Reversible autopoiesis—a foundational design principle for life's survival

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As a contribution to a general theory of living systems I here identify a foundational design principle that provides robust survival options under adverse conditions of resource limitation—*reversible autopoiesis*. Autopoiesis is the continuous process of 'self-making' that allows organisms to grow, maintain, and repair themselves, and to periodically reproduce themselves. But the technology required for maintaining and repairing cells contains within itself the ability to degrade and recycle everything that normal metabolism manufactures; otherwise the cell would rapidly become dysfunctional through waste accumulation. Because the bodies of all organisms are originally *made* out of food and water from the beginning, they can therefore *unmake* themselves in a controlled manner to *produce* food and water again in times of special need. All organisms appear to use the same strategy—focusing their efforts upon energy production to keep the protein 'wheels of life' turning. To achieve this they mobilize stored reserves, reduce their metabolic rate and their cell population numbers, and conserve essential vitamins and minerals together with the molecular mechanisms enabling recovery when conditions improve. This design principle is foundational in that it is a logical consequence of how life *must* be made from the beginning—otherwise it doesn't work.

Astrobiologist and philosopher of science Carol Cleland has recognized that we need a general theory of living systems before we can hope to explain life's origin.[1] One approach is to develop a description of life's underlying design principles. However, Cleland warns that we should not mistake the signs of life to necessarily be the essentials of life, and she is skeptical of contemporary theories of origin.[2] Creationists have identified numerous functional,[3] and aesthetic[4] design principles in living organisms, and in this article I identify one that promotes survival in the face of resource limitation—*reversible autopoiesis*. This principle is foundational in that it is a necessary and logical consequence of how life *must* be made from the beginning, and it provides robustly sustainable functionality during periods of starvation.

Autopoiesis

In 1972 Chilean biologists Maturana and Varela introduced the term *autopoiesis* to demonstrate that life's unique ability to periodically reproduce itself flows out of a more generalized and continuous process of *self-making*.[5] Philosopher Evan Thompson highlighted the importance of its recursive nature—every molecular reaction in the system is generated by the same system that the molecular reactions produce.[6] Because living organisms have this ability to routinely 'make themselves' out of nothing more than food and water, this article demonstrates how they can also reverse that process and *unmake* themselves to *produce* food and water again in times of need.

Biochemistry textbooks typically provide all the information necessary to understand how the cell makes everything it needs out of energy and nutrient sources. There will also be descriptions of how these cell commodities are degraded again, sometimes by a simple reversal of the synthetic process and sometimes via a somewhat different route. The point I want to make is that *all* the machinery necessary to both make (in their entirety) and degrade (in their entirety) *all* the cell components is present in *every* normal living cell, and it *must* be so, otherwise waste products would accumulate and destroy cell function. And it must be so from the beginning, otherwise the first generation of living cells would have died from toxic waste accumulation. The inescapable consequence is that autopoiesis is, by definition, reversible, and this situation has logical consequences for life's robustness in the face of resource limitation. This fundamental design principle—that life is *reversibly* made from food and water—becomes especially obvious in a comparison between living organisms and robots.

Robots versus cells

It is possible with current technology to make robots that emulate a wide range of life-like activities, but the crucial differences in design principles emerge most clearly when they experience stress, especially starvation. When a robot runs out of fuel it stops working. When a living organism runs out of fuel (food, water) autopoiesis goes into reverse and it begins to *unmake* itself to *produce* food and water to sustain itself until conditions improve. Robots are usually made out of plastic and metal and so have to use something entirely

different—electricity, for example—as fuel. Until robots are created that can make themselves out of the same fuel they need to run on they will never catch up with the innate abilities of living organisms. Crucially, such robots will probably need to be made largely out of water, as cells are.

