

(*hts* A, B, and C) that show homology with known heme transporter genes in other bacteria (*Yersinia enterocolytica* and *Corynebacterium diphtheriae*). Moreover, they identified a binding site for the bacterial ferric-uptake repressor protein, Fur, immediately upstream of the *HtsA* initiation codon, implying that the *hts* system is switched on in response to iron deficiency.

Although a heme-uptake system is of potential value, it would be of little use if *S. aureus* did not also possess mechanisms for liberating heme from the red blood cells where it is packaged in the form of hemoglobin (see the figure). *S. aureus* produces multiple hemolysins that breach the red cell membrane, promoting osmotic lysis of the cells (10). Once hemoglobin is released, it is not clear whether *S. aureus* liberates heme from hemoglobin with specific IsdB and IsdA enzymes (11), by secreting proteases like *Vibrio vulnificus* (12), or by oxidizing hemoglobin to promote its spontaneous dissociation into globin and heme (13). The bacteria then import the free heme, which is catabolized by the bacterial enzymes IsdG and IsdI in the same way as mammalian heme oxygenases catabolize heme, resulting in the release of iron from the heme porphyrin ring (14). Thus, *S. aureus* is a versatile pathogen that liberates heme from a vast erythrocyte repository, imports heme across its bacterial membrane, and degrades it to yield free iron (see the figure).

If the ability to take up iron is a potent virulence factor and the *hts* system is a major determinant of iron uptake, then mutational inactivation of the *hts* genes should attenuate *S. aureus* virulence. The authors analyze the pathogenicity of mutant and wild-type *S. au-*

*reus* in two model systems: the worm *Caenorhabditis elegans* and the mouse. Mutations in the *Hts* B and C genes markedly decreased mortality in worms infected 48 hours previously, and abscess formation markedly decreased in the livers and kidneys of mice 96 hours after intravenous injection of mutant compared to wild-type *S. aureus*. These results strongly imply that heme is the major source of nutrient iron in the critical early stages of *S. aureus* infection.

In response to bacterial infection and inflammation, humans restrict iron uptake and sequester iron within macrophages throughout the body. The peptide hormone hepcidin orchestrates these changes and causes a substantial decrease in serum iron levels (15). This hypoferric response may be important for host defense by making iron even less available than usual to invading pathogens. The protective effects of hypoferricemia may explain the mystery of why physicians embraced bloodletting as a therapeutic procedure for more than 2500 years. As recently as 1942, Sir William Osler's highly regarded medical textbook advocated bloodletting as a treatment for acute pneumonia: "To bleed at the onset in robust healthy individuals in whom the disease sets in with great intensity and harsh fever is good practice" (16). The development and widespread use of antibiotics in the mid-20th century obviated the need to employ questionable treatments such as bloodletting. However, the discovery that *S. aureus* depends on heme iron for growth in its animal hosts suggests that bloodletting in the pre-antibiotic era may have been an effective mechanism for starving bacterial pathogens of iron and slowing bacterial growth.

Bacteria continue to discover new ways to combat antibiotics and the race is on to discover new therapeutic targets to combat bacterial infection. The heme-uptake proteins of *S. aureus* may represent a new target for molecular therapy. Efficient lysis of erythrocytes increases the concentration of iron available to *S. aureus* by 100-fold compared to the normal concentration of transferrin-iron in serum. This liberated heme-iron apparently fuels the rapid growth of virulent *S. aureus* infection. Thus, pathogenic *S. aureus* applies principles of logic similar to those of accomplished bank robbers: They go for the heme, because that's where the iron is.

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#### MICROBIOLOGY

## Noninherited Resistance to Antibiotics

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As notorious as they may be, bacteria with inherited resistance to antibiotics are not the only reason that antibiotics fail and may not even be the major reason, at least not yet. Contributing to the humbling of these "wonder drugs" is the fact that growing populations of bacteria do not just die off when confronted with bactericidal antibiotics. Instead, their rates of mortality decline with time, and viable antibiotic-sensitive cells can be recovered even after hours of ex-

posure to the drug (see the figure, panel A) (1). This phenomenon of declining sensitivity is well established for different species of bacteria and for different classes of antibiotics (2–4). Various called "bacterial persistence" (5), "phenotypic tolerance" (6), or "adaptive resistance" (7), the phenomenon remains a mystery with respect to its mechanism as well as its contribution to treatment failure.

