Investigators Focus on Intracellular *p*H

Changes in intracellular pH often correlate with the turning on of cell activities. Researchers ponder what this means.

Within the past few years, researchers have developed a number of methods for accurately and quickly determining intracellular *p*H. The methods, which include the use of microelectrodes with tips as small as 1 micrometer in diameter, *p*H-sensitive fluorescent dyes, and phosphorus nuclear magnetic resonance, can measure the cytoplasmic *p*H with errors as low as 0.02 unit. And some can be used to follow changes in *p*H with time during significant physiological events in the life of the cell. "The last decade has given us the luxury of a variety of techniques to choose from," says Richard Nuccitelli of the University of California at Davis.

Last month, researchers gathered at the first meeting* devoted exclusively to the topic of intracellular *p*H to present some of the results made possible by those techniques. Their findings show that intracellular *p*H increases of 0.2 to as much as a full *p*H unit, depending on the system, are correlated with the turning on of a variety of cellular activities. The activities range from developmental events in newly fertilized eggs to the division of cells, including human lymphocytes, yeast, and a slime mold, to stimulation of glycolysis by the hormone insulin. In some cases it is not yet clear whether the *p*H increase is a cause of the activity or a coincidence. But the results suggest that researchers must now come to grips with the possibility that intracellular *p*H, rather than being the constant cell characteristic it was generally thought to be in the past, may instead be a dynamic regulator of activities such as development and hormonal responses.

Some of the earliest indications of an important role for intracellular *p*H came from studies of sea urchin eggs. About 5 or 6 years ago investigators, including David Epel of Hopkins Marine Station of Stanford University and Richard Steinhardt of the University of California at Berkeley, found that the *p*H of these eggs increased about 0.3 unit within the first few minutes after fertilization.

Sea urchin eggs carry out little synthetic activity before they are fertilized, but shortly thereafter undergo a great burst of protein synthesis. Matthew Winkler, who originally worked with Steinhardt and is now at the Davis campus of the University of California, explains, "The unfertilized egg is almost completely inert. After fertilization, there is a 20- to 40-fold increase in protein synthesis. Because all the [protein-synthesizing] machinery is there before fertilization, the real question is not so much how it is activated, but how it is kept inactive."

The results suggest that the lower *p*H of the unfertilized egg keeps protein synthesis turned off, and that it is turned on by the *p*H rise following sperm penetration. According to results Winkler presented at the meeting, a small portion of the stimulation comes from faster elongation of growing protein chains at the higher *p*H. But the largest portion he attributes to increased mobilization of stored maternal messenger RNA, which is needed to direct protein synthesis. Winkler concluded that "*p*H really does seem to regulate protein synthesis, mainly by regulating the availability of the messengers." He hypothesizes that the eggs contain a "masking factor" that keeps the mRNA's out of action at the lower *p*H, but releases them at more alkaline *p*H's.

The egg of the invertebrate sea urchin is not the only one to become more alkaline shortly after fertilization. According to Dennis Webb, who is also at Davis, and Nuccitelli, the internal *p*H of frog (*Xenopus laevis*) eggs increases transiently by about 0.03 unit within 2 minutes of fertilization; this is followed by a permanent increase of about 0.3 unit that begins roughly 8 minutes later. Whether the permanent alkalinization of the newly fertilized frog egg triggers increased protein synthesis here as it does in sea urchin eggs is still not known.

An issue that received much attention at the meeting concerns the possibility that a rise in intracellular *p*H may serve as a signal for cell division in either the fertilized egg or other types of cells. Some of the results presented indicated that this might be the case. Data from Steinhardt's laboratory showed that the intracellular *p*H of the slime mold *Physarum polycephalum* rises from about 7.0, 6 hours before mitosis, to a maximum value of about 7.5 at the time of mitosis. The increase is interrupted by a slight dip 2 hours before the *p*H peaks.

In earlier work, Donald Gerson of the Basel Institute for Immunology and Alan Burton of the University of Western Ontario had observed a similar increase, although the absolute values of the *p*H's they determined were lower than those seen by the Steinhardt group. This may have been due to technical problems caused by the rather blunt electrodes Gerson and Burton had to use.