Humans die within a few days without water, or even within a few hours under extreme conditions (e.g. a child left in a hot car, or an athlete exercising too hard in hot weather).[7] But some creatures can live their whole lives without drinking water. Australian wild koalas can extract all the water they need during digestion of their food—oil-rich eucalyptus leaves.[8] The leaves contain some water, and metabolic breakdown of the oils and other materials produces enough extra 'metabolic water' to meet their needs. Their sedentary lifestyle reduces energy and water use, and tree-hugging reduces body temperature (and thus water loss) during heat-waves.[9] During bush fires, however, they will run to safety and eagerly seek water to drink.[10] In humans, metabolic water yields for the most common body materials are: 107.1 g of water from each 100 g of fat; 55.1 g of water from 100 g of starch; and 41.3 g of water from 100 g of protein.[11]

Reptiles are notorious for going long periods (years, in some cases) without eating food, as do creatures that undergo seasonal hibernation or dormancy. Those that live underground in caves (troglofauna) and aquifers (stygofauna) can survive long periods (10 years in the case of the European olm, a salamander) without food.[12] An average healthy human adult can survive for about 2–3 months without food if enough water is available, but a hunger-striker in India was still alive after 116 days.[13] When robots run *low* on fuel they can *intelligently* return to a fuel source, but when they run *out* of fuel they stop dead!

Autopoiesis in reverse

The most detailed information we have on reversible autopoiesis comes from the study of humans, notably in medicine where patients may suffer nutritional stress associated with diseased or traumatized states. Famines and war zones have also produced large numbers of victims subjected to starvation and/or near-starvation over long periods of time. Anorexia nervosa is now a common cause of starvation, and ultramarathon running puts healthy human bodies under extreme nutritional stress for days at a time. Information that follows has been drawn largely from a web-based resource maintained by Duke University,[14] except where other sources are cited.

Whole-body effects of starvation

A healthy 70 kg human consists of about 38.5 kg (55%) water and 31.5 kg (45%) dry solids. Of the solids about 28 kg (40%) is organic matter and 3.5 kg (5%) minerals. The organic component includes about 6 kg of protein, of which 4 kg is in muscle and 1 kg in hemoglobin. The remainder is spread throughout the body, including 25–35 g as blood serum proteins (albumin and globulins).

A healthy person can fast (no food but with adequate water) for about three days without suffering significant physical or mental defects. Continued fasting up to 10 days will produce physical and mental symptoms including fatigue, impaired response times and loss of concentration. Blood volume reduces rapidly and the body's fluid balance turns negative as breakdown products (largely urea) are excreted at a greater rate than normal. Body weight reduces almost linearly by about 0.7 kg per day over these 10 days.[15]

Under longer-term starvation in adults virtually all soft tissues lose mass as they are deconstructed in a staged manner to maintain essential systems. The worst-affected adult survivors in European concentration camps after World War II looked like skeletons covered with skin. Similar effects were documented in Asian prisoner of war camps.[16] In children, chronic malnutrition often results in disfigurement of the body.[17] Adults generally survive longer than children during famine, both because they have larger body reserves to draw upon, but also because their systems are mature. Surprisingly, vitamin deficiencies do not usually show up in starving adults,[15] unless there is complication from disease. On the Thai-Burma railway the workers were sparingly fed mainly on white rice, which lacks, among other things, vitamin B1 and niacin, and this caused beriberi and pellagra in those who suffered dysentery. It was reported: "Their continuous passing of stools caused dehydration and drained them of vitamins essential to their survival."[18] The implication is that normal bowel motions would not have drained their vitamin stores. The starving, but otherwise healthy, adult body appears to conserve vitamins and minerals while consuming other body tissues in a controlled manner to produce essential energy. This finding is supported by research on ultramarathon runners (see below).

Psychological factors may have been important in explaining the large difference in survival rates on the Thai-Burma railway. Among Europe's Allied Powers (British, Dutch, Australians, Americans)—i.e. those who were engaging the war and believed in the cause they were fighting for—the survival rate was 80%. Among the usually smaller-bodied Burmese and Malays—who were mostly unlucky victims of foreign occupation—the survival rate was only 45–55%.[19] The amount of time these people were subjected to near-starvation and forced labour varied, but can be bracketed by the fall of Singapore in February 1942, when more than 100,000 Allied troops were captured, and the end of the war in the Pacific in August 1945—three and half years.

