Phosphate, activation, and aging

From the original article in 2013. Author: Ray Peat.

Recent publications are showing that excess phosphate can increase inflammation, tissue atrophy, calcification of blood vessels, cancer, dementia, and, in general, the processes of aging. This is especially important, because of the increasing use of phosphates as food additives.

Previously, the complications of chronic kidney disease, with increased serum phosphate, were considered to be specific for that condition, but the discovery of a phosphate-regulating gene named klotho (after one of the Fates in Greek mythology) has caused a lot of rethinking of the biological role of phosphate. In the 19th century, phosphorus was commonly called brain food, and since about 1970, its involvement in cell regulation has become a focus of reductionist thinking. ATP, adenosine triphosphate, is seen as the energy source that drives cell movement as well as the "pumps" that maintain the living state, and as the source of the cyclic AMP that is a general activator of cells, and as the donor of the phosphate group that activates a great number of proteins in the "phosphorylation cascade." When tissues calcified in the process of aging, calcium was blamed (ignoring the existence of calcium phosphate crystals in the tissues), and low calcium diets were recommended. Recently, when calcium supplements haven't produced the intended effects, calcium was blamed, disregarding the other materials present in the supplements, such as citrate, phosphate, orotate, aspartate, and lactate.

I have a different perspective on the "phosphorylation cascade," and on the other functions of phosphate in cells, based largely on my view of the role of water in cell physiology. In the popular view, a stimulus causes a change of shape in a receptor protein, causing it to become an active enzyme, catalyzing the transfer of a phosphate group from ATP to another protein, causing it to change shape and become activated, and to transfer phosphate groups to other molecules, or to remove phosphates from active enzymes, in chain reactions. This is standard biochemistry, that can be done in a test tube.

Starting around 1970, when the involvement of phosphorylation in the activation of enzymes in glycogen breakdown was already well known, people began noticing that the glycogen phosphorylase enzyme became active immediately when the muscle cell contracted, and that phosphorylation followed the activation. Phosphorylation was involved in activation of the enzyme, but if something else first activated the enzyme (by changing its shape), the addition of the phosphate group couldn't be considered as causal, in the usual reductionist sense. It was one participant in a complex causal process. I saw this as a possible example of the effect of changing water structure on protein structure and function. This view of water questions the relevance of test tube biochemistry.

Enzymes are known which suddenly become inactive when the temperature is lowered beyond a certain point. This is because soluble proteins arrange their shape so that their hydrophobic regions, the parts with fat-like side-chains on the amino acids, are inside, with the parts of the chain with water-soluble amino acids arranged to be on the outside, in contact with the water. The "wetness" of water, its activity that tends to exclude the oily parts of the protein molecule, decreases as the temperature decreases, and some proteins are destabilized when the relatively hydrophobic group is no longer repelled by the surrounding cooler water.

In the living cell, the water is all within a very short distance of a surface of fats or fat-like proteins. In a series of experiments, starting in the 1960s, Walter Drost-Hansen showed that, regardless of the nature of the material, the water near a surface is structurally modified, becoming less dense, more voluminous. This water is more "lipophilic," adapting itself to the presence of fatty material, as if it were colder. This change in the water's properties also affects the solubility of ions, increasing the solubility of potassium, decreasing that of sodium, magnesium, and calcium (Wiggins, 1973).

When a muscle contracts, its volume momentarily decreases (Abbott and Baskin, 1962). Under extremely high pressure, muscles contract. In both situations, the work-producing process of contraction is associated with a slight reduction in volume. During contraction of a muscle or nerve, heat is given off, causing the temperature to rise. During relaxation, recovering from excitation, heat is absorbed (Curtin and Woledge, 1974; Westphal, et al., 1999; Constable, et al. 1997). In the case of a nerve, following the heating produced by excitation, the temperature of the nerve decreases below the starting temperature (Abbot, et al., 1965). Stretching a muscle causes energy to be absorbed (Constable, et al., 1997). Energy changes such as these, without associated chemical changes, have led some investigators to conclude that muscle tension generation is "entropy driven" (Davis and Rodgers, 1995).

Kelvin's description (1858) of the physics of water in a soap bubble, "...if a film such as a soap-bubble be enlarged . . . it experiences a cooling effect . . . ," describes the behavior of nerves and muscles, absorbing energy or heat when they are relaxing (or elongating), releasing it when they are excited/contracting.

Several groups of experimenters over the last 60 years have tried to discover what happens to the missing heat; some have suggested electrical or osmotic storage, and some have demonstrated that stretching generates ATP, arguing for chemical storage. Physical storage in the form of structural changes in the water-protein-lipid system, interacting with chemical changes such as ATP synthesis, have hardly been investigated.

