P cycle cannot be a general mechanism for energy production, and it does not sensitize bacteria toward aminoglycosides

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In PNAS, Su et al. (1) claim that the pyruvate cycle or "P cycle," which adds three enzymes—phosphoenolpyruvate (PEP) carboxykinase, pyruvate kinase, and pyruvate dehydrogenase—to the TCA cycle, "operates routinely as a general mechanism for energy production" in *Escherichia coli*, and that glutamate generates more energy through the P cycle and sensitizes bacteria toward aminoglycosides, "resulting in improved elimination of antibiotic-resistant pathogens."

I find that none of these claims is convincing. First, the P cycle, as presented by Sue et al. (1), has several fundamental problems:

(*i*) The P cycle needs the constant input of oxaloacetate (OAA). I agree that if bacteria use some amino acids, such as aspartate, as the sole energy source, the operation of the TCA cycle may become similar to the P cycle. However, this is a special situation. Enteric bacteria survive mostly on sugars that are generated from our starchy food, and glycolysis does not generate OAA, contrary to the claim of the authors.

(*ii*) If OAA (a 4C compound) comes from outside the cycle, the cycle must release four carbons (i.e., four carbon dioxide molecules) during the cycle. Yet, figure 2 and page E1580 show the release of only three CO_2 molecules.

(*iii*) Lastly, the authors claim that the V_{max} of PEP carboxykinase in *E. coli* was 1,708 nmol (not "nM" as in the paper, which indicates concentration, not quantity), whereas that of citrate synthase was 1.19, apparently suggesting that the P cycle, rather than TCA cycle, is the predominant pathway. However, they forget that the latter enzyme is also needed in the P cycle for condensation of OAA with AcCoA. Additionally, their specific activity of citrate synthase is at least 2 orders of magnitude lower than the values reported in the literature (e.g., see ref. 2).

Second, I cannot agree with the claim that the P cycle sensitizes *Edwardsiella tarda* and *E. coli* for aminoglycosides.

(*i*) This claim is based on the drug-induced killing in M9 medium, with acetate, glutamate, citrate, etc. as the sole energy and carbon source. The authors are apparently unaware of the fact that these acidic compounds must be actively transported into cytosol before getting metabolized, and that the rate of metabolism and proton motive force generation depends strongly on the kinetics of these active transport processes.

(*ii*) Furthermore, the authors isolated their "gentamicin-resistant strain" of *E. tarda* by selection in the laboratory. Practically all aminoglycoside-resistant clinical isolates owe their resistance to the plasmid-coded aminoglycoside-inactivating enzymes, and what was seen with laboratory-isolated mutants has no relevance to the situation found in the real world (unless one is dealing with *Mycobacterium tuberculosis*) (3). Arguing that these data are relevant to our fight against multidrug-resistant bacteria is most misleading.

(*iii*) Lastly, pathogenic enteric bacteria live mostly on sugars, which are present both in the intestinal tract and in our body fluids. What happens to them in a medium containing only glutamate, without any sugars, is totally irrelevant to therapeutic situations.

1 Su Y-B, et al. (2018) Pyruvate cycle increases aminoglycoside efficacy and provides respiratory energy in bacteria. *Proc Natl Acad Sci USA* 115:E1578–E1587.
2 Lakshmi TM, Helling RB (1976) Selection for citrate synthase deficiency in *icd* mutants of *Escherichia coli*. *J Bacteriol* 127:76–83.
3 Magnet S, Blanchard JS (2005) Molecular insights into aminoglycoside action and resistance. *Chem Rev* 105:477–498.

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