In any event, Steinhardt and his Berkeley colleague Masaaki Morisawa extended the findings by showing that mitosis in *Physarum* can be blocked by preventing the *p*H increase during a critical period, about an hour before mitosis begins. Steinhardt says, "There is a cycle of *p*H during the cell cycle, and there is a critical period of alkalinization about an hour before mitosis that is necessary for mitosis. But we don't think this is necessary for all species. We think it is peculiar to certain types of cells."

In particular, they think that it is peculiar to cells that experience periods of dormancy, as the slime mold does. *Physarum*, Steinhardt said, is adapted to grow in the acid environment of the soil. By allowing its *p*H to drop when it is not dividing, the organism could save a great deal of energy that it might otherwise spend to pump hydrogen ions out of the cell.

Steinhardt does not think that a *p*H increase serves as a signal for mitosis and cell division in most other types of cells, including fertilized sea urchin and frog eggs. His group has shown that blocking the *p*H changes in these cells does not prevent them from going through mitosis.

Not everyone was willing to buy the idea that this was the final word on the matter, however. As William Moody of the University of California School of Medicine at Los Angeles pointed out, the treatments used to block the *p*H changes might have independent inhibitory effects on mitosis.

*"Intracellular *p*H: Its Measurement, Regulation, and Utilization in Cellular Functions," sponsored by the Kroc Foundation and held in Santa Ynez Valley, California, on 20 to 24 July.

0036-8075/81/0814-0745$01.00/0

Even though alkalinization of the cell interior might not serve as a direct signal for mitosis and cell division, in some cells it is linked to increased DNA synthesis, which is a prerequisite for division. Human lymphocytes, for example, are stored in the spleen in an inactive state until they are stimulated to divide by encountering an antigen. They can also be activated artificially in the laboratory by exposing them to mitogenic materials such as concanavalin A or a lipopolysaccharide isolated from bacteria. According to Gerson, the *p*H of lymphocytes stimulated in this way increases transiently by about 0.2 unit a few hours after the mitogen treatment. It then returns to normal, only to rise again between 12 and 48 hours after stimulation and again return to normal in an additional 24 hours.

The first increase corresponds in time with the first step of lymphocyte activation, which involves production of a growth factor that stimulates a subset of the population to divide. The second peak occurs at about the same time that lymphocytes begin copying their DNA as they prepare for mitosis. "We are left wondering," Gerson says, "if these are independent effects or whether one causes the other. And, if so, which is the cause and which is the effect."

Toward a Proof of Quark Confinement

A key theorem of mathematical physics—as yet unproved—states that quarks are permanently bound inside the particles of ordinary matter, that no amount of force can ever break one free. Recently, however, Stephen L. Adler of the Institute for Advanced Study in Princeton has taken an important step toward that proof. His work is published in the 15 June *Physical Review D*.

Current conventional wisdom holds that nuclear forces are best described by quantum chromodynamics (QCD), which tells how quarks interact with a group of eight photo-like particles called gluons. Simple heuristic arguments indicate that quarks governed by QCD would behave as if they were connected by springs: their mutual attraction, nearly zero in the close confines of a proton or neutron, would grow larger and larger if one tried to escape; they would be trapped, rather like marbles inside an unbreakable rubber bag. In fact, a phenomenological model postulating just such a bag was developed a decade ago by physicists at the Massachusetts Institute of Technology, and proved very successful in explaining the properties of the known nuclear particles. Unfortunately, the complexity of the mathematics has made a rigorous proof of the QCD force law impossible.