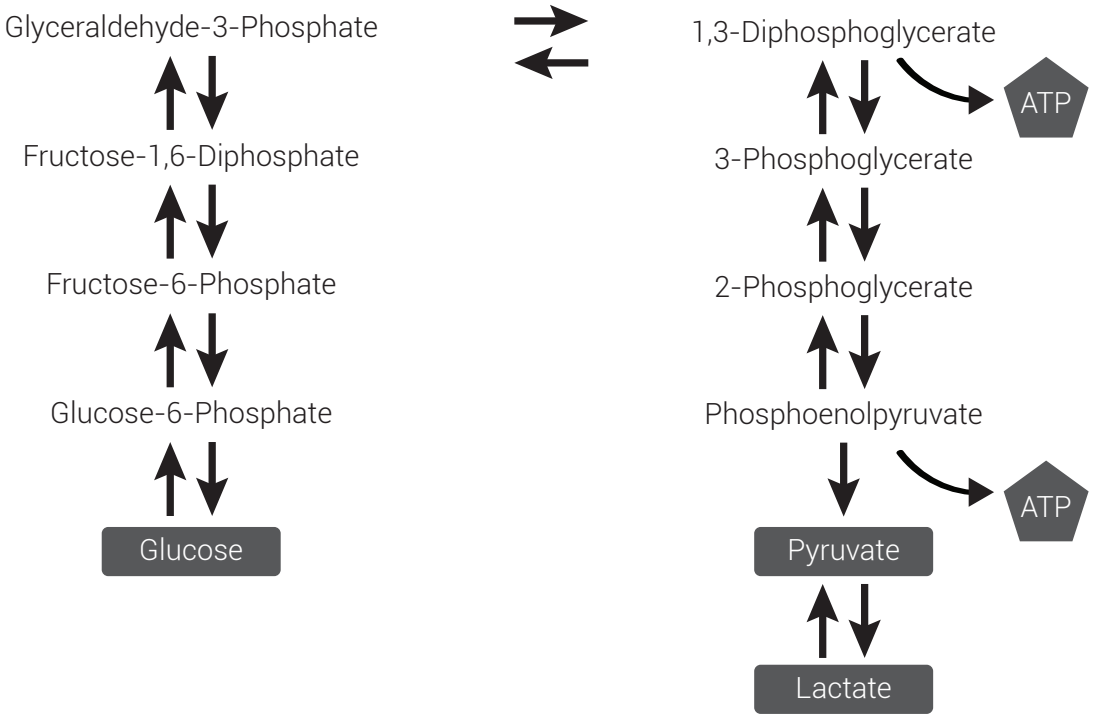

Figure 1. Glycolysis is the normal aerobic pathway for oxidative release of glucose energy as ATP. Glucose (lower left) is broken down via a series of mostly reversible reactions to produce pyruvate and lactate (lower right). This pathway can work in reverse (with three extra enzymes) to produce glucose.

Physiological effects of starvation

The brain and the central nervous system are the most critical organs that depend on glucose as their primary energy source, normally completely metabolizing 100–150 g per day to carbon dioxide and water. Irreversible brain damage can occur if glucose deprivation persists for as little as 10–20 minutes. Other glucose-sensitive tissues include renal medulla, bone marrow, red blood cells, and peripheral nerves.[14] When these tissues metabolize glucose they produce lactate and pyruvate, which are useful carbon sources that can be regenerated back into glucose via a process called *gluconeogenesis*.

The initial metabolic response to starvation does not differ physiologically from the post-absorptive phase in between meals in a well-nourished person. The body relies first upon the dietary glucose supplied by food, then excess glucose previously stored in the liver as glycogen is reduced back to glucose, then it switches to oxidation of fatty acids; proteins are initially conserved because they are not stored in the body and their loss means loss of cell function.[20] Once the glycogen store of about 120 g is used up, the body must revert to gluconeogenesis, which mostly utilizes glutamine, alanine, and glycerol to produce glucose in the liver, kidney, and intestine.[14] In parallel, production of ketones such as 3-hydroxybutyrate and acetoacetate is initiated in the liver. Ketones can directly supply energy to the brain because they are among the few substances that can cross the blood-brain barrier.

> "Starvation ensues when protein remains the only source of energy for the body. The amount of glucose usually utilized by the body is reduced to a minimum, with the metabolic rate of the cells being decreased significantly to allow for the subsistence of the organism as a whole. Individuals who suffer from chronic starvation adapt, displaying similar basal metabolic rates as healthy individuals when adjusted for fat-free body mass since the visceral organs with the highest metabolic rates such as the brain and the kidneys remain relatively unaffected."[21]

Adults can recover quite well from this state with appropriate treatment, but children suffer vitamin and nutrient deficiencies that cause stunted growth and development, poor bone quality, and earlier onset of diseases such as osteoporosis.[21]

