their proportional representation changes as a function of numerous factors, including diet. Whole-genome sequencing of culturable components of the microbiota of study participants has confirmed that strains are retained in individuals for prolonged periods and are shared among family members.

Resilience The ability of a microbiota or microbiome to rebound from a short-term perturbation, such as antibiotic administration or an infection, is defined as its *resilience*. This capacity can be visualized as a ball rolling over a landscape of local minima; essentially, the community moves into a new state and, to recover, must move through another, unstable state. In some cases, recovery will lead to the original stable state; in others, it will lead to a new stable state, which may be either healthy or unhealthy. Changes in, for example, diet or host physiologic status may introduce alterations into the landscape itself, making it easier to move from the initial state to any one of a number of other states, potentially with different health consequences. Microbial communities in our body habitats differ widely in resilience. For example, hand washing leads to profound changes in the microbial community, greatly increasing diversity (presumably because of the preferential removal of high-abundance, dominant phylotypes such as *Propionibacterium*). Within 6 h, the hand microbiota rebounds to resemble the original hand communities. The effects of repeated hand washing still need to be defined; for example, the surface microbiota on the skin (as measured by scrape biopsies) consists of ~50,000 microbial cells/cm^2, whereas the subsurface microbiota (as measured by punch biopsies) consists of ~1,000,000 microbial cells/cm^2.

In a study of three healthy adult volunteers given a short course of ciprofloxacin (500 mg by mouth twice a day for 5 days—a regimen commonly used against uncomplicated urinary tract infections), overall gut-community configuration came to resemble baseline within 6 months after treatment cessation, although some taxa failed to recover. However, the effects of the antibiotic perturbation were highly individualized. Administration of a second course of treatment months later led to altered-community states, relative to baseline, in all three volunteers; again, the extent of the alteration differed with the individual. Crucially, as shown in this and other studies, a given bacterial taxon can respond differently to the same antibiotic in different individuals; this observation suggests that the rest of the microbial community plays an important role in determining the effects of antibiotics on a per-individual basis.

In any body habitat, the microbial-community state after disturbance may be degraded. However, this degraded state may itself be resilient, and it may therefore be difficult to restore a more functional state. For example, *Clostridium difficile* infection can persist for years. The development and resilience of a degraded state may be driven by positive feedback loops, such as reactive oxygen species cascades involving host macrophages that promote the further growth of proinflammatory Proteobacteria, as well as negative-feedback loops such as depletion of the butyrate needed for promotion of a healthy gut epithelial barrier and further establishment of beneficial members of the microbiota. Consequently, microbiota-based therapies may require either (1) the elimination of a feedback loop that prevents establishment of a new community or (2) identification of a direction for change and a stimulus of sufficient magnitude (e.g., invasion and establishment of microbes from a fecal transplant or from a defined consortium of cultured, sequenced members of the human gut microbiota; see below) to overcome the resilience mechanisms inherent in the degraded state. A critical unresolved question that especially affects infants, whose microbiota is changing rapidly, is whether intervention during periods of rapid change or during periods of relative stability is generally more effective.

ESTABLISHING CAUSAL RELATIONSHIPS BETWEEN THE GUT MICROBIOTA AND NORMAL PHYSIOLOGIC, METABOLIC, AND IMMUNOLOGIC PHENOTYPES AS WELL AS DISEASE STATES

Gnotobiotic animals are raised in germ-free environments—with no exposure to microbes—and then colonized at specific stages of life with specified microbial communities. Gnotobiotic mice provide an excellent system for controlling host genotype, microbial community composition, diet, and housing conditions. Microbial communities harvested from donor mice with defined genotypes and phenotypes can be used to determine how the donors' microbial communities affect the properties of formerly germ-free recipients. The recipients may also affect the transplanted microbiota and its microbiome. Thus gnotobiotic mice afford investigators an opportunity to marry comparative studies of donor communities to functional assays of community properties and to determine how (and for how long) these functions influence host biology.

The Cardiovascular System The gut microbiota affects the elaborate microvasculature underlying the small-intestinal epithelium: capillary network density is markedly reduced in adult germ-free animals but can be restored to normal levels within 2 weeks after gut microbiota transplantation. Mechanistic studies have shown that the microbiota promotes vascular remodeling in the gut through effects on a novel extravascular tissue factor–protease-activated receptor (PAR1) signaling pathway. Heart weight measured echocardiographically or as wet mass and normalized to tibial length or lean body weight is significantly reduced in germ-free mice; this difference is eliminated within 2 weeks after colonization with a gut microbiota. During fasting, a gut microbiota–dependent increase in hepatic ketogenesis (regulated by peroxisome proliferator–activated receptor α) occurs, and myocardial metabolism is directed to ketone body utilization. Analyses of isolated, perfused working hearts from germ-free and colonized animals, together with in vivo assessments, have shown that myocardial performance in germ-free mice is maintained by increasing glucose utilization. However, heart weight is significantly reduced in both fasted and fed mice; this heart-mass phenotype is completely reversed in germ-free mice fed a ketogenic diet. These findings illustrate how the gut microbiota benefits the host during periods of nutrient deprivation and represent one link between gut microbes and cardiovascular metabolism and health.

Conventionally raised *apoE*-deficient mice develop a less severe form of atherosclerosis than their germ-free counterparts when fed a high-fiber diet. This protective effect of the microbiota is obviated when animals are fed a diet low in fiber and high in simple sugars and fat. A number of the beneficial effects attributed to diets with high proportional representation of whole grains, fruits, and vegetables are thought to be mediated by end products of microbial metabolism of dietary compounds, including short-chain fatty acids and metabolites derived from flavonoids. Conversely, microbes can convert otherwise harmless dietary compounds into metabolites that increase risk for cardiovascular disease. Studies of mice and human volunteers have revealed that gut microbiota metabolism of dietary L-carnitine, which is present in large amounts in red meat, yields trimethylamine-*N*-oxide, which can accelerate atherosclerosis in mice by suppressing reverse cholesterol transport.

Yet another facet of microbial influence on cardiovascular physiology was revealed in a study of mice deficient in Olfr78 (a G protein–coupled receptor expressed in the juxtaglomerular apparatus, where it regulates renin secretion in response to short-chain fatty acids) or Gpr41 (another short-chain fatty acid receptor that, together with Olfr78, is expressed in the smooth muscle cells present in small resistance vessels). This study demonstrated that the microbiota can modulate host blood pressure via short-chain fatty acids produced by microbial fermentation.

Bone Adult germ-free mice have greater bone mass than their conventionally raised counterparts. This increase in bone mass is associated with reduced numbers of osteoclasts per unit bone surface area, reduced numbers of CD11b+/GR1 osteoclast precursors in bone marrow, decreased numbers of CD4+ T cells, and reduced levels of expression of the osteolytic cytokine tumor necrosis factor α. Colonization with a normal gut microbiota resolves these observed differences between germ-free and conventionally raised animals.

Brain Adult germ-free and conventionally raised mice differ significantly in levels of 38 out of 196 identified cerebral metabolites, 10 of which have known roles in brain function; included in the latter group are *N*-acetylaspartic acid (a marker of neuronal health and attenuation),