical and chemical agents, such as temperature, salts or acids.**

The forces inside cells that contribute to their shape, interact with external forces and with internal and external chemical reactions. The composition of the extracellular matrix is now known to have a profound regulatory influence on the cell's genes. The cell's "phenotype" (our phenotype is what we appear to be) is regulated by the extracellular matrix, in combination with a variety of hormonal, nutritional, and metabolic factors.

A DIFFERENT PHYSICS

In the study of cohesion, adsorption, radiation, diffraction, and resonance, there has been a strong tendency to avoid questions of the "ensemble," in favor of the unique, or at most double, event or situation--one or two atoms or molecules, one or two frequencies--and beyond that, to describe things with "statistical, stochastic, colligative" assumptions and methods. Crystallography is an attempt to understand ensembles of atoms or molecules, but it represents the opposite extreme of abstraction, letting geometry replace concrete data about events in time and place. What has always been left out is the way in which long range order interacts with local, and delocalized, energy. There have been observations with relevance to these issues, but they have been "left out" because they were "anomalous."

These interactions of the parts of an ensemble over distances many times greater than the size of an atom are of special importance for biology; they are where we find all the most interesting questions in biology.

The way the various "anomalies" fit together is a matter for a general revision of science, but in biology, **the work of Alexander Rothen gives us a tool for beginning to make sense of some of the realities of biology that have seldom been thought about.**

When I began studying biology at the University of Oregon, the bulletin board near the electron microscope room displayed some photographs of crystals that professor Bajer had been making. One of them showed a quartz crystal, over which a thin film of plastic had been coated. On top of the plastic film sodium atoms had been deposited, showing that they were being aligned in a pattern conforming to that of the underlying quartz crystal. The film was much thicker than the units of silicon dioxide in the underlying crystal, so the arrangement of the sodium atoms was obviously not being determined by either the position of the plastic polymer molecules, or the relatively remote individual silicon and oxygen atoms below the film, but somehow by a projection of the pattern of the quartz crystal. Order had been projected through the disordered layer of polymer.

Earlier, I had read some of Michael Polanyi's work with long-range effects in crystals, and knew about his adsorption work, in which the adsorptive force of the underlying charcoal was able to make itself felt through several layers of atoms, continuing to cause more atoms to condense from the gas phase. Later, multilayer adsorption was explained as the result of electronic resonance.

Rothen, using the techniques he had invented for measuring the thickness of extremely thin films, found that metal-plated glass slides, coated with albumin, would bind multiple layers of antibodies which were specific for the albumin. Or, before the slide was exposed to antibodies, it could be coated with a plastic (Formvar) film 120 Angstrom units thick, and still, **layers of**

antibodies up to 80 Angstroms thick could be adsorbed.

Polanyi's multilayer adsorption was considered impossible by the leading physicists of the time, but the simple idea of electronic resonance eventually made it possible for them to accept the facts. But Rothen's adsorption of multilayers of immunologically specific proteins was just too much.

A couple of years after I had seen Bajer's micrographs, I asked him about them, and he said he didn't remember; **already, the opinion makers in science had reacted to experiments in which cells appeared to be "feeling" an artificially constructed gradient through a plastic film, by claiming that photographs had revealed tiny holes in the films, and the cells had been reaching through the holes and feeling the gradient directly. The cheating cells (like mind-readers who peek through their blindfolds) had been "exposed," and all the long-range transmembrane phenomena had fallen into disrepute.**

Even if the cells were "cheating" by reaching through holes in the film, the fact that they could sense the direction of the gradient, and move consistently "up the gradient," was never challenged, and it wasn't explained how reaching through the plastic could allow them to evaluate the gradient on the basis of a few samples--that in itself might require a more complicated explanation than the original idea, sensing the gradient through a film, required. The meaning of this episode is that the most influential scientists will seize even an irrelevant criticism as an excuse for ridiculing ideas that violate their dogma.

But pores in plastic films, that cells might poke their fingers through, had no conceivable relevance to Rothen's work (nor to Bajer's). **Rothen's technique could measure the thickness of the layers of antibodies, and show that they were continuous layers, several antibodies thick.** Antibodies to albumin aren't supposed to bind to themselves. The technique was so simple that a physicist visited Rothen's lab, learned his technique, and then claimed to have invented it as a method for simplifying immunological testing. **But he wanted only the commercial value of Rothen's discovery, and ignored its essential importance.**

Rothen's specific long-range adsorption, like Polanyi's earlier work, simply couldn't be responded to in a rational way. A rational response might have been to reason this way: If the organization of the underlying material projects itself strongly into the environment, then patterns in the environment must also make themselves felt in the underlying material. The same mechanism would amount to an action and a perception.