Important metabolic pathways for survival

Because most of the biochemical steps in normal metabolic cycles are reversible they provide ready-made pathways for regeneration of glucose from non-carbohydrate sources. The normal glucose breakdown pathway (glycolysis) is shown in figure 1, and requires enzymes that drive the forward process from left to right. Three extra enzymes are required to turn the pathway backwards into gluconeogenesis:

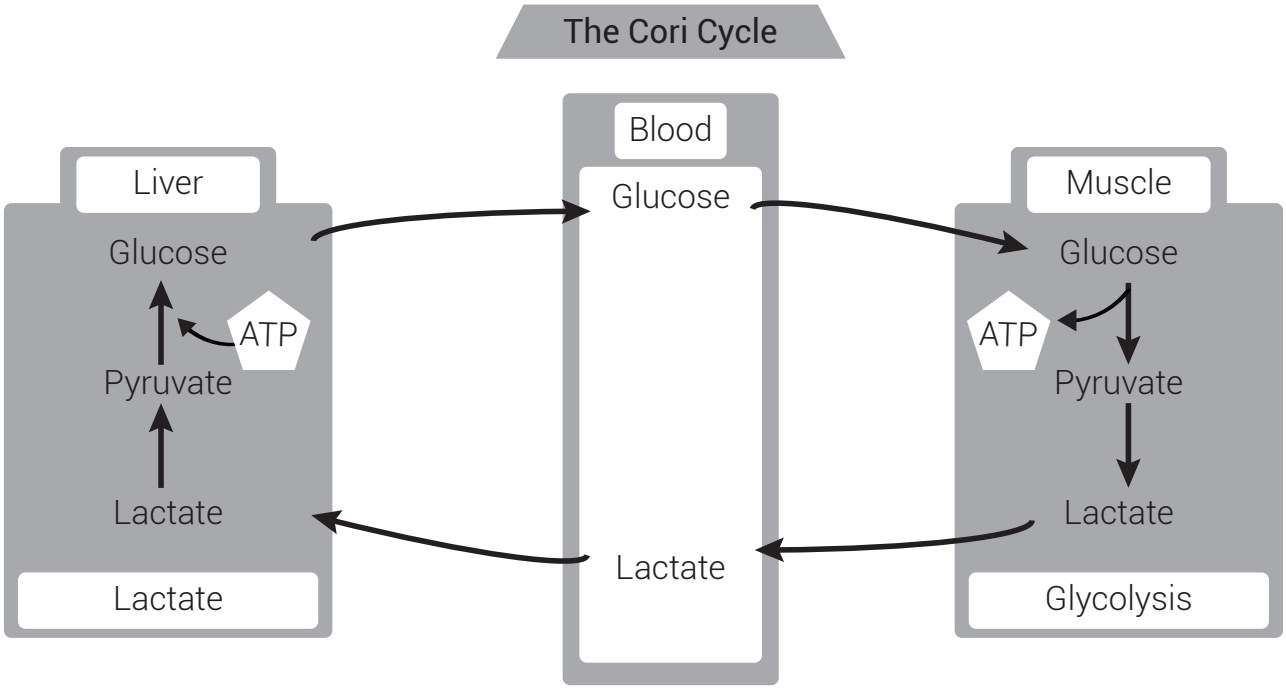

Figure 2. Cori cycle (lactic acid cycle). Muscles (right) normally use glucose to produce energy (ATP) via glycolysis. The lactate end-product is carried via the blood (centre) to the liver (left) where it is converted back into glucose via gluconeogenesis, which requires some ATP as input. Glucose is then exported back to the muscles via the bloodstream.

> "The first is a specific phosphatase capable of converting fructose diphosphate to fructose 6-phosphate. … Phosphoenolpyruvate carboxykinase catalyzes the conversion of oxaloacetate to phosphoenolpyruvate. This step permits oxaloacetate, and any substance that can be transformed into oxaloacetate, such as aspartate, to serve as substrates for gluconeogenesis. Finally, pyruvate carboxylase catalyzes conversion of pyruvate to oxaloacetate, thereby allowing lactate, alanine, serine, and similar substances to enter gluconeogenesis upon their conversion into pyruvate."[14]

Lactate is the normal end-product of glucose oxidation in muscles and it can be recycled back to glucose through the Cori cycle (lactic acid cycle, figure 2) in the liver and kidney, using energy produced by fatty acid oxidation. Pyruvate can undergo oxidation in the Krebs cycle (citric acid cycle) inside mitochondria in muscle cells to directly resupply them with ATP, or it can be transported in the bloodstream to undergo gluconeogenesis in the liver (figure 2).

