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Phage Therapy: Emergent Property Pharmacology

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Abstract

Phage therapy is the application to bodies of bacteriophages – the viruses of bacteria. This is done to combat bacterial infections. In this article we combine three themes, phage therapy, pharmacology, and the concept of emergent properties. We do this to explore what is unique along with what is not so unique about phage therapy, as viewed from a pharmacological standpoint. At the same time, we consider the place of phage therapy, and drugs generally, within the larger scheme that is biology. We make these latter considerations predominantly in terms of emergent properties, which are system characteristics that exist, qualitatively, at 'higher' but not 'lower' levels of organization. The latter is a relevant consideration especially in exploring the pharmacology of 'living' antibacterials, as are phages, which at lower levels can be viewed as collections of numerous molecules and biochemical pathways but which at higher levels become organized into somewhat complex entities that are capable of 'self amplification'. Emergent properties are also prominently relevant to pharmacology, more generally, as they give rise to requirements for animal along with clinical testing to identify otherwise unexpected drug characteristics. Our broader goal is to stimulate a better integration of issues of phage therapy and pharmacology, predominantly in light of the substantial complexity of phages in comparison with chemical drugs and towards furthering the rational development of phage-based antibacterial strategies. Our chief conclusion is that while phages, due to their complexity, potentially possess numerous emergent properties when compared with less-complex antibacterials, in fact other aspects of phages – including their relative benignness, environmental ubiquity, and that their antibacterial properties are products of evolution – can allow for certain simplifications to pharmacological development. These simplifications are not as readily achieved by chemical drugs, such as antibiotics, which can possess negative properties that are difficult to predict.

Keywords: Antibiotics; Antibacterial agents; Emergent properties; Phage therapy; Pharmacology

Introduction

"In complexity there is simplicity." (about 40 Google hits)

Biological control, or biocontrol, is the application of specific types of organisms, to environments, to combat other types of organisms. One of its earliest forms involved the introduction of pathogenic organisms into naïve human populations, that is, biowarfare [1,2]. A less belligerent and perhaps even older approach is the application of diseases or predators into populations of nonhuman targets, such as the keeping of cats for the sake of rodent control [3]. Alternatively, biocontrol includes the application of the viruses of bacteria – known as bacteriophages or phages – into populations of unwanted bacteria, so-called phage therapy [4]. In this article we consider explicitly the use of phages to combat bacterial diseases associated with humans or animals, phage therapy *sensu stricto*, reserving the term biocontrol for all other phage uses as antibacterial agents [5].

A large number of reviews have addressed phage therapy generally as well as historically; see Abedon and Thomas-Abedon [6] along with Abedon et al. [7] for references. Here we focus less on the actual practice of phage therapy, or its history, and more on how the use of phages to combat bacterial infections can be considered in pharmacological terms. Since phage therapy, as well as biocontrol in general, is a form of applied ecology, we additionally integrate these ideas into a more general biological framework, focusing on the concept of emergent properties. This article also can be viewed as an extension of a number of recent efforts to consider the biology of phage therapy from a pharmacological perspective [5,6,8-11].

Overall, the use of phages as antibacterial agents can be contrasted with that of antibiotics. An urgency to these considerations exists because, for various reasons, antibiotics as antibacterial agents appear to possess less utility [12,13] or benignness [14,15] than once had been expected. For example, "at least 25,000 patients in Europe die per year because their bacterial infections are not treatable with available antibiotics" (p. 68) [12]. As possible solutions to this world-wide 'antibiotic crisis', phages – as semi-autonomous genetic entities which, to a first approximation, would appear to be too complex to develop as antibacterial "drugs" – might not seem promising. Conversely, in practice phages appear to be both efficacious and relatively easily developed, including within the context of human treatment [7,16]. In this article it is this paradox in particular that we seek to address. That is, how does what would appear to be an extreme in pharmacological complexity instead beget a relative pharmacological simplicity? To address this question we begin by defining the concept of emergent properties, indicating how this notion is relevant to pharmacology in general and phage therapy pharmacology in particular.

Emergent Properties

"In drug development, emergent properties can be thought of as contributing to the high failure rate of new chemical entities in clinical trials. The behaviour(s) of most candidate drugs in humans is not predicted by their activities in the assays used in typical drug discovery

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efforts, explaining, in large part, the high failure rate of new drugs in clinical testing." Berg et al. [17], p. 204.

The goal of pharmacological research is the development of safe and effective drugs that are useful in the treatment or prevention of disease. Though to a degree these efforts can be theory driven, in practice pharmacology requires substantial empirical testing, including in animals along with human patients. Animal testing as well as subsequent clinical trials are important because pharmacological data from chemical assays, from *in vitro* models, or even from complex systems biology approaches – given less than complete knowledge – cannot effectively capture all emergent properties associated with the systems that drugs interact with [18-20]. As a consequence, whether or not a candidate drug becomes clinically useful is dependent upon factors that are not readily predicted. These factors instead are discovered, often at great expense, in the course of both animal and human testing. A key bottleneck in pharmacological development thus is the potential for drugs in combination with biological systems to display emergent properties.

Emergent or "nonreducible" properties [21] can be defined as higher-level aspects of systems that, qualitatively, are not directly exhibited by lower-level components of the same systems. These properties *emerge* because the resulting higher-level features are not apparent within lower-level aspects until those components interact as a more cohesive whole. Salt [22], considering emergent properties as an ecological concept, provides this definition (p. 145), "…the property of the whole is produced by properties of the parts but is not qualitatively similar…", which is a quote of Harré [23].

Ecological properties in particular emerge from an organism's interaction with its environment, and among environmental components are those aspects that enter into an organism's body from the outside world. The field of ecophysiology considers explicitly how these extraorganismal factors impact the organism internally, that is, physiologically. Body-drug as well as body-toxin interactions equivalently can also be viewed as inherently ecological and thereby at least potentially possessing emergent properties. Drugs, as extraorganismally sourced 'environmental' components, in other words, often have substantially different properties as viewed chemically within the laboratory, or even within the context of complex *in vitro* or *in silico* models, versus physiologically within experimental animals or human patients.

Appreciation of emergent properties typically requires an empirical component inasmuch as emergent properties, essentially by definition, are difficult to predict; this especially is observation biased towards more-complete assemblages, such as approximations of whole ecosystems, versus analyses of system components as they may be studied in relative isolation. Thus, in Salt's own words (p. 145): "'An emergent property of an ecological unit is one which is wholly unpredictable from observation of the components of that unit.' The corollary is: 'An emergent property of an ecological unit is only discernable by observation of that unit itself.'" Furthermore, the idea of *emergence* can be contrasted with that of *collective* [22]. Pharmacologically, additive drug interactions might be deemed collective ('sum of the parts') whereas, for example, synergistic or antagonistic interactions between drugs – which typically represent far more complex phenomena, that is, more, or less, than the sum of the parts – we could describe instead as emergent.

Drug-body interactions can be difficult to predict in part because of the complexity of animal bodies but also because of the variance in characteristics that exist between individuals. New understandings of body systems as a consequence can 'emerge' upon animal or clinical testing. Antimicrobial drugs take this issue to an additional level. Here not only is complexity and variability inherent in the larger organism but so too the properties of target parasites, or pathogens, are complex. Thus, it is difficult to fully anticipate an antimicrobial drug's impact on the body, such as its toxicology, or the body's impact on the drug (e.g., its rate of clearance). So too one must study living microorganisms, rather than just their components (e.g., genes), to ascertain a drug's antimicrobial properties even in greatly simplified environments such as test tubes or Petri dishes. Bacteria present within bodies, for example, are often able to resist antibiotics upon formation of biofilms, that they nonetheless are sensitive to *in vitro* [24]. More generally, bacteria are able to evolve various biofilm-independent mechanisms of antibacterial resistance including those resulting from horizontal gene transfer [13,25]. As a consequence, while pharmacology to a substantial extent can involve the elucidation of drug-body emergent properties, such emergent properties can be prevalent particularly among antimicrobial drugs.