There were fields such as metallurgy, in which long-range forces, and the effects of surfaces on crystal structure, were "normal science," but in biology, physics had been reduced to the ideas that were dominant in 1915.

The conformation of a large molecule is part of its biological or immunological specificity. The specificity of conformation of any molecule must participate in the specific nature of the ensemble, and that of the ensemble, in the individual molecule. For example, certain enzyme molecules are known to arrange themselves one way in the cytoplasm, and another, random way when they are examined in the unnatural environment of biochemist's test-tube.

Since Polanyi's multilayer adsorption was explained as a resonance phenomenon, the same term could be applied to Rothen's specific adsorption, without implying that we understand exactly how it works.

Rothen also showed that enzymes can act across the films. To me, the main importance of this would be to highlight the extent to which an archaic physical view has governed biochemical thinking. The action of an enzyme at a distance means that its catalytic region might be larger than previously supposed, but it also suggests that we can't generalize from the behavior of an enzyme dissolved in water, to its behavior in the cell, where it would be subject to the reciprocal effects of many other components of the complex system. Biochemists have claimed that "biochemistry couldn't exist if you doubted that the function of enzymes in solution is identical to the function of enzymes in the cell," but other

biochemists have demonstrated that **some of the most basic assumptions of biochemistry were mistaken (Bernhard, 1988), including the assumption that the enzymes of glycolysis act on substrates that are floating around in solution.**

Digestive enzymes, added to a solution containing the food materials normally digested in the intestine by those enzymes, failed to alter the food materials at a rate that could be compared to that of normal digestion. But when a formalehyde-fixed piece of intestine was added to the mixture, the reactions occurred at approximately the rate that is normal for digestion. Although in young infants digestion does happen in the fluid contents of the intestine, after the age of one, most of the action of the digestive enzymes occurs at or near the surface of the intestine, under the influence of the adsorbed enzymes. This is another clear example of the idea that enzymes are dependent on their environment.

Whether or not cells are able to "feel" what is beneath a plastic film, recent evidence indicates that the thickness and composition of the material they sit on does make a difference. Their genes are turned on by the structures that are sensed in the extracellular matrix, and the longevity, growth, function and death of the cells are determined by the nature of the substances outside the cell.

The implication of much current research is that if all of the specific protein filaments can be identified that form links between the structural filaments inside cells and the matrix materials outside the cells, the powerful influence of the external matrix on the cells' functions will somehow be explained without having to think in terms of physical ensembles. **"This protein on one side of the [imaginary] cell membrane tugs on a protein on the other side," and then maybe the little homunculus who counts molecules will decide the time is right for him to carry a message to the correct gene to turn it on.**

It is probably true that a general picture can be completed after all of the details are present, but it isn't necessarily true that the specific interpretation will be meaningful. But at the present, we can see some of the meanings of the interaction between matrix and cell.

MUCUS, MUCINS, AND MYOPIA

The importance of carbon dioxide for respiration has been established, and its essentiality for even single-celled organisms has been reported. Whenever there is respiration, carbon dioxide is produced. By maintaining the effective concentration of Krebs cycle material, it protects the efficiency of respiration. Carbon dioxide is very soluble in water, but it is even more soluble in living tissue, so soluble that it will move from a low concentration in bath water into the body, where its concentration is already much higher.

Its high concentration in bone is not usually appreciated, but calcium carbonate is the form in which calcium is first deposited in new bone. Carbon dioxide reacts spontaneously with ammonia, and with other amines. The reaction of ammonia with carbon dioxide is the first step in the formation of urea, protecting against the potential toxicity of ammonia. Proteins probably bind a little more carbon dioxide on their amino groups than the amount that dissolves in a similar quantity of water, but biologists generally talk only about the carbamino content of hemoglobin, since the binding of carbon dioxide to this protein regulates the blood's ability to deliver oxygen to the tissues.

Carbon dioxide strongly influences the way in which water interacts with proteins, and it participates in the ability of iron-binding molecules to carry iron.