Early studies of muscle chemistry and contraction found that adding ATP to a viscous solution of proteins extracted from muscle reduced its viscosity, and also that the loss of ATP from muscle caused its hardening, as in rigor mortis; if the pH wasn't too acidic, the dead muscle would contract as the ATP content decreased. Szent-Gyorgyi found that a muscle hardened by rigor mortis became soft again when ATP was added.

Rigor mortis is an extreme state of fatigue, or energy depletion. Early muscle studies described the phenomenon of "fatigue contracture," in which the muscle, when it reaches the point at which it stops responding to stimulation, is maximally contracted (this has also been called delayed relaxation). Ischemic contracture, in the absence of blood circulation, occurs

when the muscle's glycogen is depleted, so that ATP can no longer be produced anaerobically (Kingsley, et al., 1991). The delayed relaxation of hypothyroid muscle is another situation in which it is clear that ATP is required for relaxation. (In the Achilles tendon reflex test, the relaxation rate is visibly slowed in hypothyroidism.) A delayed T wave in the electrocardiogram, and the diastolic contracture of the failing heart show the same process of delayed relaxation. Supplementing the active thyroid hormone, T3, can quickly restore the normal rate of relaxation, and its beneficial effects have been demonstrated in heart failure (Pingitore, et al., 2008; Wang, et al., 2006; Pantos, et al., 2007; Galli, et al., 2008).

A large part of the magnesium in cells is bound to ATP, and the magnesium-ATP complex is a factor in muscle relaxation. A deficiency of either ATP or magnesium contributes to muscle cramping. When a cell is stimulated, causing ATP to release inorganic phosphate, it also releases magnesium. Above the pH of 6.7, phosphate is doubly ionized, in which state it has the same kind of structural effect on water that magnesium, calcium, and sodium have, causing water molecules to be powerfully attracted to the concentrated electrical charge of the ion. Increasing the free phosphate and magnesium opposes the effect of the surfaces of fats and proteins on the water structure, and tends to decrease the solubility of potassium in the water, and to increase the water's "lipophobic" tendency to minimize its contacts with fats and the fat-like surface of proteins, causing the proteins to rearrange themselves.

These observations relating to the interactions of water, solutes and proteins in muscles and nerves provide a coherent context for understanding contraction and conduction, which is lacking in the familiar descriptions based on membranes, pumps, and cross-bridges, but I think they also provide a uniquely useful context for understanding the possible dangers of an excess of free phosphate in the body.

A few people (M. Thomson, J. Gunawardena, A.K. Manrai) are showing that principles of mass-action help to simplify understanding the networks of phosphorylation and dephosphorylation that are involved in cell control. But independently from the phosphorylation of proteins, the presence of phosphate ion in cell water modifies the cell's ion selectivity, shifting the balance toward increased uptake of sodium and calcium, decreasing potassium, tending to depolarize and "activate" the cell.

About 99% of the publications discussing the mechanism of muscle contraction fail to mention the presence of water, and there's a similar neglect of water in discussions of the energy producing processes in the mitochondrion. The failure of mitochondrial energy production leads to lipid peroxidation, activation of inflammatory processes, and can cause disintegration of the energy producing structure. Increased phosphate decreases mitochondrial energy production (Duan and Karmazyn, 1989), causes lipid peroxidation (Kowaltowski, et al., 1996), and activates inflammation, increasing the processes of tissue atrophy, fibrosis, and cancer.

For about twenty years it has been clear that the metabolic problems that cause calcium to be lost from bones cause calcium to increase in the soft tissues, such as blood vessels. The role of phosphate in forming calcium phosphate crystals had until recently been assumed to be passive, but some specific "mechanistic" effects have been identified. For example, increased phosphate increases the inflammatory cytokine, osteopontin (Fatherazi, et al., 2009), which in bone is known to activate the process of decalcification, and in arteries is involved in calcification processes (Tousoulis, et al., 2012). In the kidneys, phosphate promotes calcification (Bois and Selye, 1956), and osteopontin, by its activation of inflammatory T-cells, is involved in the development of glomerulonephritis, as well as in inflammatory skin reactions (Yu, et al., 1998). High dietary phosphate increases serum osteopontin, as well as serum phosphate and parathyroid hormone, and increases the formation of tumors in skin (Camalier, et al., 2010). Besides the activation of cells and cell systems, phosphate (like other ions with a high ratio of charge to size, including citrate) can activate viruses (Yamanaka, et al., 1995; Gouvea, et al., 2006). Aromatase, the enzyme that synthesizes estrogen, is an enzyme that's sensitive to the concentration of phosphate (Bellino and Holben, 1989).