An additional level of emergence can occur when the "drug" itself is also somewhat complex. One sees this with the use of live-attenuated vaccines (e.g., MMR) as well as whole-killed vaccines, particularly against the highly variable influenza virus. These reagents are not just interacting with living organisms – directly with ourselves and indirectly with would-be infecting pathogens – but themselves are organisms or at least somewhat intact derivatives of organisms. Our ability to predict the physiological impact of these entities on humans, from knowledge of their chemical composition alone, is quite small. Vaccine development, like pharmacology in general, therefore possesses a substantial empirical component: At best we apply educated guesses to drug design, and then always brace ourselves for the *emergence* of unexpected properties upon animal and clinical testing. Another category of live as well as naturally attenuated "drugs" [10] are a group of viruses, called phages, which can be used as antibacterial agents in the guise of what is known as phage therapy.

Phages

Phages are viruses that infect members of domain *Bacteria*, which contains all known pathogenic bacteria [26]. The majority of phages are both lytic and tailed, the former a description of how phage virions are released from infected bacteria and the latter a description of virion morphology. A tail is a capsid appendage that is responsible for virion adsorption along with certain aspects of DNA translocation into bacterial cells. Furthermore, all tailed phages have double-stranded DNA genomes. In lytic infections, mature phages are released from infected bacteria via degradation of the bacterial cell envelope. In the course of this lysis the infected bacterium is physiologically and to a large extent also structurally destroyed. See Figure 1 for summary of selected characteristics of various phage types.

The problem of lysogeny

Many lytic phages are also temperate. In isolating *Staphylococcus aureus* phages for biocontrol purpose from dairy products, for example, García et al. [27] reported that eight of the eight phages obtained by plating a milk-derived enrichment culture without explicit bacterial

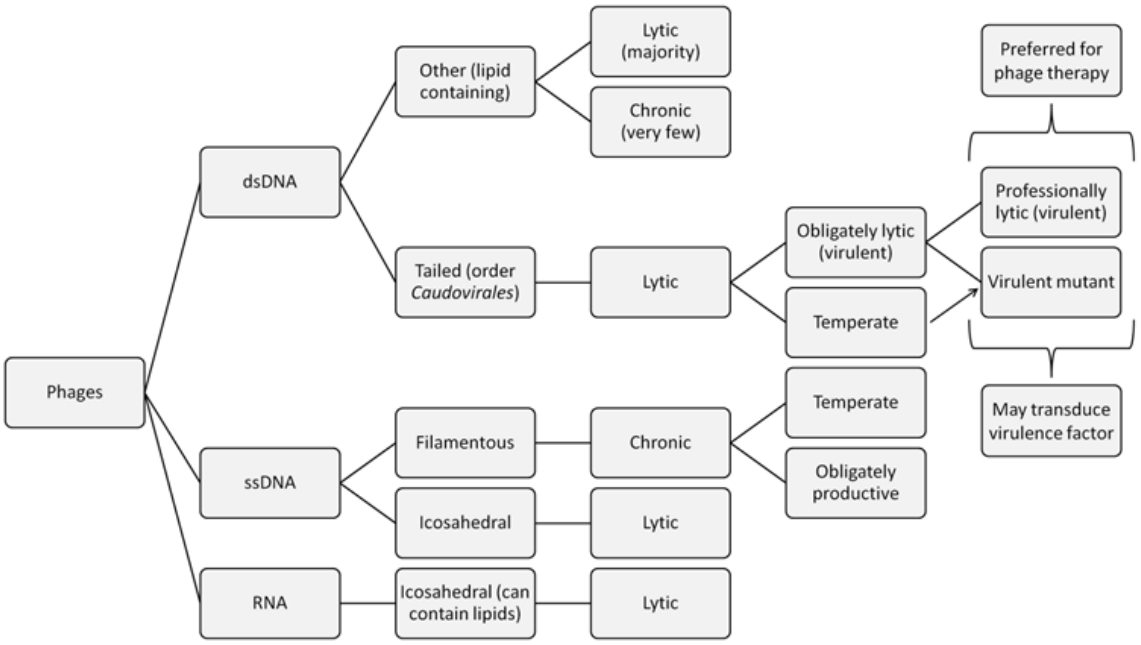

Figure 1: Types of phages. Phage therapy, as indicated, is preferably achieved employing professionaly lytic phages, that is, phages that are both obligately lytic and otherwise not substantially genetically similar to temperate phages. The arrow between "Temperate" and "Virulent mutant" is meant to indicate descent while "Virulent mutant" as well as "Professionally lytic" are both types of "Obligately lytic" phages.

induction were temperate. A temperate phage is able to display lysogenic as well as productive cycles. In a productive cycle, phage progeny are produced and subsequently released, such as through the above-described lytic process. In a lysogenic cycle, phages persist mostly as genetic material, termed prophages, replicating intracellularly in tandem with their bacterial hosts.

Prophages, through the process of induction, can also display productive cycles. Indeed, a majority of the infections initiated by temperate phages give rise to lytic, productive cycles rather than lysogenic cycles. Temperate phages nonetheless are problematic for phage therapy for a number of reasons, and consequently should be avoided for that purpose. These reasons include the display of what is known as superinfection immunity [28], which in practical terms means that bacteria that are lysogenically infected (a.k.a., which are lysogens) can no longer be killed by the same phage types. It is as though the majority of an applied antibacterial were to succeed in killing the encountered bacteria but with some fraction instead providing an 'antidote' or 'vaccine' to the bactericidal activity.

Many temperate phages in addition carry genes that encode bacterial virulence factors including such infamous exotoxins as diphtheria toxin, cholera toxin, and Shiga toxin [29]. One can mostly avoid virulence factor genes by limiting phage choice for phage therapy to what can be described as "professionally lytic" phages [30]. These, defining the term narrowly, are phages that not only are "obligately lytic" (that is, unable to display lysogenic cycles) but which also have not recently descended from temperate phages. In phage therapy, the phages of choice tend to be professionally lytic as well as tailed. See Figure 1 for summary.

Phage environmental microbiology

Any description of phages would be remiss if it did not mention their ubiquity. This issue is relevant to phage therapy pharmacology in the sense that most substances that we live our lives awash in tend to be relatively benign, with phages seemingly *not* an exception. It has been estimated based on combinations of known phage prevalence, along with considerations of likely ratios of phages to bacterial hosts within environments, that perhaps 10^{31} or 10^{32} phage virions exist on Earth at any given moment, e.g., as discussed in Abedon [31]. This means that any time that one goes swimming in either the ocean or a less than oligotrophic lake, then a million or so phages are also bathing us for every ml surrounding our bodies [32]. A liter of water can easily contain a billion individual virions! Further, food, including especially bacteria-fermented products, e.g., [33,34], tend to be full of phages, as too can animal bodies, in particular colons [35]. We are born into a world of phages and develop through our adulthoods experiencing a constant phage barrage. Indeed, animals have been so exposed presumably for as long as animals have been in existence, with phages an unseen and at times underappreciated but nevertheless omnipresent aspect of our world.

Phage Therapy

Phage therapy is the application of phage virions to bacteria-containing environments with the goal of bacterial control. In most instances with phage therapy "control" translates as "kill" and the bactericidal nature of obligately lytic phages can be well suited to this task. Phage therapy, strictly, is a form of biocontrol, with one organism (here, phages) employed to control another, typically pathogenic or nuisance organism (here, bacteria). We concentrate in this article on issues relevant to the phage therapy of animals, particularly as may be applied to humans [7,16]. Others have considered, by contrast, the phage-mediated biocontrol, for example, of foods [36,37], of food animals such as to reduce loads of zoonotic pathogens [38], or of plants [39].

Active versus passive treatments

Phage treatments whether phage therapy or more generally biocontrol can be distinguished into two basic types. These are those treatments that are dependent on phage *in situ* replication to achieve bacterial control and those that do not require such replication. The former has been dubbed an "active" treatment after the idea that it requires "active" phage replication to be effective; see Abedon and Thomas-Abedon [6] for discussion. By contrast, phage therapy that does not require active phage replication has been dubbed passive or, alternatively, inundative phage therapy.