Although carbon dioxide probably associates with most of the amino groups in the body, only a few of these reactions have been studied. For example, it is known to bind to insulin, affecting its conformation. I think this is likely to explain some of the effects of the thyroid hormone in diabetes, since thyroid increases the production of carbon dioxide.

Mucins, or mucopolysaccharides, are combinations of proteins and polysaccharides, which make up a large part of the extracellular matrix; the blood–type substances are mucopolysaccharides, as are chondroitin and hyaluronic acid. The

carbohydrate chains of the mucins are made up of alternating sugars and amino sugars. When the amino groups are free, they will tend to associate with carbon dioxide, and so the way they interact with water, with each other, and with proteins, will be modified according to the amount of carbon dioxide present.

The simplest way to visualize the effect of carbon dioxide on mucopolysaccharides is to think of its action as an expectorant, in which it decreases the viscosity of bronchial mucus, allowing it to be reabsorbed or expelled. Since iodide also has a long history of use as an expectorant, we should compare the effects of carbon dioxide and carbonic acid with the effects of iodide in other situations.

The transparent structures of the eye are interesting places for considering the effects of carbon dioxide. I think carbon dioxide has a role in maintaining the clarity of the lens, as I discussed in the cataracts newsletter, **by preventing swelling**. The connective tissues of the eye's sclera and cornea are rich in mucopolysaccharides, and the vitreous body and the aqueous humor are very much like more dilute solutions of the same materials--the cornea is the most dense, and the aqueous is the most dilute. The fluidity of the aqueous is important because it circulates nutrients and oxygen from the blood, to sustain the lens and the cornea. The individual molecules in the aqueous are larger than those in the vitreous, but because there are fewer of them, the aqueous isn't very viscous. If carbon dioxide affects these molecules as it does the mucins in the bronchial secretions, then we can infer that it is important in maintaining the fluidity of the aqueous humor; **this would suggest that hypothyroidism, leading to a substitution of lactic acid for carbon dioxide, might contribute to the development of glaucoma by increasing the viscosity of the aqueous humor.**

Among mountain climbers, it has been reported that after staying several hours at a very high altitude, there is a decrease in the degree of myopia. Because of the Haldane-Bohr effect, in which carbon dioxide is retained when less oxygen is available, I think the high altitude effect on myopia involves a condensation of the connective tissue. In experimenters wearing gas tight goggles, and in scraped corneas kept in tissue culture, carbon dioxide has been found to have an **anti-swelling effect on the cornea.**

When rats have their thyroid glands removed, they become nearsighted, but if their pituitary glands are removed at the same time, they don't develop myopia. Hypothyroidism is classically associated with "myxedema," in which the tissues become overloaded with the glycoproteins or mucins. Several pituitary hormones are known to stimulate the overproduction of mucins.

In several of my classes, I asked students if they remembered when they became nearsighted. Although puberty is the usual time for myopia to begin, several girls mentioned that they had needed glasses within a month of beginning the oral contraceptive pill, and some of them found that their vision returned to normal when they stopped the pill, or began using thyroid supplements. High prolactin (associated with high estrogen or low thyroid) may be one of the factors in myopia.

Another experimental method for producing myopia is to cover the eye of a growing animal, or to keep the animal in constant darkness. In darkness, the eye grows larger than normal, causing the refractive error of myopia, and the sclera is soft and weak. Since stimulated nerves consume oxygen and emit carbon dioxide, I think darkness might mimic hypothyroidism, allowing the connective tissues to swell.

The sea cucumber has been used to study the physical properties of connective tissue, and it has been found that certain salts tend to soften the connective tissues, but that iodide doesn't. The well-established use of iodide to resolve granulomas, even when it doesn't eliminate the infectious agent, might suggest that it is protecting against something which is disrupting the connective tissue structure.

The only publications I have seen that presented clear evidence of the disappearance of arteriosclerosis involved treatment with iodides. In the retina, blood vessels can be seen to return to their normal appearance following a course of iodide treatment. Besides its possible direct effects on the mucins, iodide might help to

eliminate calcium from the walls of blood vessels, since calcium iodide is very soluble.

In aging, connective tissue becomes hardened by chemical cross-linking of the large molecules. If amino groups are well saturated with carbon dioxide, this type of reaction should be inhibited. (Carbon dioxide also inhibits the production of free radicals, which are involved in some types of cross-linking reactions.) The waterlogged condition seen during shock or stress in blood vessels, lungs, and other organs, and the edema of the brain and cataracts of the lenses that follow metabolic impairments of various sorts, seem to involve the uptake of "free" water, at the same time that "bound" (unfreezable) water is lost. Carbon dioxide seems to promote the retention of bound water, and protects against the edematous conditions. The swelling of muscles during hypoxic stress probably represents the basic process, in which lactic acid and pH increase, while CO_2 is lost.

