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Comment on "A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus"

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Wolfe-Simon *et al.* (*Science* Express Research Article, published online 2 December 2010; 10.1126/science. 1197258) reported that bacterium GFAJ-1 can substitute arsenic for phosphorus in its macromolecules, including DNA and proteins. If such arseno-DNA exists, then much of the past century of work with arsenate and phosphate chemistry, as well as much of what we think we know about metabolism, will need rewriting.

Wolfe-Simon *et al.* (*1*) reported that a bacterium isolated from Mono Lake, California, can grow by using arsenic instead of phosphorus. Their hypothesis that this microorganism contains DNA and other standard biomolecules in which arsenate atoms replace phosphorus atoms would, if true, set aside nearly a century of chemical data concerning arsenate and phosphate molecules, revolutionize our view of bacterial metabolism, and radically alter our understanding of microbial adaptation (*2*). This does not mean, of course, that the hypothesis should be discarded out of hand. Automatically discarding results inconsistent with decades of work, if generally applied, would cause us to overlook most valid advances in science (*3*). Nor does it mean that the primary conclusion of Wolfe-Simon *et al.*, a bacterium that can grow in the presence of high arsenate, is incorrect. On the contrary, this particular conclusion appears secure based on the data published. However, neither the report itself nor the accompanying news article (*4*) conveyed the extent to which this hypothesis, if true, would require rewriting of many conclusions that we have hitherto accepted based on data from many laboratories. We consider just two of these here.

Any hypothesis that arsenic replaces phosphorus in biomolecules such as DNA (where arsenate diesters would replace phosphate diesters) must manage well-measured rates for the hydrolysis of arsenate esters. For example, Baer *et al.* (*5*) measured the rate of hydrolysis of arsenate triesters, reporting a second-order rate constant for the hydrolysis of trimethyl arsenate in methanolic sodium hydroxide (73 $M^{-1}sec^{-1}$ at 25°C, pH ≈ 12.5). Extrapolating this directly to pure water (~55 M) gives a pseudo first-order rate constant of 4015 s^{-1}, corresponding to a half-life measured in fractions of a millisecond. Extrapolation of this to the pH of Mono Lake (pH ≈ 10) gives a pseudo first-order rate constant ≈ 10 s^{-1}; extrapolation to pH ≈ 7, perhaps the pH inside of the cell, gives a rate constant ≈ 0.01 s^{-1}, corresponding to a half-life for each linkage in the hypothetical arseno-DNA of approximately 1 minute. Thus, if the genomic DNA band shown in figure 2A in (*1*) is, in fact, arsenate-linked DNA, then either (i) that DNA contains very few arsenate linkages, (ii) the kinetic data reported in (*5*) [and elsewhere; see review in (*6*)] are incorrect, (iii) the extrapolation fails by many orders of magnitude, (iv) the analogy between model arsenate esters and arseno-DNA is similarly imperfect, or (v) the band must be associated with additional compounds that stabilize arsenate diester linkages. Explanation (iv) runs afoul of measurements made for many other arsenate esters (*6*). Explanation (v) would require those additional compounds to remain associated with the hypothetical arseno-DNA through extractions by phenol and chloroform, ethanol precipitation, and the time required to prepare the sample and run the gel electrophoresis experiment (*1*). Nor is it clear why the band in the gel would be so sharply defined if it were arseno-DNA associated with stabilizing molecules. In contrast, the base-catalyzed rate of hydrolysis of typical phosphate diesters is too slow to measure easily. Nevertheless, estimates suggest that the phosphate diester in DNA has a half-life in water of ~30 million years (*7*).

The hypothesis that phosphate DNA might "morph" into arseno-DNA upon going from a phosphate-rich environment to a phosphate-poor environment creates an interesting paradox with respect to metabolism. The phosphorus atoms that end up in DNA come from the "alpha" phosphate group of one of four deoxynucleoside triphosphates (*8*). These are produced in many Gammaproteobacteria by a ribonucleotide reductase that acts on the corresponding ribonucleoside triphosphates. Thus, the phosphorus atom having, as its fate, to serve as a linking phosphorus in DNA meets that fate early in the biosynthesis of ribonucleoside phosphates, averaging about eight steps (depending on the nucleobase) earlier in standard metabolism. If the biosynthetic pathway for the

hypothetical arseno-DNA is analogous, we must assume that the arsenic atom fated to replace phosphate originated in the five-position arsenate ester of ribose, the sugar. Despite its hydrolytic instability, that arsenate must be maintained for many steps as the purine and pyrimidine rings are forged. Alternatively, we must envision an entirely novel way of making triphosphates so that an arsenic atom can be tucked away within a chain of atoms, or a novel way of assembling DNA. Either way, if the arseno-DNA hypothesis proves true, then it will lead to discoveries in metabolism that lie outside metabolic precedent.

The actual numbers reported by Wolfe-Simon *et al*. (*1*) describing the ratio of arsenic to phosphorus in various subcellular fractions do not allow us confidently to rule out an alternative hypothesis, that the reported microbe aggressively scavenges phosphorus from the environment and uses it where it is most important—in its DNA. Unfortunately, the level of contaminating phosphate in the arsenate that was used to grow these microbes is unknown. Thus, it remains possible that the arsenate added in the growth mixture was itself a source of phosphate that the microbe might have scavenged.

For the reasons outlined here, the hypothesis of arseno-DNA would seem to fall into the category of "exceptional" that, as Carl Sagan remarked, requires exceptional support. Processes are well known to guide experiments to manage such hypotheses. For example, the molecules isolated in the band boxed in lane 2 of the gel in figure 2A in (*1*) should be easily subject to standard tools used in chemistry to determine molecular structure. Is the band degraded by acid or base? The rate of such degradation should be consistent with rates of other arsenate-containing compounds. Is it phosphorylated by DNA kinase? A positive answer would confirm a structure consistent with the specificity of DNA kinase and provide a labeled species useful for further downstream analysis. Is the kinased DNA then digested by exonuclease? One would expect arsenate linkages to behave differently from phosphate linkages during digestion. And above all, does the band in the gel become radioactive above background if the microbe is fed an isotope of arsenic that is radioactive? This would provide a direct confirmation that the band contains arsenic, without indicating its linkage.

References

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