Though seemingly a somewhat intimidating approach to antibacterial therapy, in fact inundative treatment is the phage equivalent of conventional antibiotic therapy, i.e., where enough drug is supplied via traditional approaches to dosing that bacterial infections are brought under control. In any case, unless phages are either engineered to kill bacteria without producing infectious phage particles [40], or otherwise are unable to reproduce upon infection of bacteria due to limitations in their 'spectrum of activity', then phages should still display some degree of *in situ* "self amplification" or "auto dosing" [41] in the course of their antibacterial action.

Phages as emergent property pharmaceuticals

Note that the property of phage replication, in the course of their antibacterial action, can be considered to be emergent since it is a phage aspect that would not be easily predicted were we to analyze phage components, such as individual proteins and nucleic acids, solely in isolation. Indeed, numerous genes are found in the genomes particularly of larger phages for which no function has been assigned, e.g., Hendrix [42], but which in many cases presumably can contribute to phage population growth in some manner. Nevertheless, the phage property of replication and subsequent population growth is an *expected* consequence of phage-bacterial interactions given our understanding of phage biology. *Pharmacologically*, therefore, phage population growth is *not* an emergent property in the sense of requiring association with bodies to become apparent any more than the antibacterial activity of most chemical antibiotics is manifest solely within the context of treating bacterial infections within animals: Bacterial killing and phage amplification are both readily observed outside of the body so therefore do not emerge *pharmacologically* within bodies.

Absence of phage replication or antibacterial action, *in situ*, could be described as pharmacologically emergent—a pharmacodynamic property that *emerges* only upon characterization of the larger system. Here this larger system would be phages plus target bacteria plus the body environment rather than phages plus target bacteria within a simpler laboratory environment. See, for example, the results of Bull et al. [43] as well as Abedon [10], along with references therein cited, for a broader discussion of what can 'go wrong' in terms of bacterial killing during phage therapy animal testing. To clarify: Phage replication when in the presence of target bacteria certainly can be viewed as a phage 'behavior' that emerges from the lower-level aspects of phage biochemistry, but that replication is less emergent in pharmacological terms because it is something that is expected to occur prior to the performance of animal or clinical testing.

On the one hand, phages within the context of phage therapy may thus be considered to be emergent property pharmaceuticals, given the impressive complexity from which their population growth and even antibacterial activity in fact emerge. On the other hand, and a prominent theme within this article, the chemical complexity of phages as "drugs" – that is, the multitude of genes and gene products that together make up phages – in certain ways conspire to *simplify* rather than *complicate* their pharmacological properties. Such pharmacological simplification, as we will consider, may be most prominently observed from the perspective of phage safety.

Phages are used therapeutically mostly "off label"

In principle phage therapy can be used against any bacterial disease, though in practice the diseases that are targeted tend to be ones against which alternative treatments are less available. This, particularly in Western medicine, can be against antibiotic-resistant bacteria. In the former Soviet Union, by contrast, phage therapy has been used against bacterial infections in general, owing to a tradition of phage use that developed in that country prior to widespread antibiotic availability or, for that matter, before the development of a robust understanding of phages as biological entities. In Poland phages have been employed on an "experimental" basis to treat chronically infected wounds that have not responded to conventional antibiotic treatment. What is fascinating about these efforts is that they seemingly have been quite successful and, especially in the former Soviet republic of Georgia as well as Wrocław, Poland, have been ongoing. Reviews by Kutter and colleagues [7,16,44] have explored these various international aspects of phage therapy use.

Though phage therapy has been employed extensively outside of North America, the technique has *not* been subject to a great deal of double-blind clinical trials. Phages consequently, as a class of "drugs", have been employed almost entirely "off label". Widespread double-blind clinical testing of phage therapy likely will only happen given substantial increases in the availability of resources to the field. In addition, phage therapy, as a field, appears to be hampered by a variety of peculiar issues. First is unfamiliarity with phage use by Western physicians – which is not to say that there is a lack of Western physicians who are enthusiastic of the idea, but instead that obtaining and then using phage therapeutics is difficult in environments where their use effectively is not even off label. A second issue is the question of intellectual property rights and particularly the patenting of technologies that have been in existence for nearly 100 years. Furthermore, in those countries most familiar with phage therapy the therapeutic use of phages already represents a standard of care, a situation that for ethical reasons can deter double-blind study.

Notwithstanding these considerations, it is our opinion that it would be helpful to the field to adopt a more pharmacologically rigorous perspective on phage therapy research and development. Though various principles of phage therapy pharmacology have been considered elsewhere [5,6,8-11], here we consider those principles particularly in light of constraints that are imposed on pharmacological development by drug emergent properties. See also Table 1 and [41] for discussion of advantages of using phages as antibacterial drugs including in comparison to chemical antibiotics.

Toxicity and Side Effects

Toxicity or side effects can be described as negative pharmacodynamic consequences, contrasting the directly sought after positive pharmacodynamic effects such as bacterial control. Pharmacodynamically, therefore, the key phage characteristic that contributes to the potential for phage therapy to serve as an alternative to antibiotic treatment, other than the phage ability to kill bacteria, is the generally low toxicity of especially professionally lytic phages. The

Property[2]	Comment
Bactericidal	Reduced potential for bacterial development of resistance
Auto "dosing"	*In situ* activity increases numbers (though only given sufficiently high bacterial densities)
Low inherent toxicity	Virions consist of only proteins and DNA
Low normal flora impact	Low likelihood potential for superinfection by endogenous flora, e.g., *C. difficile*
Narrow resistance evolution	Selection for resistance limited mostly to within populations of targeted bacteria
Lack of cross-resistance	Antibiotic-resistant bacteria tend to retain phage sensitivity
Rapid discovery process	Phages with large therapeutic windows[3] are often simple to isolate
Potential for modification	Phages can be easy to molecular characterize and manipulate
Use with other agents	Versatility in formulation development and combination with other drugs
Dosing versatility	Phage formuations can take many forms and can be delivered via many routes
Biofilm clearance	Certain phages, unlike most chemical antibiotics, can be relatively good at this
Favorable pharmacokinetics	Delivery to targets or persistence *in situ* often is either good or improvable
Single-dose potential	Can provide dosing convenience; this is an auto-dosing consequence
Low-dosage potential	Of possible economic or safety utility; this is an auto-dosing consequence
Single-hit killing kinetics	But nonetheless effectively multi-hit since phages still multiply adsorb bacteria
Engineered lower toxicity	Particularly elimination of bacterial lysis, but auto-dosing advantage as a result is lost
Low environmental impact	Due to a combination of narrow spectrum of anti-bacterial activity, lability, and low inherent toxicity
Not antibiotics	Fewer societal concerns with use, such as in agriculture; avoids antibiotic allergies
Natural products	Potential appeal to natural medicinals market
Relatively low cost	As drugs, reasonable production costs
Public perception	Public perception of the use of phages as antibacterials seemingly is positive

[1]Advantages are as seen relative particularly to the use of chemical antibiotics as antibacterial agents.
[2]Line break between upper and lower portions of the table is indicative of what we feel are greater (upper) versus lesser (lower) advantages to phages as antibacterials.
[3]Ratio of drug dosage that gives rise to toxic effects to that which gives rise to efficacy. The larger the ratio, the safer the drug and/or the easier it is to work with.

Table 1: Advantages Associated with Phage Use as Antibacterials[1].

advantage of low-toxicity pharmaceuticals is not just their safety but also a freedom from patient monitoring to prevent toxicity as well as an ability to lengthen intervals between dosing, which together can greatly reduce costs while increasing both patient convenience and compliance. Low toxicity also can allow *in situ* levels to exceed a drug's minimum effective densities, resulting in antibacterials with increased potential to control infections. This means that the phage ability to replicate *in situ* in the course of killing bacterial targets can be a dosing ally, allowing phage densities to increase precisely at their site of action and do so without simultaneously serving as a toxic hindrance.

Pharmacokinetics, contrasting pharmacodynamics, describes the impact of the body on drugs. Low toxicity can result in a reduction in certain pharmacokinetic concerns since higher doses, or phage self amplification, can be used to counter mechanisms of drug loss or dilution in the course of what is known, pharmacokinetically, as drug absorption and distribution. More generally, toxicity and side effects represent the most troubling types of pharmaceutically emergent properties and their relative absence would presumably simplify pharmacological development. It is somewhat surprising therefore that phages, with their multitude of *biologically* emergent properties, can lack many of the side effects observed with seemingly simpler, *chemical* antibacterial drugs. We thus begin our discussion of pharmacology as it applies to phages by focusing on phage safety since much of how phages are or could be used as pharmaceuticals derives from this property.