Whatever the cell's "membrane" structure may be, Rothen's demonstration of the deep interactions of adjacent layers showed that the conditions on either side of the surface of a cell are very sensitive to conditions on the other side.

Since the mucopolysaccharides regulate cell division, cell shape and motility, cell longevity and cell death, and carbon dioxide has very powerful structural effects on the mucopolysaccharides, the cell, by producing carbon dioxide, is stabilizing its regulatory framework, the extracellular matrix, at the same time that it stabilizes the intracellular proteins and systems of metabolism..

The thyroid hormone is the most important promoter of carbon dioxide formation.

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attributable to sialic acid, chondroitin sulphates, hyaluronic acid, and heparan sulphate." "Cell growth was inhibited 40% when the fibroblasts were cultured on the fixed sheets of late passage cells. Treatment of the fixed cell sheets with heparitinase or nitrous acid resulted in complete recomvery from the growth inhibition. Cell growth on sheets of fixed cells derived from young, middle, and senescent fibroblasts showed that the surface of the senescent cells had the greatest inhibitory effect. These inhibitory effects of fixed cell sheets correlated well with both the amount of heparan sulphate relative to the total GAGs on the surface and to the saturation density of cell crowth at each passage. These findings strongly suggest that heparan sulphate, or its complex, on the cell surface in involved in the regulation of cell proliferation."

"The intracellular equilibrium thermodynamic and steady-state concentrations of metabolites," Bernhard SA, Institute of Molecular Biology, University of Oregon, Eugene 97403. Cell Biophys, 1988 Jan-Jun, 12:, 119-32 "A new model for the organization and flow of metabolites through a metabolic pathway is presented. The model is based on four major findings. (1) The intracellular concentrations of enzyme sites exceed the concentrations of intermediary metabolites that bind specifically to these sites. (2) The concentration of the excessive enzyme sites in the cell is sufficiently high so that nearly all the cellular intermediary metabolites are enzyme-bound. (3) Enzyme conformations are perturbed by the interactions with substrates and products; the conformations of enzyme-substrate and enzyme-product complexes are different. (4) Two enzymes, catalyzing reactions that are sequential in a metabolic pathway, transfer the common metabolite back and forth via an enzyme-enzyme complex without the intervention of the solvent environment. The model proposes that the enzyme-enzyme recognition is ligand-induced. Conversion of E2S and E2P results in the loss of recognition of E2 by E1 and the concomitant recognition of E2 by E3. This model substantially alters existent views of the bioenergetics and the kinetics of intracellular metabolism. The rates of direct transfer of metabolite from enzyme to enzyme are comparable to the rates of interconversion between substrate and product within an individual enzyme. Consequently, intermediary metabolites are nearly equipartitioned among their high-affinity enzyme sites within a metabolic pathway. Metabolic flux involves the direct transfer of metabolite from enzyme to enzyme via a set of low and nearly equal energy barriers."

Cytoarchitecture and cell growth control. Slack JK; Higgins PJ Cell Motil Cytoskeleton, 1996, 33:2, 83-7 Appropriate cell-to-substrate adhesion together with SGF stimulation is **necessary to initiate and continue cell cycle progression of growth arrested cells. Adhesion-dependent signaling events, which likely occur through integrin receptors specifically organized with cytoskeletal components within focal contacts, can induce expression of specific genes and stimulate quiescent cells into the growth cycle.** The mechanisms as to **how:** (1) cell-to-substrate adhesion complexes are formed and maintained, (2) adhesion-dependent signal transduction events interface with SGF initiated signalling events, (3) adhesion influences expression of growth-state regulated genes, and (4) an appropriate **cytoarchitectural environment may coordinate these events to regulate cellular growth are unclear.** While it is apparent that defining these mechanisms would be critical to understanding the basic events which control cell growth, many of the mechanisms are just beginning to be addressed and understood."