Humans depend mainly on gluconeogenesis in the liver to meet energy requirements early in starvation, but in later stages the kidneys provide up to half of the total. When muscle protein breaks down it produces a range of amino acids, one of which is glutamine, and several other amino acids are converted into glutamine by a process called *transamination*. Glutamine then becomes the major amino acid in the bloodstream and it enters the gluconeogenesis pathway in the kidney (figure 3). The main nitrogenous by-product is ammonia (NH_3) which is partly excreted in the urine and partly recycled back into making new proteins.

The Cahill cycle (figure 4) provides a major pathway for gluconeogenesis in the liver, where its main amino acid substrate is alanine.[22]

> "In muscle, pyruvate is generated during anaerobic breakdown of glucose. The nitrogen moiety of branched-chain amino acids (valine, leucine, and isoleucine) is transaminated to pyruvate, forming alanine … [plus] additional ATP for local use. The alanine released from muscle is taken up by the liver, where the nitrogen is split off. The resultant pyruvate is recycled to glucose via gluconeogenesis. Most of the nitrogen is excreted in the urine as urea; however, some is re-utilized in protein synthesis."[14]

> "Early in starvation, approximately 75 g of body protein and 160 g of adipose tissue are metabolized each day … . All [body] proteins are utilized, … [and blood s]erum albumin is used in the ratio of 1 g albumin per 30 g tissue protein lost. The most clinically evident protein loss is from skeletal muscles. [Fat breakdown] releases free fatty acids and glycerol. Although free fatty acids cannot participate directly in gluconeogenesis, they can serve as an energy source in the liver for the Cori cycle and generate acetyl-CoA, which enhances the conversion of pyruvate to

oxaloacetate. Glycerol is readily converted to glucose."[14]

Prolonged starvation results in a lowered metabolic rate, which results in "diminished muscle activity, increased sleep, and decreased core temperature."[14] The need for gluconeogenesis diminishes because the central nervous system changes over from using glucose to using ketones as its primary energy source. The stimulus for this change seems to be a rise in serum levels of D-β-hydroxybutyrate,[23] a product of ketone metabolism.[24] "Protein catabolism [breakdown] falls from 75 to 20 g/day, with a marked decrease in excretion of urea nitrogen to 3–5 g/day."[14]

When the body has reached its lowest level of adaptation to starvation, energy comes mostly (60%) from fat metabolism (cell membranes begin to disintegrate), 25% from ketone metabolism, 10% from conversion of free fatty acids into ketones, and 5% from protein breakdown. [14] Ketones in urine are a signal of prolonged starvation. [14] Death usually ensues after 60 to 70 days without food.