Toxicity as well as phage-immune system interactions

Drugs can be mutagenic or teratogenic, they can activate or inactivate receptors, they can be cytotoxic or mimic hormones, etc. Given appropriate phage choice, which generally means employing only professionally lytic phages for phage therapy (Figure 1), then what are presented to bodies by phages are mostly proteins and DNA that display little toxicity to body tissues. An alternative perspective is that in order for phages to display an evolved toxicity to human tissue they would have to be able to interact with human cells similarly to how they interact with target bacterial cells. Inasmuch as the processes inside a human cell are vastly different from the ones occurring inside a bacterial cell, such tissue toxicity generally is not even a potential concern nor has it been demonstrated to be upon empirical observation; for review of phage-human- or animal-body interactions, see Merril [45].

Though not necessarily acting directly as toxins, protein-based drugs still can interact with host immune systems, in some cases to disastrous effect [46]. More common is simply the production of anti-drug antibodies (ADAs). ADAs are usually benign, but when protein-based drugs resemble endogenous molecules then ADA cross reactivity and subsequent auto-immune response can result in severe complications [47]. As phages typically are *not* expected to be similar to endogenous molecules, and in fact are typical components of animal environments, such complications would be expected to be less likely in phage therapy application. Low likelihoods of complications stemming from phage-immune system interactions could be due, perhaps, either to bodies acquiring a tolerance to ubiquitous phages found in their environments or, instead, to phage types that are normally found as animal commensals evolving to minimize their potential to destructively impact their overall environment, i.e., our bodies. This low likelihood of complications, however, is dependent on phage choice, that is, the use in phage therapy of professionally lytic phages and/or phages that have been shown to not carry bacterial virulence factor genes.

In practice there has been little documentation that phages used therapeutically will produce substantial side effects, or even reductions in phage therapy effectiveness due to phage interactions with the adaptive immune system [7]. At least in part this general lack of evidence of negative phage-body interactions may stem from typical phage application practices, which is topical for chronic infections,

or systemic use over only relatively short spans against more acute infections. That is, the impressive extent to which phages appear to safely serve as antibacterial agents upon application to bodies may not be a wholly unexpected consequence of phage-patient interaction [45]. Safety as well as efficacy, however, has been achieved even given treatments of chronic wounds over substantial spans of time, such as many weeks [48-50].

Bacterial dysbiosis

Besides direct interaction with animal tissues, including immune systems, a second means by which an antibacterial can negatively impact treated individuals is via the destruction of non-target bacteria, particularly beneficial members of normal flora. The loss of these non-target bacteria can result in antibiotic-associated superinfections such as vaginal yeast infections or *Clostridium difficile*-associated colitis. Generally this kind of side effect is more likely to occur when broad-spectrum rather than narrow-spectrum antibacterials are employed. Most phages, though, display narrow spectra of activity [51], a property that may be of utility even among chemical antibacterials [52]. Indeed, as Blaser [15] notes (p. 394), "We… need new, narrow-spectrum antibacterial agents to minimize collateral effects on the microbiota. …and, importantly, better diagnostics that rapidly identify the problematic agent."

In considering the narrowness of phage spectra of activity, one may conceptualize the different steps of phage-bacterial interactions as "sieves" that generally result in prevention of non-targeted cell death. The first sieve eliminates all cells that do not carry the cognate receptor for the phage. The second eliminates all cells that are capable of resisting phage DNA uptake. Another can reduce the phage-mediated elimination of those cells that possess restriction enzymes against phage DNA or other mechanisms of active resistance by bacteria to phage infections [51,53-55], and so forth. Even when multiple phages are combined to form cocktails, the result typically is still an overall spectrum of activity that is no wider than that of the narrowest spectrum antibiotics currently employed clinically [11]. Phages thus may be applied even prophylactically (below) with little concern of generation of bacterial dysbioses.

Drug-drug interactions

Many drugs are metabolized in the liver by a class of enzymes known as cytochromes. When pharmaceuticals, including antibiotics, interact with these enzymes, inhibition or activation can occur. As a consequence, harmful drug-drug interactions can result, including increased toxicities that are due to a build up of drug densities within bodies following less effective drug removal or, alternatively, loss of drug potency due to too rapid elimination [56]. The characteristics of drug-drug interactions that can result from such hepatic system emergent properties can be difficult to predict or observe in the course of *in vitro* toxicity or metabolism screening. A concern with using quinolones, for instance, is that their interaction with cytochrome P450 isoform CYP1A2 decreases the liver's ability to metabolize other drugs, including antidepressants and antipsychotics [57]. If patients are not carefully monitored, then harmful levels of the interacting drug can accumulate.

For phages, to at least a first approximation, such potentially harmful drug-drug interactions should not be a concern since these same liver enzymes will not play as large a role in phage elimination any more than they do in the elimination of body proteins and DNA in general. Another way of stating this is that body metabolism of phage particles should have little impact on normal mechanisms of body homeostasis. Phages thus may be more readily used in conjunction with other drugs and not just other antibacterials but other drugs that a patient may already be consuming. We conjecture, in other words, that phage-drug interactions do not substantially give rise to emergent properties in terms of the functioning of these other drugs, though with the caveat that this issue to our knowledge has not been systematically studied.

Release of bacterial toxins

Most phages, via a complex series of steps, bring anti-cell wall hydrolases into the immediate vicinity of bacterial peptidoglycan layers. The resulting bacterial lysis results in the solubilization of bacteria-associated molecules, such as endotoxin or certain exotoxins, which can be directly or indirectly toxic to body tissues. One consequence of this lysis is that, particularly with systemic phage application, there can be a need for substantial purification of phage virions [58], i.e., as protein-based drugs in general require purification prior to use, e.g., Magalhães et al. [59].

This issue is also a concern with regard to the lysis of bacteria that can occur *in situ*, during phage therapy, and motivates occasional efforts to engineer phages that are bactericidal without being lytic [40]. Phages, though, are not unique in being lytic antibacterials since that characteristic tends to be shared with small-molecule antibiotics that target cell walls such as penicillins. As a narrow-spectrum lytic antibacterial, however, the number of bacteria lysed by phages should be fewer. Indeed, much of the complexity of phages as bacterial lysing agents can be seen as contributing to the selectivity of their lytic behavior.

This latter advantage should be directly relevant only to the extent that potentially lysed non-target bacteria are found in locations in which their lysis could be harmful to the body, particularly within the blood such as during the treatment of septicemias. It is helpful, though, to recall that it is the bacteria being treated that ultimately are the problem, more so than the impact of antibacterials employed to eliminate them. That is, the elimination of systemically infecting bacteria is not necessarily going to occur without harming the integrity of those bacteria and thereby releasing at least some bacterial toxins, though as noted phages can be engineered to reduce this damage [40].

Safety in numbers

Phages only replicate when they are efficacious and the result, from a pharmacodynamic standpoint, is a wide breadth of possible dosing ranges during phage treatment. This range extends from low doses in anticipation of *in situ* phage self amplification – which has a low likelihood of releasing toxic bacteria-associated molecules unless bacterial targeting is successful – to high doses if rapid bacterial lysis is *not* a concern and/or if phage self amplification is less likely. Notwithstanding this potential for a wide breadth of dosing ranges, a standard approach towards phage therapy dosing, for both safety and economic reasons, should be the use of relatively low phage densities, with either repeated dosing or application of greater phage densities or volumes should phage access to bacteria prove limiting. This suggestion is rather than to strive under all circumstances to inundate bacteria using substantial excesses of phages, such as $>>10^8$ phages/ml.