"Three-dimensional analysis of the substrate-dependent invasive behavior of a human lung tumor cell line with a confocal laser scanning microscope," Strohmaier AR; Spring H; Spiess E Biomedizinische Strukturforschung (0195), Deutsches Krebsforschungszentrum, Heidelberg, Germany. Histochem Cell Biol, 1996 Mar, 105:3, 179-85 Matrigel and collagen G gels were used as models for basement membrane and interstitial space-collagen, respectively, to study the invasive behavior of cells of the human lung tumor cell line EPLC 32M1, which was derived from a squamous cell carcinoma. For three dimensional analysis of the invasive process, cells were seeded onto the gels in a slide chamber and observed with a confocal laser scanning microscope. Optical sectioning in the xy and xz directions and image reconstruction with computer programs allowed us readily to obtain a three-dimensional overview of the invasive process in situ. Both types of gel showed a smooth surface. Matrigel had a granular structure whereas collagen G revealed a fiber-like morphology. The tumor cells showed a matrix-dependent behavior. On Matrigel, within 24 h of incubation, a network of cells appeared on the surface, which developed further within 72 h to interconnected multicellular cords also invading the gel. Tumor cells seeded on collagen G remained individual. They formed pseudopodia and achieved tight contact with the matrix, eventually also invading the gels in a time-dependent manner. **Therefore, the composition of the substrate crucially influences the invasion path,"**

"Modulation of protein tyrosine phosphorylation by the extracellular matrix," Corbett SA; Schwarzbauer JE J Surg Res, 1997 Apr, 69:1, 220-5 Fibronectin (FN) cross-linked to fibrin following injury provides the provisional matrix required for cells to begin tissue repair. Our previous work has demonstrated that fibroblasts adherent to multimeric FN within the context of a fibrin matrix (FN-fibrin) **exhibit clear phenotypic differences from those adherent to a dimeric FN-coated surface.** We hypothesize that this response to multimeric FN may be mediated by altered protein tyrosine phosphatase activity following integrin activation. Methods: NIH 3T3 cells were plated in the presence or absence of pervanadate (PV), a phosphotyrosine phosphatase inhibitor, on wells coated with FN or FN-fibrin matrix. Spread cell areas were measured after increasing incubation times and are recorded as mean cell area (mm2) +/- SEM. Alternatively, cells were lysed and equal amounts of protein were analyzed by immunoblot using a monoclonal antibody

specific for phosphotyrosine. Results: PV significantly inhibited cell spreading on FN-fibrin matrices. In contrast, PV treatment had little effect on cell area on FN alone. Analysis of cell lysates revealed that protein tyrosine **phosphorylation events differ in a substrate-dependent manner**. Conclusion: **Cell attachment to a FN-fibrin matrix induces distinct cell shape and cytoskeletal organization.** Inactivation of tyrosine-specific phosphatases enhances this distinction and inhibits the spreading of cells attached to this substrate. The phosphotyrosyl protein content of treated cells on FN-fibrin matrix is also diminished. These results suggest that **cell-extracellular matrix interactions affect the tyrosine phosphorylation balance of the cell, thus modifying cytoskeletal organization** and related signaling events."

"Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells," Re F; Zanetti A; Sironi M; Polentarutti N; Lanfrancone L; Dejana E; Colotta F Borgomainerio, Milano, Italy. J Cell Biol, 1994 Oct, 127:2, 537-46 When cultivated on substrates that prevent cell adhesion (the polymer polyhydroxyethylmethacrylate, bovine serum albumin, and Teflon), human endothelial cells (EC) rapidly lost viability with a half-life of approximately 10 h. Dying EC showed the morphological and biochemical characteristics of apoptosis. The apoptotic process of suspended EC was delayed by the protein synthesis inhibitor cycloheximide. To obtain information as to the mechanism involved in the apoptosis of suspended EC, we investigated whether **adhesion to matrix proteins or integrin occupancy in EC retaining a round shape** may affect EC suicide. EC bound to **low coating concentration** of either fibronectin or vitronectin, **retaining a round shape and failing to organize actin microfilaments, underwent to rapid cell death;** by contrast, cells on **high substrate concentrations became flattened, showed actin microfilament organization,** and retained viability. Addition of saturating amounts of soluble vitronectin to suspended round-shaped EC did not reduce the process of apoptosis. Finally, when suspended EC bound Gly-Arg-Gly-Asp-Ser-coated microbeads (approximately 10 microbeads/cell), yet retaining a round shape, the apoptotic process was not affected. Oncogene-transformed EC in suspension were less susceptible to cell death and apoptosis than normal EC. Overall, these data indicate that cell attachment to matrix or integrin binding per se is not sufficient for maintaining cell viability, and that cells need to undergo some minimal degree of shape change to survive. Modulation of interaction with the extracellular matrix can, therefore, be an important target for the control of angiogenesis.