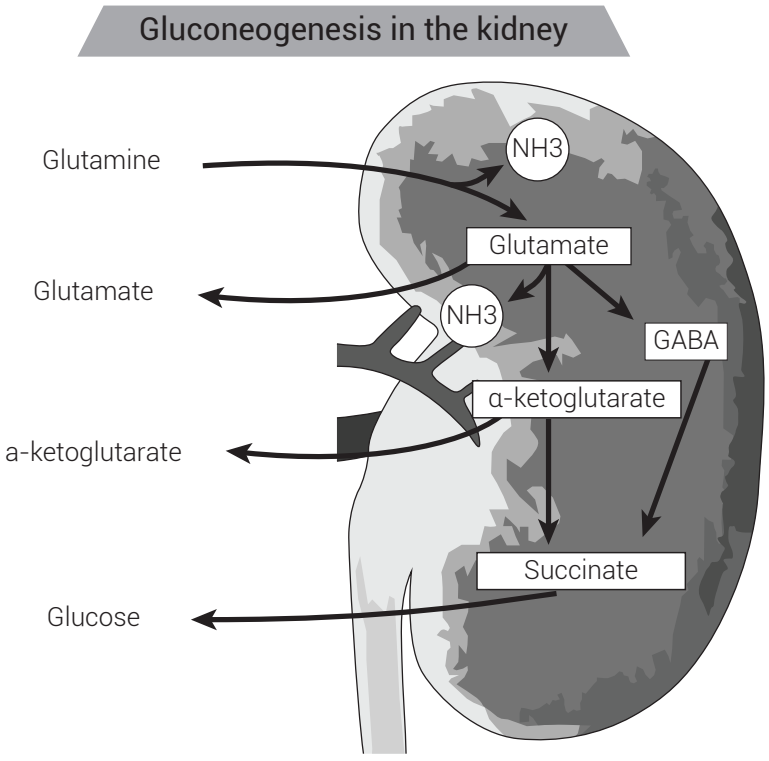

Figure 3. Glutamine is a major substrate for gluconeogenesis in the kidney, particularly in the later stages of starvation.

Recovery from starvation

Recovery from 10-day starvation is generally rapid from day one onwards, and after six weeks the subjects are largely back to normal weight.[15] Recovery from longer starvation is dependent on body reserves and the severity of caloric deficit. Some survivors of European concentration camps were so weak they could not eat. In such cases alternative energy sources are required. Some survivors who could not eat but were given sugar cubes to suck did recover, but many who were given army rations died because their depleted bodies could not cope with rich food. Other survivors fared much better for various reasons.[25]

United Nations experience with famine relief highlights the special needs of children, pregnant women, and breast-feeding mothers.[26] In the early stages of famine, human lactation is relatively unaffected so breast-feeding is an important built-in method of protecting offspring. Famine-relief foods for children and pregnant and lactating women need to be rich in calories, protein, and vitamins. Infectious disease also complicates famine deaths, especially among children, so maternal and child healthcare is required.

Once an adult victim of starvation has had adequate energy resupply, normal

> "… total body protein synthesis is usually restored at a rate of 18 to 30 g/day. … During recovery, essential amino acid, total protein, and caloric requirements are all greater than normal. After nitrogen [i.e. protein] losses have been restored, fat is gained almost exclusively for several weeks or months until the normal body fat stores are regained. In this phase, nitrogen balance is zero, although carbon balance is positive." [14]

Starvation affects adult bone much less than soft tissues. Bone morphology remains largely intact, but bone density and quality may decline. Animals well adapted to annual starvation (e.g. hibernating bears) recover well, but others, such as moose, that are not adapted to starvation because they migrate to find food, are prone to becoming ill and/or lame through osteoporosis. Chinese women 65 and older who had experienced famine at some time in their lives showed a 5% increase in the frequency of osteopenia and osteoporosis compared with those who had not been so affected. Female survivors of the Holocaust who were 60 and older, compared with a contemporary control group of European Jewish women, showed a 200% increase in the frequency of osteoporosis.[21] Child Holocaust survivors (under 16 at the end of World War II) suffered the highest incidence of osteoporosis and osteopenia in later life. Anorexia nervosa sufferers have demonstrated similar effects.[21]

Long-term complete recovery is never certain. Symptoms observed in surviving prisoners of the war in Asia 10 years after their release included easy fatigability, profuse sweating