The relatively low toxicity of phages in combination with their potential for self amplification thus provides substantial leeway in terms of what phage densities may be applied in the course of dosing,

with neither phage densities that are potentially too low for passive treatment, such as doses with phage densities of less 10^7/ml [6,9,10,36], nor somewhat high phage densities (e.g., $>10^9$/ml) necessarily resulting in poor phage pharmacodynamic effects. Note in any case that what are being considered are phage *densities* rather than total phage numbers applied per dose. The reason for this perspective is that the goal of dosing is one of achieving sufficient densities within the vicinity of target tissues. The phage *densities* applied therefore should be viewed as limiting the maximum phage densities that may be achieved unless phage *in situ* amplification serves to boost phage *in situ* densities [9,10]. Since such self amplification in fact can occur, the result is great latitude for phages in terms of what densities may be supplied from circumstance to circumstance in the course of conventional dosing.

Less latitude exists in terms of what peak phage densities in the vicinity of target bacteria are required to achieve effective bacterial killing. Because phages display low toxicities, however, generally the problem is more one of not achieving sufficient phage densities rather than providing excessive doses. Note that sufficient peak phage densities, that is, minimally effective phage densities delivered to or generated within the immediate vicinity of target bacteria, may be assumed, as a rule of thumb, to be approximately 10^8 or more active phage particles per ml [6,9,10].

Types of Treatment

Treatments with drugs can be differentiated into a number of different approaches including prophylaxis, empirical treatment, and pathogen-driven therapy. In this section we introduce these various concepts in terms of phage therapy.

Prophylaxis

The utility of prophylaxis as a treatment approach is that it can be easier to prevent an infection rather than treat an ongoing disease. Furthermore, *in situ*-generated bacterial lysis products should be much less abundant given the associated (or expected) lower bacterial densities. Thus, phage safety should be further enhanced in primary prophylaxis, increasing the reasonableness of such an approach and indeed potentially allowing for phage use under circumstances where application of chemical antibiotics might be avoided due to the possibility of bacteria-unrelated side effects. In addition, phages tend to not interfere with wound healing, e.g., Soothill [60].

To the extent that prophylaxis is against bacteria existing at low densities, then active treatment, that is, *in situ* phage self amplification, may not be possible as threshold bacterial densities required for successful phage auto dosing will not be present [6,9,10]. Prophylactic treatment also will often be empirical (below) since the pathogen to

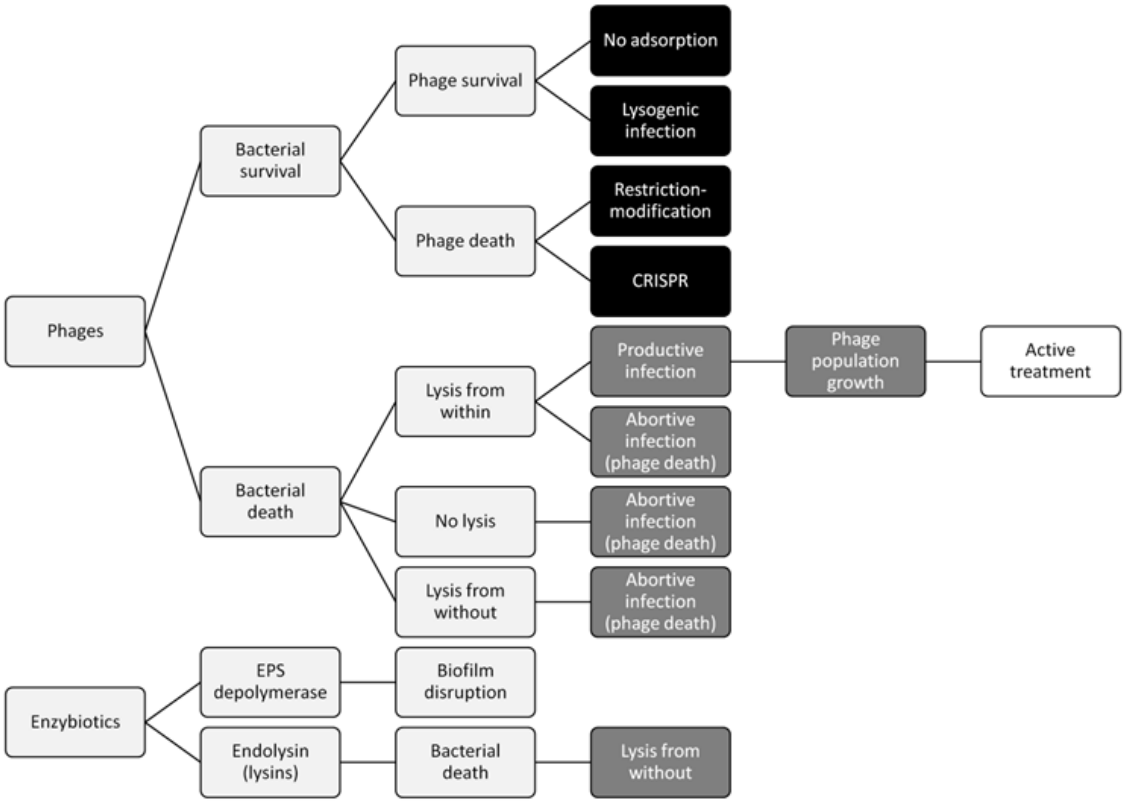

Figure 2: Phage impact on bacteria. "Phages" refers to intact phage particles whereas "Enzybiotics" are purified phage products. Treatment failure is associated with substantial bacterial survival (black outcomes with white text). Passive or inundative treatment occurs when sufficient phage numbers are provided that substantial bacterial losses are achieved without reliance on phage population growth (darker gray endpoints with white text , all but "Productive infection" and "Phage population growth", give rise to obligately passive treatments). Active treatment, indicated as a white outcome with black text, cannot substantially eradicate target bacterial populations unless sufficient phage population growth occurs *in situ*. Phage infections in which bacteria die and no phage progeny are produced we define as abortive. Note that CRISPR systems do not necessarily result in bacterial survival in combination with phage death but rather can also give rise to abortive infections [68].

which an individual might be exposed in many instances will not be known. The use of phages prophylactically nonetheless has occurred, and with success, both experimentally in animals and clinically particularly in terms of war wounds; see, for example, Abedon et al. [7] for limited review.

Empirical therapy

Empirical therapy, as we employ the term here, is the initiation of treatment prior to full diagnosis, particularly in terms of the diagnosis of antibiotic susceptibility of an infectious agent (synonym: presumptive treatment). Although phages lack the same dysbiosis or superinfection concerns of antibiotics, their spectrum of activity generally is much narrower, making successful empirical therapy by specific phages less probable. Phage cocktails – formulations containing combinations of potentially efficacious phage isolates – may help to overcome this concern. Empirical treatment, that is, is more likely to be successful to the extent that a cocktail is sufficiently well formulated to cover most bacteria that would be encountered, per infection type, in a given geographic area.

In Georgia and the rest of the former Soviet Union phage cocktails have been continuously modified in response to changing spectra of locally prevalent bacterial pathogens [7]. Though the practitioners of this approach of ongoing formulation modification certainly would appear to have found it to be beneficial, an obvious concern is that such a strategy could be inconsistent with standard approaches to drug development or regulatory approval (such as by the U.S. Food and Drug Administration). Alternatively, continuous surveillance along with periodic formulation revision is the hallmark of influenza vaccine implementation [61].

Pathogen-directed therapy

In pathogen-directed therapy the infectious etiology is not just identified prior to the initiation of treatment but also is characterized in terms of its antibacterial susceptibility. Because of phage specificity, pathogen-directed therapy is the type of treatment that best suits phage therapy. Such an approach, as typically practiced in Poland, usually employs what can be described as the phage bank approach, where isolated bacterial pathogens are tested for susceptibility to pre-prepared phage stocks [11,16]. Indeed, bacteria can be retested periodically, given extended phage treatment such as of chronic infections, with the phages employed in the treatment of specific individuals revised if there is evidence of bacterial resistance [49]. It is also possible to enrich phages from environmental samples against specific bacterial strains that have been isolated from patients, though obviously this can introduce additional delays as well as costs. The development of phage cocktails can be viewed as employing just such techniques, only performed well in advance of patient treatment [11].