One mechanism postulated to account for the declining sensitivity and survival of bacteria confronted with bactericidal antibiotics is that growing populations of genetically identical bacteria continually gen-

erate subpopulations that are less sensitive to killing by antibiotics because they either are not growing or are dividing at very low rates (8). On page 1622 of this issue, Balaban *et al.* provide evidence for the existence of these refractory bacterial subpopulations and explain how they may account for the persistence of antibiotic-sensitive bacteria (9). Meanwhile, on page 1629, Miller *et al.* present a mechanism by which this kind of noninherited resistance to antibiotics can be generated. This mechanism unexpectedly involves the SOS response, which blocks cell division during the repair of DNA damage (10).

Balaban and colleagues used a really cool combination of microfluidics and optical microscopy to make intimate movies of the replication of individual *Escherichia coli* bacteria, under normal conditions and when treated with the antibiotic ampicillin. In this way, the investigators were able to distinguish at least two distinct cell types in exponentially growing clones of *E. coli*: "nor-

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mal” rapidly dividing cells and slowly dividing “persisters.” By combining mathematical modeling, data fitting, and competition experiments, they present a compelling argument that the decline in the mortality rate of growing populations of *E. coli* exposed to ampicillin can be attributed to switching from a normal to a persister phenotype. Ampicillin treatment enriches the more antibiotic-tolerant persister subpopulations, thereby reducing the average susceptibility of the population to ampicillin.

In a complementary study, Miller and co-workers used classy molecular genetics to show that ampicillin (as well as some, but not all, other  $\beta$ -lactam antibiotics) induces an SOS response in *E. coli* that halts cell division. They demonstrate that ampicillin induces a temporary block in *E. coli*

genes needed for induction of the SOS response—*dpiA*, *recA*, and *lexA*—are required for the production of the Balaban *et al.* persisters. Of course, it is possible that these bacterial persisters are the product of stochasticity (leakiness) in the induction process (11). Whether produced constitutively or stochastically, persister populations should be enriched by all bactericidal antibiotics that are ineffective against slowly dividing or nondividing cells.

From the perspective of classical genetics, phenotypic variation in genetically homogeneous populations of the sorts considered in these studies is an inconvenience. Who wants to deal with characters that are leaky, incompletely penetrant, or variable in their expressivity? On the other hand, from a clinical, ecological, and evolution-

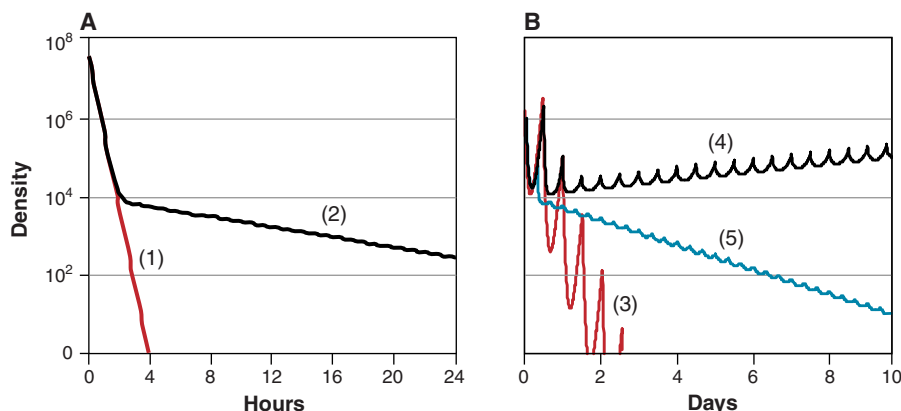
4) that includes phenotypic switching of the sort considered by Balaban *et al.* (see the figure, panel B). Similar results have been obtained with a model of antibiotic-mediated induction of the tolerant state in bacteria akin to that observed by Miller *et al.* (1).