"EFFECT OF CARBON DIOXIDE ON THE THERMODYNAMIC STATE OF WATER IN COLLAGEN," JOURNAL OF FOOD SCIENCE 53(4). 1212-1215, 1988. "Thermodynamic activities of polar sites of collagen in the presence of CO2 were observed by inverse gas chromatographic techniques using water as a probe. The interactions between collagen and the water probe were evaluated by determining the specific retention volume (Vg.degree.) and partition coefficient (Kp) at 25.degree. C, 30.degree. C, and 35.degree. C. Thermodynamic parameters were determined from these data. CO2 exhibited a significant effect on the water binding of collagen as shown by increased Vg.degree. and Kp values as compared to N2- and He-treated collagen. The thermodynamic parameters of partial molar Gibb's free energy...partial molar enthalpy...and partial molar entropy**...indicated CO2 significantly increased the average energy of water binding by collagen."**

Kinetics of decreased LPS-stimulated cytokine release by macrophages exposed to CO2. West MA; Baker J; Bellingham J, J Surg Res, 1996 Jun, 63:1, 269-74. **"The mechanisms responsible for the lack of inflammation after laparoscopic surgery remain unknown. Peritoneal macrophages (M phi) incubated in carbon dioxide (CO2) but not air or helium (He), had significant, reversible inhibition of lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF) and interleukin-1 (IL-1)** release. In these experiments the kinetics of these C02-induced alterations in cytokine secretion were examined. Murine peritoneal Mphi were stimulated with LPS for 4 hr and incubated in different test gases (95% air/5% CO2,80%CO2/20%O2,80% He/20% O2) for intervals between 0.25 and 4 hr. Time between gas incubation and LPS stimulation was varied to determine the persistence of CO2 inhibition. Parallel M phi groups received LPS stimulation 24 hr later. Supernatant TNF and IL-1 were measured by bioassay and polymerase chain reaction was used to examine cytokine mRNA. Significant reversible inhibition of TNF and IL-1 was seen with CO2, but not He or air. **Inhibition of IL-1 occurred 15 min after CO2 exposure,** was associated with decreased IL-1 mRNA, and was rapidly lost following incubation in the control atmosphere. TNF inhibition was seen despite normal levels of TNF message, required more than 30 min of CO2 exposure, and persisted after CO2 removal. **CO2 produced profound, reversible, inhibition of LPS-stimulated cytokine release by peritoneal Mphi. The transient inability to secrete inflammatory cytokines after CO2 exposure may explain the lack of systemic inflammation after laparoscopic surgery with CO2."**

"Do plasma and serum have different abilities to promote cell growth?" Gospodarowicz D; Ill CR Proc Natl Acad Sci U S A, 1980 May, 77:5, 2726-30 "The abilities of plasma and serum to support the growth of vascular smooth muscle cells maintained on uncoated tissue culture dishes or dishes coated with an extracellular matrix (ECM) have been compared. Vascular smooth muscle cells maintained on plastic dishes and exposed to plasma proliferate poorly; when exposed to serum they proliferate actively. Addition of fibroblast growth factor (FGF) incrases the growth rate of the cultures in both cases. In contrast, when vascular smooth muscle cells are maintained on an ECM, they proliferate

equally well exposed to either plasma or serum. Because the cultures had an average doubling time (15 hr) that was already at a minimum, FGF no longer had an effect on vascular smooth muscle cell proliferation." "These results raise the possibility that the lack of response of vascular smooth muscle cells, as well as that of other cell types in vitro, to plasma factors is **not an intrinsic property of the cells but is rather due to the substrate upon which the cells rest. Because cells maintained on an ECM respond to plasma factors, it is likely that the close contact of the cells with the ECM restores their sensitivity to physiological factors present in plasma."**