Adler does not claim to have a complete proof either. "But I do claim to have found the right mechanism," he says. Internal consistency checks lead him to believe that the approximations used in his derivation are "robust," in the sense that the exact solution to the QCD equations will differ from his solution only in the details, not in the overall structure. Moreover, he believes it may well be possible to do away with the approximations and establish a rigorous proof of quark confinement. His work has drawn a good deal of favorable comment from fellow theorists, most recently at a meeting in Baltimore.*

Adler begins by treating the QCD equations in strict parallel with the "mean field" approximation of quantum electrodynamics, the theory of electrons and photons. The approximation assumes that electrons are heavy and slow-moving, and furthermore, that quantum fluctuations in the electromagnetic field are unimportant. The field can safely be replaced with its average, or "mean" value and calculated according to the standard nonquantum field equations. The method is quite accurate in the tame environs of systems such as the hydrogen atom. And since the confining force only takes hold when the quarks are far apart and slow-moving, says Adler, the method should also be valid in the case of quark confinement.

He finds that the whole procedure can indeed be applied to QCD but that there are two crucial differences. First, QCD implies that the gluon fields within a real nuclear particle would average out to zero; to extract a useful mean field theory one has to average the product of the field with certain matrices representing the internal states of the quarks. (In technical terms, the expectation values of the gauge potentials are zero in a color singlet state; the expectation values of the product of the gauge potentials with the color charge matrices are not.) Second, in QCD, quantum fluctuations in the gluon field *are* important, largely because of mutual interactions among the gluons. Fortunately, well-established "renormalization group" methods can be used to approximate these effects. Building on earlier work by Heinz Pagels of Rockefeller University, Elestherios Tomboulis of Princeton University, and G. K. Savvidy of the Soviet Union, Adler derives a set of differential equations for the gluon field that resembles the equations of electrostatics in a continuous medium—except that the "medium" is not water or air, but empty space, and the "dielectric constant" of this medium is a nonlinear function of the gluon field strength.

Adler is currently developing computer codes to solve these equations in the relatively simple case of a quark bound to an antiquark inside a meson. Two other groups are working on deriving the corresponding equations for three quarks bound inside a proton or neutron. Once the field equations are solved it will be a simple matter to compute (within the approximations) the binding energy of the quarks as a function of their separation, and thence to find the force between them.

But some things can already be learned just from the form of the equations. For example, the "dielectric constant" of empty space is such that the gluon field is confined and compressed, rather like a bubble under water. Quark and antiquark move freely within the bubble when they are close together; as they are separated, however, the bubble stretches out in a thin tube between them. It is easy to show that the potential energy then grows linearly with the separation and that the quarks cannot escape. Such behavior has long been expected. But Adler is the first to derive a confining potential from the basic QCD equations (albeit with approximations) without having to impose it artificially.—M. MITCHELL WALDROP

*The Fifth Johns Hopkins Workshop on Current Problems in Particle Theory, 25 to 27 May 1981.

0036-8075/81/0814-0746$01.00/0

At least in regard to the increased DNA synthesis, it appears that the *p*H change might be causative. Gerson treated dividing lymphocytes to alter their internal *p*H and found that the rate of DNA synthesis increased as the *p*H went up. In a converse experiment, he applied a chemical that inhibits DNA synthesis to cells that had already been stimulated to divide, but the *p*H rise still occurred in these cells. He concludes, "I can change the rate of DNA synthesis by changing the *p*H, but cannot change the *p*H by stopping the DNA synthesis."

Gerson hypothesizes that the *p*H increase may stimulate DNA polymerase, the enzyme that catalyzes DNA synthesis. This enzyme works best around *p*H 9, but its activity shows a marked stimulation as the *p*H rises from 7 to 8, a range that encompasses the increase seen in stimulated lymphocytes. Even though the *p*H increase may be required to facilitate DNA synthesis, it is not sufficient to cause it, however. Mitogen stimulation is also needed.

Robert Gillies, who works in Robert Shulman's laboratory at Yale University, presented evidence from that group showing that *p*H increases also occur at specific times in the cell cycles of the single-celled organism *Tetrahymena* and of brewer's yeast. Again, the increases appeared to be linked to DNA synthesis and not to actual cell division.