Mechanisms of Action

Antibiotics possess a wide array of mechanisms of action. Penicillins inhibit cell wall formation [62], for example, while quinolones inhibit DNA gyrase [63] and topoisomerase [64] in bacteria. By contrast, phage infections are multistep processes with the genetical killing of bacteria generally occurring well prior to their physiologically termination via lysis [65-67]. In this section, for the sake of illustration of the diversity of phage antibacterial actions, we provide brief overviews of general modes of phage-mediated bacterial killing. See Figure 2 for illustration of the relationship of these different approaches to bacterial killing as seen with passive versus active treatments.

Lysis from without

Certain phages, at high multiplicities of adsorption, can display the emergent property of being able to lyse bacteria without first infecting [69]. The result can be viewed as a type of abortive infection, that is, a phage infection that results in the death of both adsorbed bacterium and adsorbing phage(s). Claims of lysis from without should always include empirical evidence that phages can lyse bacteria in a manner that (i) occurs soon after phage adsorption, (ii) does not result in phage production, and (iii) is more likely, in terms of bacterial lysis, given higher versus lower phage multiplicities of adsorption, e.g., 50 versus only 5. By contrast, and despite numerous publications which appear to suggest otherwise, simply showing that phages kill bacteria following high multiplicity adsorption does not represent evidence for lysis from without, and this is so even if phage progeny production also does not occur.

Abortive infection

When phage infection productivity is completely blocked but bacteria nonetheless die, the result can be described as an abortive infection. These can be a consequence of expression of specific bacterial anti-phage resistance genes [51,53]. Abortive infections also can result from (i) an absence in bacteria of specific factors that are required for productive phage infection in combination with (ii) a presence of those factors necessary to support phage-mediated bacterial killing. As with lysis from without, phage therapy mediated by phages that kill bacteria, but which do so abortively in terms of phage replication, by definition represents a form of passive rather than active treatment.

Though bacterial genome sequence determinations, including those of bacterial plasmids, in principle can allow identification of abortive infection-effecting genes, the interactions between bacterial and phage gene products can be difficult to predict. Thus, whether presumptive bacterial abortive-infection genes will give rise to functional abortive-infection systems, ones that block the infection of specific phage types, must be confirmed experimentally including in terms of what phages are susceptible to these systems. In practice, as with elucidation of other phage-replication- or bacteria killing-associated emergent properties, one simply tests phage isolates against bacterial strains to determine bacterial susceptibility. Due to abortive infection systems, though, it is possible for otherwise obligately lytic phages to inhibit bacterial replication while at the same time failing to productively infect those same bacteria. The potential for phage replication in the presence of target bacteria, rather than simply killing bacteria, therefore also must be experimentally determined, such as via plaque assays or broth-culture lysis, particularly following low-multiplicity phage application (Figure 2).

Lysis from within

The more typical scenario that results in the death of a bacterium, in the course of phage therapy, occurs in association with a phage lytic cycle. The resulting lysis is described as a lysis from within to distinguish it from the above-noted lysis from without [70]. Given multiple mechanisms by which phages can mortally impact infected bacteria during productive infections (above), the cell lysis event may be viewed simply as an additional layer of bacterial killing. Nonetheless, lysis from within can be an important aspect of phage therapy since

bacterial killing *without* associated progeny release, that is, absent virion release as mediated by the lysis from within of infected bacteria, is effectively an abortive infection and therefore cannot support active treatment.

Enzybiotics

Certain phage molecules that possess antibacterial activity can act from without rather than from within, that is in the absence of phage presence rather than in the course of phage infection. The harnessing of purified forms of these molecules as a means of antibacterial treatment has been described as the use of enzybiotics [71,72]. The two most prominent enzybiotics are phage endolysins and phage extracellular polymeric substance (EPS) depolymerases.

Endolysins are the enzymes that effect lysis from within. Typically, as noted, they are not what actually kills bacteria though certainly are key to bacterial destruction along with phage progeny release from infected cells. Endolysins can, however, be used in a purified form to kill especially Gram-positive bacteria, which lack the protection afforded by the Gram-negative outer membrane. This process also can be described as a lysis from without, though it is a form of lysis from without that differs from the more traditionally described phenomenon discussed above [69]. Of interest, whereas penicillin binding proteins, i.e., the targets of penicillin and related antibiotics, can be easily mutated to no longer bind penicillin while still performing their peptidoglycan synthesis function [73], the actual bonds of peptidoglycan are not as easily modified. The result is a low potential for bacterial populations to evolve endolysin resistance.

EPS depolymerases particularly affect bacterial biofilms though are not directly bactericidal [74,75]. They exert their impact on extracellular polymers associated with retaining biofilm integrity. Upon lysis, these enzymes as released from phage infections can both increase phage penetrating ability into biofilms and possibly make biofilm bacteria more susceptible to antibacterial treatment (including, potentially, to antibiotic treatment). See Figure 2 for partial comparison of enzybiotic therapy to phage therapy.

Phage Virions as Antibacterial Delivery Agents

Most targets of antibiotics are bacterial molecules that are found inside of cells. This typically means that an antibacterial, to reach its target, must be able to pass through the cell wall and/or cell membrane. The first molecule contacted by phages during the infection process, however, is found on the outside of the cell and phages subsequently have an inherent ability, within the limits of their host ranges, to penetrate through bacterial cell walls and membranes. Consequently, there is no need to design or even explicitly select phages for this ability. Rather, a phage that has been shown to replicate in the presence of a specific bacterial type too must possess a bacterial cell-envelope penetrating ability. In this section we consider this phage ability in terms of virion modularity, that is, phage possession of a delivery component (virion proteins) in combination with one or more bactericidal mechanisms (encoded by phage DNA).

Naturally occurring emergent-property antibacterial agents

It is feasible to design or discover molecules that penetrate into body compartments as well as into bacteria, ones that interfere with bacterial metabolic processes, that are relatively lacking in side effects, that can target particular species of bacteria, or are agents possessing reasonable stability during storage as well as following administration to patients. Identifying molecules that have all of these properties at once, however, can be quite difficult. This is why isolating natural antibacterial products has been a standard approach to antibacterial discovery since the introduction of antibiotics to clinical medicine. That is, antibiotics traditionally are natural, microorganism-produced molecules with an evolved ability to penetrate into and then either kill bacteria or reversibly inhibit their growth. Once discovered, antibacterial molecules are then chosen based upon their other desirable properties, as listed above. Numerous antibiotic features that are useful in terms of pharmacological functioning thus to a large extent are emergent properties which may be evident only upon application to bodies. As discussed above, however, the bacteria-killing aspects of antibiotics themselves do not generally require antibiotic-body interactions to become manifest so are not similarly emergent in the larger scheme of drug pharmacology. Indeed, useful antibiotics almost by definition are chemicals that display antibacterial activities in a manner that is somewhat independent of whether they have been administered to a body environment.

Phages, too, are evolved antibacterial agents though substantially more complex than naturally occurring chemical antibacterials. Perhaps most notably, and as mentioned above, phages are both antibacterial delivery systems (virions) and antibacterial synthesizing agents (phage infections). Phages therefore can be naturally benign until they are presented with target bacteria and, of course, are even capable of increasing in number once bacteria have been acquired. As a consequence, and despite their underlying complexity, professionally lytic phages tend to be highly safe antibacterials. In addition, from phage interactions with bacteria emerge the relatively straightforward phage properties of bacterial lysis and production of new, progeny phages, though the details of these infections themselves can be somewhat complex [76]. Natural selection, in part as a consequence of phage modularity, thus has resulted in substantial constraints on phage emergent properties, which for professionally lytic phages typically are limited to bacterial acquisition, phage progeny production, and bacterial lysis from within. These constraints exist despite or perhaps even as a consequence of the substantially greater complexity that phages display relative to naturally occurring antibiotics.

Another way of making this point is to describe professionally lytic phages as possessing relatively complex genetic programs whose primary function is the production of new phages rather than a general interference with metabolisms, other than the metabolisms of bacteria they are directly infecting (and even then, only as such interference is useful towards the endpoint of making and then releasing new, progeny virions). Furthermore, when not actively infecting bacteria, the primary phage function is one of bacterial acquisition, which is accomplished in a mostly non-metabolically active manner. Phage killing power thus is focused both metabolically and spatially on target bacteria, resulting, with professionally lytic phages, in only chance interference with animal physiologically along with seemingly little likelihood of even inadvertent adverse consequences given the non-xenobiotic nature of phage molecules. Antibiotics, by contrast, often are more general disrupters of metabolic processes and thereby can have a greater potential to disrupt human or animal physiologies.