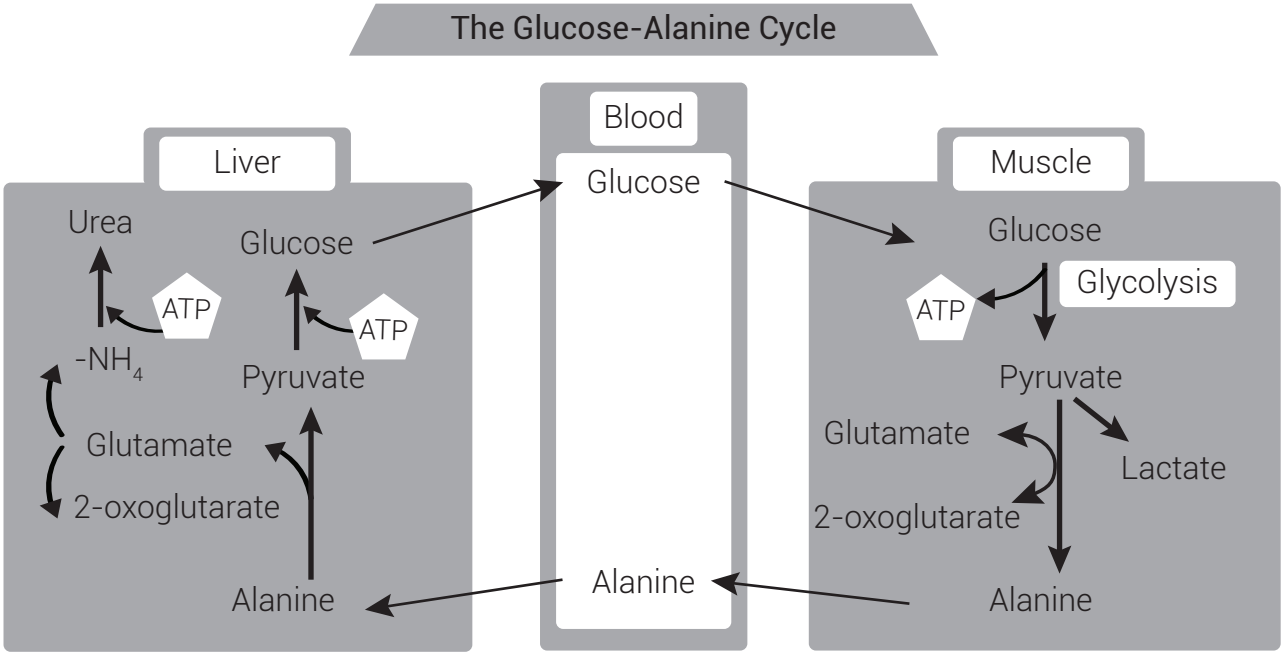

Figure 4. The Cahill (glucose-alanine) cycle, a major source of gluconeogenesis in the liver

for no apparent reason, numbness and cramps in calf muscles, loss of ambition, poor vision, oedema, dyspnoea on even the slightest exertion, depression, tachycardia, anorexia, nausea, restlessness, irritability, and insomnia.[15] Many, however, recovered sufficiently to live long and fruitful lives.

Evidence from ultramarathon running

The effects of starvation and recovery in the human body reported above are confirmed in the literature on ultramarathon running. An ultramarathon course is longer than a standard marathon of 42 km and includes the 100 km course, double marathons, 24-hour races, and multi-day races of 1,600 km or longer.[27]

Endurance athletes who have a short recovery time (8–24 hours) before the next event need only eat optimal amounts of carbohydrate and protein.[28] The carbohydrate replenishes glycogen stores in the muscles, with any excess going to fat storage, and the protein is used in rebuilding depleted muscles. Vitamin and mineral supplements are not necessary as the body can conserve these components while using up carbohydrates, fats and proteins in gluconeogenesis. Regular rehydration is essential, but the need for electrolyte supplements varies among individuals.[29] Some women may need calcium, iron, and/or vitamin D supplementation.[30]

While post-event dietary needs are relatively uniform, the preparation required before an ultramarathon is extremely variable, largely due to individual differences in physiology, culture, and taste. Ultramarathon runner Mark Woolley (Ph.D. in physical chemistry) argues that because the body uses carbohydrate reserves first, but it gains more energy from fat metabolism, he trains on a low-carbohydrate and moderately high-fat diet. This, he believes, trains his body to make more general use of fat metabolism. On race day he eats a high-carbohydrate diet up to the 12-hour point in the race then switches to sandwiches soaked in olive oil.[31] Supervising doctors say that you should do whatever works for you.[29]

Microbial responses to starvation

Many microbes are well adapted to boom-and-bust environments. Gut microbes in warm-blooded vertebrates can feast on a regular food supply at optimal body temperatures (e.g. 37°C in humans), but when passed out in excreta conditions can change dramatically. Shallow lakes and floodplains in monsoonal climates are likewise subject to a yearly cycle of lush growth in the wet season and high-temperature drought in the dry season so their microbes must have a variety of strategies for survival.[32] Marine microbes in the varied habitats of shallow seas in arid regions are also subject to extreme environmental fluctuations.[33]

Bacteria display a characteristic *stringent response* in reaction to various stress conditions, including "amino-acid starvation, fatty acid limitation, iron limitation, heat shock, and others".[34] The stringent response is communicated throughout the cell by a special signalling molecule called an *alarmone*, "which modulates transcription of up to a third of all genes in the cell. This in turn causes the cell to divert

resources away from growth and cell division" and towards energy production.[31]