Stimulation of synthetic reactions needed for cell division or development is only one kind of activation in which *p*H changes may play a role. Richard Moore of the State University of New York in Plattsburgh has proposed that a rise in intracellular *p*H, which is evoked by insulin, serves as the signal for the hormone-induced stimulation of glycolysis, one of the biochemical pathways by which the cell breaks down glucose to obtain energy. Despite the wealth of information on insulin's effects, exactly what happens inside the cell once the hormone has bound to its receptors on the outer membrane is something of a mystery.

Moore observed that frog muscle cells respond to insulin binding with a *p*H increase of 0.2 unit. Many cells have in their membranes a transport system that moves hydrogen ions out of the cell as sodium ions move in. Moore's results suggest that insulin binding produces the *p*H increase by activating this exchange system. If so, the effect may also help to explain the well-known stimulation by insulin of the sodium pump, an energy-requiring transport system that moves sodium ions out of, and potassium ions into, the cell. The pump speeds up in response to an increase in the internal concentration of sodium ions, and would thus keep that concentration relatively constant even though the *p*H was going up.

As to how the *p*H increase produces stimulation of glycolysis, Moore's data suggest that it activates the enzyme phosphofructokinase, which controls the rate of the glycolytic pathway. This enzyme responds to small increases in *p*H in the range observed by Moore with large increases in activity. He concludes, "The change in *p*H is the intracellular signal for increased glycolysis."

Bringing the insulin story full circle, Caroline Pace of the University of Alabama in Birmingham presented evidence that suggests that the stimulation by glucose of insulin release from pancreatic cells may itself involve a *p*H increase, this time in the secretory granules in which insulin is stored.

Another secretory response that may involve a *p*H increase is the release of serotonin by platelets activated by thrombin, the normal activator of these tiny cells. According to Elizabeth Simons of Boston University Medical Center, thrombin treatment of platelets produces a change in the electrical properties of the membranes and an increase in intracellular *p*H. These responses and the serotonin release depend on the thrombin concentration in the same way, all reaching a maximum at a dose of 4.5 micromoles per milliliter, which is equivalent to the thrombin concentration in actively clotting blood.

Finally, changes in internal *p*H can affect the electrical properties of the membranes of excitable cells, such as nerve or muscle cells. Moody has shown that decreasing the intracellular *p*H makes crayfish muscle more active. He had previously found such an effect during periods of oxygen deprivation, when the internal *p*H also decreases. And David Spray of the Albert Einstein College of Medicine showed that a decrease in intracellular *p*H could profoundly decrease electrical conductance through gap junctions. In nerves, these connections serve as electrical synapses between cells. They also occur in other cells, where their functions are poorly understood.

These effects should not be too surprising because nerve firing and muscle contraction depend on the movement of one or more ions across cell membranes. These movements could be easily affected, either directly or indirectly, by changes in the hydrogen ion concentration.

A simple listing of all the cellular activities now being linked to changes in intracellular *p*H makes it appear as if this parameter might affect virtually every such activity. But investigators must keep a watchful eye on the possibility that the *p*H change they have just correlated with some important cellular event is secondary to another change that is in fact the causative one. The internal ionic environment of the cell has been characterized by Howard Rasmussen of Yale University School of Medicine as an "ionic network" in which changes in the concentration of one ion evoke alterations in the concentrations of one or more additional ions. For example, some cell types recover from intracellular acidification by exporting hydrogen ions in exchange for the inward transport of sodium ions.

A simple listing of all the cellular activities now being linked to changes in intracellular *p*H makes it appear as if this parameter might affect virtually every such activity.

The evidence for a causative relationship between increased internal *p*H and stimulation of protein synthesis in the fertilized sea urchin eggs is relatively strong. But in other cases, where investigators are just now beginning to see correlations, the data are less firm. Mindful of the possible land mines in the research, Roger Thomas of the University of Bristol Medical School cautioned the meeting participants by saying, "We may be falling into the trap of believing that because we can measure it [intracellular *p*H], it must be doing something interesting." But equally mindful of the progress that has been made recently, Moore retorted, "But we don't want to fall into the trap of taking it for granted that it doesn't."—JEAN L. MARX