More generally, increased dietary phosphate increases the activity of an important regulatory enzyme, protein kinase B, which promotes organ growth. A high phosphate diet increases the growth of liver (Xu, et al., 2008) and lung (Jin, et al., 2007), and promotes the growth of lung cancer (Jin, et al., 2009). An extreme reduction of phosphate in the diet wouldn't be appropriate, however, because a phosphate deficiency stimulates cells to increase the phosphate transporter, increasing the cellular uptake of phosphate, with an effect similar to the dietary excess of phosphate, i.e., promotion of lung cancer (Xu, et al., 2010). The optimum dietary amount of phosphate, and its balance with other minerals, hasn't been determined.

While increased phosphate slows mitochondrial energy production, decreasing its intracellular concentration increases the respiratory rate and the efficiency of ATP formation. A "deficiency" of polyunsaturated fatty acids has this effect (Nogueira, et al., 2001), but so does the consumption of fructose (Green, et al., 1993; Lu, et al., 1994).

In a 1938 experiment (Brown, et al.) that intended to show the essentiality of unsaturated fats, a man, William Brown, lived for six months on a 2500 calorie diet consisting of sucrose syrup, a gallon of milk (some of it in the form of cottage cheese), and the juice of half an orange, besides some vitamins and minerals. The experimenters remarked about the surprising disappearance of the normal fatigue after a day's work, as well as the normalization of his high blood pressure and high cholesterol, and the permanent disappearance of his frequent life-long migraine headaches. His respiratory quotient increased (producing more carbon dioxide), as well as his rate of resting metabolism. I think the most interesting part of the experiment was that his blood phosphate decreased. In two measurements during the experimental diet, his fasting plasma inorganic phosphorus was 3.43 and 2.64 mg. per 100 ml. of plasma, and six month after he had returned to a normal diet the number was 4.2 mg/100 ml. Both the deficiency of the "essential" unsaturated fatty acids, and the high sucrose intake probably contributed to lowering the phosphate.

In 2000, researchers who were convinced that fructose is harmful to the health, reasoned that its harmful effects would be exacerbated by consuming it in combination with a diet deficient in magnesium. Eleven men consumed, for six months, test diets with high fructose corn syrup or starch, along with some fairly normal U.S. foods, and with either extremely low magnesium content, or with slightly deficient magnesium content. The authors' conclusion was clearly stated in the title of their article, that the combination adversely affects the mineral balance of the body.

However, looking at their results in the context of these other studies of the effects of fructose on phosphate, I don't think their conclusion is correct. Even on the extremely low magnesium intake, both their magnesium and calcium balances were positive, meaning that on average their bodies accumulated a little magnesium and calcium, even though men aged 22 to 40 presumably weren't growing very much. To steadily accumulate both calcium and magnesium, with the calcium retention much larger than the magnesium, the minerals were probably mostly being incorporated into their bones. Their phosphate balance, however, was slightly negative on the "high fructose" diet. If the sugar was having the same effect that it had on William Brown in 1938 (and in animal experiments), some of the phosphate loss was accounted for by the reduced amount in blood and other body fluids, but to continue through the months of the experiment, some of it must have represented a change in the composition of the bones. When there is more carbon dioxide in the body fluids, calcium carbonate can be deposited in the bones (Messier, et al., 1979). Increased carbon dioxide could account for a prolonged negative phosphate balance, by taking its place in the bones in combination with calcium and magnesium.

Another important effect of carbon dioxide is in the regulation of both calcium and phosphate, by increasing the absorption and retention of calcium (Canzanello, et al., 1995), and by increasing the excretion of phosphate. Increased carbon dioxide (as dissolved gas) and bicarbonate (as sodium bicarbonate) both increase the excretion of phosphate in the urine, even in the absence of the parathyroid hormone. Below the normal level of serum bicarbonate, reabsorption of phosphate by the kidneys is greatly increased (Jehle, et al., 1999). Acetazolamide increases the body's retention of carbon dioxide, and increases the amount of phosphate excreted in the urine.

Much of the calcium dissolved in the blood is in the form of a complex of calcium and bicarbonate, with a single positive charge (Hughes, et al., 1984). Failure to consider this complexed form of calcium leads to errors in measuring the amount of calcium in the blood, and in interpreting its physiological effects, including its intracellular behavior. Hyperventilation can cause cramping of skeletal muscles, constriction of blood vessels, and excitation of platelets and other cells; the removal of carbon dioxide from the blood lowers the carbonic acid, changing the state and function of calcium. Hyperventilation increases phosphate and parathyroid hormone, and decreases calcium (Krapf, et al., 1992).

