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Commentary

Smaller fleas . . . *ad infinitum*: Therapeutic bacteriophage redux

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So, naturalists observe, a flea
Hath smaller fleas that on him prey;
And these have smaller still to bite 'em,
And so proceed *ad infinitum*.

Jonathan Swift (1)

Since the discovery of the bacteriophage, by Frederick W. Twort in 1915 and Felix d'Herelle in 1917—a complicated story (2)—medical scientists have dreamed that it might be used to counter bacterial infections. That hope was the central theme of Sinclair Lewis's famous fictional romance "Arrowsmith" (3), also made memorable by Ronald Coleman's cinematic enactment of life at the McGurk (read Rockefeller) Institute in the 1920s. Martin Arrowsmith, scooped in the discovery of phage by d'Herelle's publication, leaps to a clinical trial for the control of plague on a mythical St. Hubert's Isle. But deranged by his wife's death from an accidental infection, he abandons experimental controls: "Oh damn experimentation!"; and he administers the phage to all comers. Hence we cannot know whether it played any role in the subsidence of the epidemic. Perhaps a few hundred more lives were saved by the indiscriminate distribution of the remedy; but thousands more are at risk for lack of clear scientific knowledge. And confused we are to the present day.

Nevertheless, for larger pests, biological control, by the use of hyperparasites (4), has become one of the favored methods of control. And biochemical antagonisms in the soil, the chemical competition among soil microbes, have been the successful forerunner of contemporary antibiotics (5).

In actual history, phage was studied more intensively for its role in cholera, with some hope of sanitizing contaminated water supplies, and perhaps for therapeutic intervention, even by such well-regarded investigators as Asheshov, Topley, and Wilson (6). Despite sporadic encouragements, their eventual verdict was inconclusive. Since the 1940s, antibiotics have held center stage in antibacterial therapeutics. As for bacteriophage, its study has veered towards congruency with molecular genetics and opened the door to more comprehensive syntheses of the concept of virus as a genetic entity (7, 8). So we hardly think today of bacteriophage as a parasite in its ecological relationships with bacteria, and very little is known of its role in the natural history and ecology of microbes.

Just now, Merril *et al.* (9) have again addressed one of the limitations of bacteriophage in therapeutics: its rapid uptake (and presumed disposition) by spleen cells (reticuloendothelial system; RES). This had been demonstrated by Geier, Trigg, and Merril over 20 years ago (10). Their new approach was elegantly simple: to select for phage variants that survive in the circulation of the mouse after i.p. inoculation. After 10 serial passages, mutant strains of phages λ and P22 were achieved that would sustain titers of 10^9 pfu (plaque-forming units) after 18 hr. A long-circulating variant also helped protect mice from heavy lethal doses of *Escherichia coli* administered i.p. The λ variants were found to have an amino acid substitution, Glu → Lys, in the capsid E protein.

Albeit in a rather artificial infection model, this is an ingenious surmounting of one of the hurdles to the use of phage in therapy. Needless to say, many new questions are opened up. What is the uptake mechanism for wild-type phage that has been bypassed by this mutation? The entrapment occurred even in germ-free mice presumably unburdened by immune reactions. Does it nevertheless involve autoantibodies or other humoral mediators? The capsid mutation evidently does not interfere with the phage life cycle *in vitro* or in the mouse: what sustains the wild-type, RES-vulnerable phenotype in nature? Will many phages be found to occur naturally that evade the RES? Or is RES uptake a hint of viable intracellular reservoirs of phage in nature, perhaps in the soil amoebae that harbor *Legionella* and other bacteria (11)? Going beyond RES tolerance, their work may stimulate broader renewed interest in the prospects for phage therapy, perhaps emulating their approach of getting assistance from contrived natural selection to achieve the best strains.

A number of intrinsic difficulties also quickly come to mind—above all, that the phages will promptly induce neutralizing antibodies interfering with attack on bacteria, and opsonins that will restore their vulnerability to the RES. With enteric pathogens, probable prior exposure is also likely to accelerate an immune reaction to therapeutic phage, and the possible toxicity of phage–antibody complexes will have to be watched. With most phages, bacterial mutations for resistance occur fairly frequently: these might be delayed empirically by the use of a cocktail of phages with different bacterial receptors. In these days of genetic engineering, other tricks come to mind.

Phages will certainly not diffuse as readily from the circulation and through infected tissue spaces as do lower-molecular-weight antibiotics. Besides mechanical barriers, tissue components and breakdown products may also interfere with rapid phage action. The authors have already commented on the need to separate the phage from toxic by-products and phage-gene-controlled toxins.