Like higher organisms, the gut microbe *Escherichia coli* survives famine by lowering its metabolic activity to conserve energy but many individuals die. A comparative utilization study of 95 different energy substrates by starved versus non-starved cultures of a toxic *E. coli* strain from New Zealand cattle found that usage of nine substrates, including fructose-6-phosphate, glucose-1-phosphate, pyruvic acid, and thymidine, was not affected by starvation.[35] These substrates are commonly found in forage plants recently ingested by ruminants and show that the starved microbes had conserved their ability to detect when conditions improve so they can rapidly re-establish their population numbers. Survival outside the host depends strongly upon temperature. At 15°C (summer in New Zealand) 97% of the populations died in the first three weeks on low-nutrient supplies, but most of the remainder persisted throughout the 84-day experiment. At 4°C (winter) 99.9% of the population died in the first six weeks on low-nutrient supplies, and most of the remainder also persisted throughout the 84-day experiment. The summer populations were ~100 times larger than those in winter.

Bacillus subtilis is a common soil and gut bacterium that belongs to a special group noted for their ability to escape starvation by forming a dormant and highly resistant *endospore*. Endospore formation is usually triggered by a lack of nutrients and it reverts back to its normal reproductive habit when conditions improve.[36] A comprehensive molecular analysis of protein usage in *B. subtilis* during glucose starvation showed a general shift away from protein synthesis and towards carbon metabolism. In contrast, during heat stress most resources were used to increase the amount of chaperones and proteases.[37] So, during starvation, energy production had top priority, and, during heat stress protein conformation had top priority. Both responses are rational strategies for keeping the machinery of life working properly under stressed conditions.

Bacterial colonies that develop in complex natural environments are notoriously variable in both phenotypic and genomic diversity compared with those in sterile culture media. Sputum samples of 44 morphologically identical *Pseudomonas aeruginosa* isolates taken from a single patient with cystic fibrosis illustrate this complexity.[38] Phenotypic analyses revealed large variances and trade-offs in growth, virulence factors, and quorum sensing signals. Whole genome analysis of 22 isolates revealed high levels of intra-isolate diversity ranging from 5–64 single nucleotide polymorphisms. Recombination, however, and not spontaneous mutation was the dominant driver of this diversity. Phenotypic differences between isolates were likewise not linked to mutations but were correlated with recombination events. Antibiotic resistance was greater in mixed populations,[38] quite possibly because of greater opportunities for horizontal gene transfer (natural genetic engineering).[39]

When taken together with the very large population numbers in typical bacterial habitats (billions, compared to the 44 isolates reported in the study quoted here) we can see the enormous power that microbes have in generating diversity that can contribute towards survival. The *E. coli* experiments cited earlier[32] showed a rapid death rate among most bacteria, but then a surprisingly long persistence time for the survivors. Both experiments began with 500 million cells/ml of substrate and under winter and summer conditions the populations stabilized at about 100,000 and 13 million cells/ml respectively. This dual strategy of lowered metabolic rate and differential individual survival helps to explain why bacteria inhabit all known ecological niches on Earth.

Conclusions

How important is autopoiesis for a general theory of living systems? How important is the fact that it is reversible? The answer to both questions is that they are essential parts of any such theory because they must be present from the beginning. If they are not present from the beginning then the very first cell would rapidly malfunction due to waste accumulation. But once the machinery for synthesis, maintenance, and repair of life's mechanisms is in place then the life system *automatically* becomes robust under conditions of resource limitation. Having been *made* out of food and water to begin with, the cell can (to a limited and controlled extent) *unmake* itself to produce food and water again when needed. Living organisms, from the smallest prokaryotes to the largest mammals, all have the same strategy for coping with resource limitation—they redirect their metabolism to focus upon energy production so that the protein 'wheels of life' keep turning. Bacteria have a three-pronged strategy—they reduce their metabolic rate and their population numbers, and they conserve their mechanisms for sensing when conditions improve so they can rapidly rebuild their population numbers. Humans likewise have built-in mechanisms for re-orienting their metabolism from normal activities to intense focus upon energy production, they reduce their metabolic rate, mobilize stored energy reserves, conserve essential vitamins and minerals, and reduce cell numbers in all soft tissues. Reversible autopoiesis is a foundational design principle that *must* be present at the beginning in any theory of life's origin. No simple-to-complex Darwinian scenario can meet such a standard and, once again, Genesis-style fiat creation is the only rational explanation.

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