The reports by Balaban, Miller, and their colleagues remind us once again that eukaryotes and bacteria such as streptomycetes and myxobacteria are not the only organisms that undergo cellular differentiation. Spores and the stationary phase of growth are differentiated cellular states that enable bacteria to survive resource deprivation and other hardships. One interpretation of the two new studies is that cellular differentiation survival mechanisms akin to those induced during times of famine operate during times of feast.

It is easy to concoct just-so stories to explain the evolution of a mechanism that, like the SOS response, produces quiescent cells that are refractory to lethal agents. Yet it seems unlikely that ampicillin was the original selective force responsible for the evolution of the induction mechanism observed by Miller and colleagues. A bigger challenge to those in the evolution business is to account for the generation of lower fitness cell types when they do not provide an advantage to the collective, like the persisters of Balaban *et al.* in the absence of antibiotics. Then again, just like people, bacteria do some seemingly perverse things that are not easy to account for by simple stories of adaptive evolution (14).

#### References and Notes

1. The mathematical models, computer simulations, and parameter values used to generate these figures and explore these dynamics are described at [www.ecdf.net](http://www.ecdf.net) (Tolerance models). The Berkeley Madonna software programs used for these simulations can also be accessed from this Web site.
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15. Dedicated to the memory of John A. Barrett, a friend and the founder of the EcLF (*E. coli* Liberation Front). I thank R. Regoes, C. Wiuff, and D. Rozen for helpful comments. Supported by NIH grants GM33782 and AI40662 and by the British Wellcome Trust (IPRAVE project).



**Bugs bite back.** Computer simulations map changes in the density of a viable bacterial population exposed to an antibiotic. (A) Kill curve over time for a sensitive bacterial population confronted with a constant concentration of a bactericidal antibiotic: (1) the exponential decline we would like, (2) the declining rate of mortality and tail-off that actually occur. (B) Simulation of antibiotic treatment of a bacterial infection with the kill curve depicted in (A) but with an exponentially decaying antibiotic that is periodically replenished by a fixed dose every 8 or 12 hours: (3) no phenotypic switching, (4 and 5) phenotypic switching with antibiotic doses administered every 12 and 8 hours, respectively.

cell division by inactivating a penicillin-binding protein, thereby activating the DpiBA two-component signal transduction system that regulates not only transcription but also DNA replication. The result is the production of an ampicillin-tolerant subpopulation of bacteria that is functionally similar to the persisters observed by Balaban and colleagues.

Although both sets of experiments used *E. coli* and ampicillin, I don’t believe the two studies present different aspects of a single process. Rather, I propose that they describe two mechanisms that could operate synergistically to produce the same result: bacterial subpopulations that are tolerant to antibiotics. In accord with the phenotypic switch hypothesis (but unlike SOS induction), tolerant subpopulations are generated in the absence of ampicillin. Whether these processes are in fact different could be tested genetically by ascertaining whether the

any perspective, such characters raise important as well as intriguing questions.

Although the phenomena of bacterial persistence in vitro and antibiotic tolerance in vivo are well established (12), they have been largely ignored in the “rational” design of antibiotic treatment protocols (13). In theory, the declining rates of mortality and the “tail” observed in antibiotic kill curves (see the figure, panel A) could have a substantial effect on treatment efficacy. Initially, the density of a susceptible bacterial population is cut back by the antibiotic treatment, but, as time elapses, increasing fractions of the population become refractory to that drug. When the concentration of the antibiotic wanes between doses, the bacterial population becomes increasingly sensitive, but that may not be sufficient for the antibiotic to clear the infection. This can be seen with a computer simulation of a simple model of antibiotic treatment (3,

# ERRATUM

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Owing to an error at *Science*, an incorrect version of this PDF, containing typographical errors, was posted when the article was first published (the print and full-text HTML versions of the article, however, are correct). The current version of the PDF corrects the errors in that original version.