Weighing against all these anticipated obstacles is the idea of harnessing the host specificity and rapid, exponential proliferation of the phage as a therapeutic intervention. These studies also remind us how little is known of the interaction of bacteriophages with the mammalian host, even while they are universally present together with their bacterial hosts in the lower gut. Phages are commonly found in bovine sera used for cell culture (12), and will then potentially contaminate vaccines subsequently harvested from these cultures. The provenience and biological consequence of these phages need more study. Along with the excitement of a new approach to antibacterial therapy, at a time when many antibiotics have run out, should be a renascence of study of bacteriophages as smaller fleas on their bacterial hosts in nature. Better hints as to the utility of phage in therapy might come from observations of the natural history of bacterial infections where phage might play a role, a subject scarcely mentioned since d'Herelle's time. We are also reminded of another set of medium-sized fleas, the *Bdellovibrios* (13) which are bacteria parasitic on other bacteria.

Abbreviations: RES, reticulo-endothelial system; IP, intra-peritoneal; pfu, plaque forming units.

What are the most likely targets for the application of phage therapy? We think, first of all, of organisms recalcitrant to the usual antibiotics or species in which high-level resistance has emerged (14) and is being promiscuously disseminated by plasmid vectors. Here some pseudomonads and other opportunistic invaders are notorious. Then there are settings in which individual therapy may be less than feasible, or too costly. If the transmission of cholera or dysentery could but be mitigated, the refugee camps bordering Rwanda might be less terrifying. Yes, d'Herelle had enunciated these dreams (15), and today we are somewhat put off by the anecdotal quality of the evidence that he assembled. Even so, it is ambitious but not preposterous to suggest that newer knowledge might yet engender some workable weapons for the medical armamentarium.

Moving from pestilence to famine, a more likely arena for early application is in agriculture, to deal with bacterial infestations like citrus canker (*Xanthomonas citri*) and Erwinia fire blight. There have already been suggestions that the synecology of bacteriophage and bacterial pathogens may account for fluctuations in their outbreaks (16, 17). Genetic engineering has been applied to the refinement of entomophagous viruses (18) and of bacteria to produce insect toxins (19) but not yet to bacteriophage control. An almost unknown terrain is occupied by fungal pests, which have the potential to wipe out a year's crop of potato, maize, or wheat, and have done so with historic consequences. Virus-like agents are known in many fungi (20), but they are not (or are not readily) now transmitted by horizontal exogenous cross-infection (21): there would be a worthy challenge to contemporary biotechnology. Hopefully that would also be accompanied by the most prudent inquiry about what might go wrong, but the long-term prospects of earth's food supply are not so robust that we can afford to ignore such opportunities.

1. Swift, J. (1942) in *A New Dictionary of Quotations on Historical Principles from Ancient and Modern Sources*, ed. Mencken, H. L. (Knopf, New York), p. 1712.
2. Duckworth, D. H. (1976) *Bacteriol. Rev.* **40,** 793–802.
3. Bloom, H. ed. (1988) *Sinclair Lewis's Arrowsmith, Modern Critical Interpretations* (Chelsea House, New York).
4. Sullivan, D. J. (1987) *Annu. Rev. Entomol.* **32,** 49–70.
5. Waksman, S. A. (1954) in *My Life with the Microbes*, (Simon & Schuster, New York), p. 364.
6. Wilson, G. S. & Miles, A. A., eds. (1955) *Topley & Wilson's Principles of Bacteriology and Immunity* (Arnold, London), p. 1106.
7. van Helvoort, T. (1996) *Am. Soc. Microbiol. News* **62,** 142–145.
8. Kellenberger, E. (1995) *FEMS Microbiol. Rev.* **17,** 7–24.
9. Merril, C., Biswas, B., Carlton, R., Jensen, N. C., Creed, G. J., Zullo, S., Adhya, S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 3188–3192.
10. Geier, M. R., Trigg, M. E. & Merril, C. R. (1973) *Nature (London)* **246,** 221–223.
11. Cirillo, J. D., Falkow, S. & Tompkins, L. S. (1994) *Infect. Immun.* **62,** 3254–3261.
12. Merril, C. R., Friedman, T. B., Attallah, A., Geier, M. R., Krell, K. & Yarkin, R. (1972) *In Vitro* **8,** 91–93.
13. Tudor, J. J., McCann, M. P. & Acrich, I. A. (1990) *J. Bacteriol.* **172,** 2421–2426.
14. Tomasz, A. (1994) *N. Engl. J. Med.* **330,** 1247–1251.
15. d'Herelle, F. (1926) *The Bacteriophage and Its Behavior* (Williams & Wilkins, Baltimore), translated by Smith, G. H., p. 629.
16. Erskine, J. M. (1973) *Can. J. Microbiol.* **19,** 837–845.
17. Alippi, A. M. (1989) *Microbiologia* **5,** 35–43.
18. Tinsley, T. W. (1979) *Annu. Rev. Entomol.* **24,** 63–87.
19. Kirschbaum, J. B. (1985) *Annu. Rev. Entomol.* **30,** 51–70.
20. Wang, P. & Nuss, D. L. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11529–11533.
21. Ghabrial, S. A. (1994) *Adv. Virus Res.* **43,** 303–388.