Modularity as a simplifying characteristic

Phage properties can be distinguished into those that operate extracellularly versus those that operate instead intracellularly. The extracellular aspects determine formulation stability, most phage pharmacokinetic characteristics, and target-bacterium acquisition

properties including host-range specificity. Indeed, a phage virion in principle is simply a bacterium-acquisition devise whose sole function is the delivery of the intracellular acting agents to bacterial cytoplasms. To reach bacterial targets, by contrast, chemical antibiotics must inherently cross membranes by being relatively small and hydrophobic, have targets that are found external to membranes such as cell walls, be able to pass through porins found in the Gram-negative outer membranes, or otherwise must be associated with supplied bacteria-penetrating mechanisms. Bacteria-penetrating properties, however, are not necessarily benign within the body environment, particularly to the extent that they can also give rise to eukaryotic cell penetration or simply plasma membrane disruption. Thus, the phage potential to penetrate bacterial envelopes without first dissolving in bacterial membranes likely contributes greatly to their relative dearth of negative pharmacodynamic aspects. Phages as antibacterial agents, that is, may be relatively safe in part because they are large and complex rather than in spite of these properties.

Once DNA has been delivered to a bacterium's cytoplasm, the potential to encode and produce antibacterial agents is almost limitless.

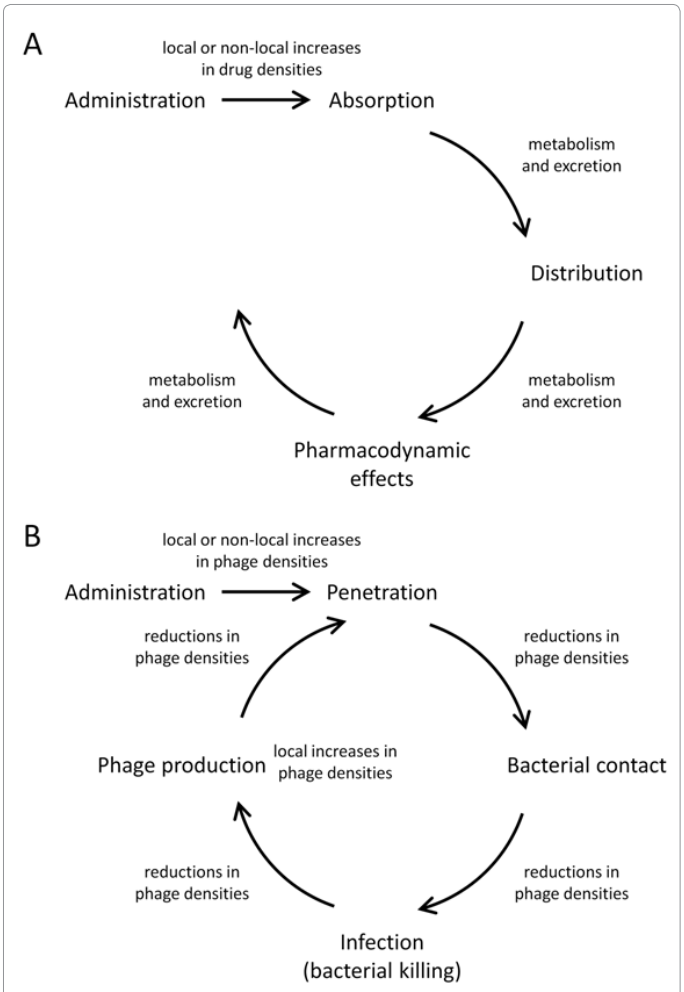

Figure 3: Pharmacokinetics basics (panel A) as compared with phage therapy basics (panel B). Phage therapy as typically practiced often differs in two basic ways from the 'life cycle' of most drugs (panel A). First, absorption, that is, uptake into the blood, is not an issue for the sake of positive pharmacodynamic effects unless systemic treatment is sought. Second, most drugs, unlike phages, do not self amplify *in situ* following contact with target tissues.

Indeed, not only can phages encode bacteria-toxic substances that further phage reproduction but so too phages can be easily engineered to produce antibacterial agents that have nothing to do with phage propagation, such as producing restriction endonucleases [77]—all while preserving their inherent delivery mechanisms. By contrast, what *is* difficult to engineer are novel antibacterial activities that do not also interfere with the production of new phage virions. Fortunately, there often is little need to improve upon the antibacterial activity of naturally occurring phages as phages with novel properties typically may be isolated in the course of standard laboratory enrichment protocols.

In addition to this separation of extracellular and intracellular properties, phages are capable of varying their properties in response to different situations such as differences in target bacterium genotype and physiology. A result of these various phage emergent properties is that it is generally unnecessary to fully characterize every phage in terms of the mechanistic underpinnings of their antibacterial activity. A phage must be demonstrated to be safe through a combination of avoiding the use of temperate phages and otherwise steering clear of bacterial virulence factors such as via bioinformatic analysis. Phage isolates otherwise can be treated as essentially bacteria-killing black boxes that vary most importantly in terms of their individual antibacterial spectra of activity. Phages thus can be viewed as possessing assorted emergent properties that we need not fully appreciate mechanistically to effectively employ phages as antibacterial agents.

Modularity and the thwarting of bacterial resistance mechanisms

The intricacies of the phage infection cycle requires that many reactions successfully proceed for phage infections of bacteria to be productive or at least bactericidal. As a result, there exist many opportunities for things to go wrong. Bacteria as a consequence have numerous steps available to them that they may interfere with to effect a resistance to specific phage types. Similarly, the specificity required for these various phage actions typically will limit which bacteria are susceptible to specific phage isolates.

A second issue is the question of whether resistance mechanisms are limited in their actions to only phages, and therefore whether antibiotic-resistance mechanisms might result also in phage resistance. This concern is in part because bacterial resistance to phage-mediated killing, analogous to mechanisms of antibiotic resistance, can be a consequence of both phage inactivation and modification of phage targets [51,53]. Nevertheless, the targets that bacteria modify to prevent antibiotic-mediated killing, or factors involved in antibiotic inactivation, are typically not the same as bacteria employ for resisting phages. The result, minimally, is that bacterial evolution of antibiotic resistance typically is not expected to translate into cross resistance to phages.

Bacterial resistance to phages that is acquired mutationally typically involves loss or alteration of the bacteria-encoded phage receptor molecules that are found on the surfaces of bacterial cells. A crucial difference between phages and antibiotics as antibacterial agents, however, is that adsorption blocks to phages act only on the extracellular, bacterial acquisition step of phage adsorption, which is highly variable among phages [78]. Restoration of phage antibacterial ability in light of such resistance simply requires modification of the extracellular step, with little need to modify the intracellular infection/bactericidal program. Such modification of adsorption affinity often

can be attained via simple laboratory adaptation experiments [79]. Alternatively, and more conceptually similar to rational-based drug design, phages can be genetically engineered so that they display affinities for new phage receptors [40]. In actual practice the solution to the occurrence of bacterial resistance often is far simpler: One simply isolates new phages, ones which vary in both extracellular and intracellular characteristics. See Chan and Abedon [11] for additional consideration of such issues.

To summarize: (i) Phages possess narrow spectra of activity, that is, their host range [51,80]; (ii) they are affected by resistance mechanisms that are not thought to share specificities with antibiotic-resistance mechanisms; (iii) it is difficult for bacteria to acquire a general resistance to a substantial majority of potentially infecting phages [51,80]; and, as a consequence, (iv) it often is relatively straightforward to isolate from the environment new, professionally lytic phages. That is, phages which are specific for target bacterial pathogens, while at the same time unaffected by a given spectrum of bacterial anti-antibiotic as well as anti-phage resistance mechanisms, often can be relatively easily obtained. Substantial exploitation of naturally occurring anti-resistance properties found among phages in phage therapy, however, is highly dependent on a regulatory framework that allows for or even encourages the development of what could potentially be an embarrassing wealth of both safe and diverse antibacterial agents, that is, a regulatory framework that views phages as drugs categorically rather than as individually unique pharmaceutical agents. Indeed, if we simply redefined antibiotics to include phages, and then took advantage of the diverse potential for phages to overcome bacterial resistance mechanisms to both phages and antibiotics, the antibiotic-resistance crisis likely would be much smaller in scope [6].