Since estrogen tends to cause hyperventilation, lowering carbon dioxide, its role in phosphate metabolism should be investigated more thoroughly. Work by Han, et al. (2002) and Xu, et al. (2003) showed that estrogen increases phosphate reabsorption by the kidney, but estrogen also increases cortisol, which decreases reabsorption, so the role of estrogen in the whole system has to be be considered.

This calcium solubilizing effect of bicarbonate, combined with its phosphaturic effect, probably accounts for the relaxing effect of carbon dioxide on the blood vessels and bronchial smooth muscles, and for the prevention of vascular calcification by the thyroid hormones (Sato, et al., 2005, Tatar, 2009, Kim, et al., 2012). Distensibility of the blood vessels and heart, increased by carbon dioxide, is decreased in hypothyroidism, heart failure, and by phosphate.

While fructose lowers intracellular phosphate, it also lowers the amount that the intestine absorbs from food (Kirchner, et al.,2008), and the Milne-Nielsen study suggests that it increases phosphate loss through the kidneys. The "anti-aging" protein, klotho, increases the ability of the kidneys to excrete phosphate (Dërmaku-Sopjani, et al., 2011), and like fructose, it supports energy production and maintains thermogenesis (Mori, et al., 2000).

Lowering the amount of phosphate in the blood allows the parathyroid hormone to decrease. While the parathyroid hormone also prevents phosphate reabsorption by the kidneys, it causes mast cells to release serotonin (and serotonin increases the kidneys' reabsorption of phosphate), and possibly has other pro-inflammatory effects. For example, deleting the PTH gene compensates for the harmful (accelerated calcification and osteoporosis) effects of deleting the klotho gene, apparently by preventing the increase of osteopontin (Yuan, et al., 2012).

Niacinamide is another nutrient that lowers serum phosphate (Cheng, et al., 2008), by inhibiting intestinal absorption (Katai, et al., 1989), and also by reducing its reabsorption by the kidneys (Campbell, et al., 1989). Niacinamide's reduction of free fatty acids by inhibiting lipolysis, protecting the use of glucose for energy, might be involved in its effect on phosphate (by analogy with the phosphate lowering action of a deficiency of polyunsaturated fatty acids). Aspirin is another antilipolytic substance (de Zentella, et al., 2002) which stimulates energy production from sugar and lowers phosphate, possibly combined with improved magnesium retention (Yamada and Morohashi, 1986).

A diet that provides enough calcium to limit activity of the parathyroid glands, and that is low in phosphate and polyunsaturated fats, with sugar rather than starch as the main carbohydrate, possibly supplemented by niacinamide and aspirin, should help to avoid some of the degenerative processes associated with high phosphate: fatigue, heart failure, movement discoordination, hypogonadism, infertility, vascular calcification, emphysema, cancer, osteoporosis, and atrophy of skin, skeletal muscle, intestine, thymus, and spleen (Ohnishi and Razzaque, 2010; Shiraki-Iida, et al., 2000; Kuro-o, et al., 1997; Osuka and Razzaque, 2012). The foods naturally highest in phosphate, relative to calcium, are cereals, legumes, meats, and fish. Many prepared foods contain added phosphate. Foods with a higher, safer ratio of calcium to phosphate are leaves, such as kale, turnip greens, and beet greens, and many fruits, milk, and cheese. Coffee, besides being a good source of magnesium, is probably helpful for lowering phosphate, by its antagonism to adenosine (Coulson, et al., 1991).

Although increased phosphate generally causes vascular calcification (increasing rigidity, with increased systolic blood pressure), when a high level of dietary phosphate comes from milk and cheese, it is epidemiologically associated with reduced blood pressure (Takeda, et al., 2012).

Phosphate toxicity offers some interesting insights into stress and aging, helping to explain the protective effects of carbon dioxide, thyroid hormone, sugar, niacinamide, and calcium. It also suggests that other natural substances used as food additives should be investigated more thoroughly. Excessive citric acid, for example, might activate dormant cancer cells (Havard, et al., 2011), and has been associated with malignancy (Blüml, et al., 2011). Nutritional research has hardly begun to investigate the optimal ratios of minerals, fats, amino acids, and other things in foods, and how they interact with the natural

toxicants, antinutrients, and hormone disrupters in many organisms used for food.

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