"Factors controlling the proliferative rate, final cell density, and life span of bovine vascular smooth muscle cells in culture," Gospodarowicz D; Hirabayashi K; Giguère L; Tauber JP J Cell Biol, 1981 Jun, 89:3, 568-78 "Low density vascular smooth muscle (VSM) cell cultures maintained on extracellular-matrix(ECM)-coated dishes and plated in the presence of either plasma or serum will proliferate actively when serum-containing medium is replaced by a synthetic medium supplemented with three factors: high density lipoprotein (HDL, 250 micrograms protein/ml); insulin (2.5 micrograms/ml) or somatomedin C (10 ng/ml); and fibroblast growth factor (FGF, 100 ng/ml) or epidermal growth factor (EGF, 50 ng/ml). The omission of any of these three factors from the synthetic medium results in a lower growth rate of the cultures, as well as in a lower final cell density once cultures reach confluence. When cells are plated in the total absence of serum, transferrin (10 micrograms/ml) is also required to induce optimal cell growth. The effects of the substrate and medium supplements on the life span of VSM cultures have also been analyzed. Cultures maintained on plastic and exposed to medium supplemented with 5% bovine serum underwent 15 generations. However, **when maintained on ECM-coated dishes the serum-fed cultures had a life span of at least 88 generations. Likewise, when cultures were maintained in a** synthetic medium supplemented with HDL and either FGF or EGF, an effect on the tissue culture life span by the substrate was observed. Cultures maintained on plastic underwent 24 generations, whereas those maintained on ECM-coated dishes could be passaged repeatedly for 58 generations. These experiments demonstrate the influence of the ECM-substrate only in promoting cell growth but also in increasing the longevity of the cultures."

"Determination of cellular shape by the extracellular matrix and its correlation with the control of cellular growth." Gospodarowicz D; Greenburg G; Birdwell CR Cancer Res, 1978 Nov, 38:11 Pt 2, 4155-71 **"Although the problem of cellular proliferation may seem at first glance to be tremendously complex, the mechanisms which control it may be extremely simple. One of the primary factors which regulates the mitogenic response of a given cell type to a given class of mitogenic agents seems to be the cellular shape.** We have found that corneal epithelial cells, for example, adopt a flattened configuration when maintained in vitro on plastic and are very sensitive to fibroblast growth factor, but not to epidermal growth factor. When maintained on collagen, on the other hand, they become tall and columnar and respond primarily to epidermal growth factor. The cellular shape is dictated in vivo by the extracellular material upon which the cells rest and in vitro by the substrate upon which the cells are maintained. The substrate itself may, in turn, induce the cells to manufacture their extracellular material and specific cell surface proteins which control the cellular shape."

"The control of mammalian cell proliferation by growth factors, basement lamina, and lipoproteins. Gospodarowicz D J Invest Dermatol, 1983 Jul, 81:1 Suppl, 40s-50s "The effect of growth factors such as fibroblast growth factor on the production of a basement lamina by cultured endothelial cells has been investigated. The ability of these cells to grow and differentiate properly correlated with their ability to produce a basement lamina. The effect of such a substrate on the growth, differentiation, and aging of cells in vitro, as well as its use for the long-term culture of either normal diploid cells or tumor cells, is reviewed.

Effect of high and low density lipoproteins on proliferation of cultured bovine vascular endothelial cells." Tauber JP; Cheng J; Gospodarowicz D J Clin Invest, 1980 Oct, 66:4, 696-708 "Bovine vascular endothelial cells maintained on dishes coated with an extracellular matrix and exposedto medium supplemented with lipoprotein-deficient serum (LPDS) require the presence of lipoprotein to proliferate optimally. High density lipoprotein (HDL) seems to be the major factor involved in the proliferation of vascular endothelial cells. This is mostly due to its lack of toxicity when added at high concentration, as well as to its nondependence on LPDS to exhibit its mitogenic properties. Therefore, HDL hysiological concentrations (1,000--1,500 microgram protein/ml) can fully replace serum. Low density lipoprotein, unlike HDL, has a biphasic effect. Although mitogenic for vascular endothelial cells when added at low concentration, once physiological concentrations are reached it becomes toxic for the cells. Moreover, and in contrast with HDL, the mitogenic effect of low density lipoprotein was found to be a function of the LPDS concentration to which cultures were exposed. The substrate upon which cultures are maintained has been found to be an important factor if a mitogenic effect of HDL is to be observed. When maintained on plastic, cells proliferate poorly in response to HDL unless fibroblast growth factor is added to the medium. **In contrast, when maintained on extracellular matrix, an optimal growth rate is induced by HDL, even in the absence of fibroblast growth factor.** This **suggests that, in vivo, the integrity of the basement membrane upon which endothelial cells rest and migrate is an important factor in determining the cells response to lipoproteins present in plasma. "**