Pharmacokinetics

Pharmacokinetics is the study of the impact of bodies on drugs where body can be defined to include not just animal cells and tissues but also associated microflora. Typically this impact is differentiated into various mechanisms that together describe barriers to the achievement of effective densities at a drug's site of action. Drugs thus are subject to absorption, distribution, metabolism, and excretion (together, ADME). These are drug movement into the blood, particularly from the gut though also by more direct routes; drug movement from the blood to other body compartments; drug chemical modification; and the movement of intact drug out of the body, respectively. Antimicrobial drugs thus are administered to patients and in some manner reach bacterial targets where they can have positive effects. All the while, drugs are also blocked in their movement, diluted, chemically destroyed, removed from the body, or otherwise sequestered away from bacterial targets. For additional discussion of these ideas as they pertain to phage therapy, see Abedon and Thomas-Abedon [6] along with Abedon [10]. In this section we consider additional, more specific aspects of pharmacokinetics as this subject can be applied to phages. See Figure 3 for summary of pharmacokinetics in general along with associated pharmacodynamics, both as compared with those of phage therapy.

Topical versus systemic administration

Topical phage administration, which includes application to both wounds and lungs, does not require absorption nor necessarily substantial distribution. With topically applied phages one can use the term *penetration* instead [10], as in phage penetration into bacterial biofilms. Such biofilm penetration by phages [74,75] may be more effective than the impact of chemical antibiotics [81]. Indeed, we can apply the phrase "active penetration" to describe the possibility that phage-induced bacterial lysis, particularly of bacteria found in the outer layers of bacterial biofilms, may aid phage penetration into biofilms [6]. This lysis either supplies phages directly to underlying bacteria or allows nutrients to diffuse to already established phage infections [74,75].

Oral phage delivery too can involve phage penetration without accompanying absorption or distribution. High stomach acidity can inactivate phages, however, and thus "penetration" through gastric juices should always be a concern given oral phage administration. Poorly understood, though, is the issue of penetration of phages to gastrointestinal bacteria once past the stomach. In particular, the question of whether or when phages can consistently display efficient active therapy within the gastrointestinal lumen is somewhat open and efforts to reduce bacterial densities using relatively low phage densities and/or few doses are unlikely to be effective unless active treatment is in fact possible. Thus, not only is phage inactivation in the course of passage through the stomach a concern but so too phage dilution upon mixing with food, drink, or gastric secretion as dilution can also have the effect of reducing overall phage densities [10].

Phage application to the gastrointestinal tract of animals can result in uptake into blood, a process that has been dubbed bacteriophage translocation [82]. Unfortunately, little is understood of the mechanism of this translocation, except that it is not always demonstrable [83-85]. When such absorption is anticipated following oral administration, then the application can be described as *per os*. By design, most antibiotics employed systemically can be delivered in this manner. Since oral dosing can be highly convenient for patients, including in a non-clinical setting, it would be helpful were phages similarly deliverable. Consequently, a clear limitation to the potential of phage therapy as a general strategy of bacterial disease control is the substantial uncertainty that surrounds the phage facility to reach systemic circulation following oral delivery.

More direct approaches of administration to systemic circulation of course also exist. These, however, can be more problematic due to a combination of greater invasiveness, lower convenience, and a need for greater phage purification [58]. Contrasting *per os*, though, delivering phages directly to systemic circulation may be more reliably achieved. In addition, passive treatment also is possible with direct administration whereas *per os* phage delivery would appear to be wholly dependent on active treatment, that is, phages entering systemic circulation in low numbers that are then bolstered upon contact with target bacteria. Human treatment using these more direct approaches to systemic delivery have been used though such efforts are not nearly as common as more local applications [16]. A reasonable assumption is that the phage therapy niche as an alternative to chemical antibiotics may be biased towards local administration, probably including oral administration for gastrointestinal treatments, with phage treatment of systemic infections perhaps limited to circumstances in which antibiotic use is less permissible, for instance against antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant enterococci (VRE) [13]. Indeed, a number of studies have either demonstrated in humans [86,87], shown in animals [88], or proposed [89] the phage therapy or biocontrol [90]

of MRSA. Phage-based treatment of MRSA may even be less expensive than treatment with chemical antibiotics [91].

Distribution

Distribution is the movement of drugs out of the blood and into other body tissues. Achievement of this movement is a function of drug molecular properties and it probably is reasonable to speculate that the phage potential for such movement occurs for reasons that are somewhat independent of the mechanisms employed by small-molecule drugs. The means by which phages achieve such movement, though, is poorly understood [10,92]. What is crucial to keep in mind, however, is that issues of drug distribution towards positive pharmacodynamic effects only matter to the extent that drug application is systemic and intended to reach tissues other than blood. Another key issue is that disease, including bacterial infections, can increase tissue permeability such as to phages; see Abedon [10] for discussion. Also relevant is that the pharmacokinetic property of self amplification to at least some degree can compensate for inefficiencies in phage distribution: If phages can reach bacterial infections then they can have some impact on those infections. If their ability to replicate is sufficiently robust then they may be able to achieve a *substantial* impact.

Metabolism

Metabolism in a pharmacokinetic sense describes the chemical modification of drugs. Though this often is a means to the end of drug elimination from the body, in some cases metabolism instead can convert a prodrug into its active form. With chemical pharmaceuticals most metabolism occurs in the liver. There cytochrome P450 family enzymes make lipophilic xenobiotics more polar, that is, molecules not normally found within the body. Thus modified, these molecules are more easily excreted through the renal system [93]. As phages consist mostly of proteins and DNA, they generally are not xenobiotic. In addition, in terms of phage pharmacokinetics, phage sequestration such as due to immune system action need not result in subsequent phage chemical modification to have phage levels reduced, so long as phages otherwise have been permanently separated from their bacterial targets.

Phages must be resupplied on an ongoing basis to the vicinity of target bacteria for phage therapy to succeed in the face of phage elimination from tissues. If effective phage densities are, for example, 10^8/ml, and phages are inactivated at a rate of, say, 1% per minute, then 10^6 new phages must be added to the system per minute just to sustain phage densities at effective levels. With active treatment not only must phages be sustained at effective levels but so too must those relatively high phage densities be achieved in the first place, all of which must occur in the face of ongoing phage losses. If phages nonetheless are easily delivered, or otherwise can replicate sufficiently robustly *in situ*, then inactivation by immune system action need not be a substantial issue. Note that phage self amplification *in situ* too may be described as an action of metabolism [6,10] though of course not one associated with the action of liver enzymes.

Excretion

Phage elimination from bodies is mostly due to immune system-mediated sequestration or inactivation rather than excretion, which is elimination of a drug in the same form as it was administered. Phages do have some access to urine, which may be useful for treatment of urinary tract infections following systemic phage administration [10]. Other than phage delivery to such sites, excretion is not terribly relevant to phage therapy and particularly less so given topical rather than systemic application.

Conclusion

The impact of a standard chemical drug is difficult to predict due to the emergent properties that are associated with the complexity of the organisms they are treating, such as ourselves. With small-molecule antimicrobial drugs, that complexity is further increased owing to the multifaceted nature of the parasitic organisms being targeted. This means that negative impacts on target pathogens (i.e., positive pharmacodynamic effects) along with negative impacts on the body itself can be difficult to predict. When phages are properly chosen, by contrast, their negative impact on bodies can be both minimal and somewhat predictable, and this predictability can occur in spite of a lack of specific knowledge of the biochemical processes that phages encode. The result can be a much simpler path towards antibacterial development than one typically sees with chemical antibacterials, where toxicity testing often serves as a major road block [17].

Because their negative impact is mostly limited to target bacteria, professionally lytic phages can possess biases towards pharmacologically desirable rather than undesirable effects. More generally, phages, like antibiotics, are naturally occurring antibacterial agents whose positive pharmacodynamic effects have been honed by billions of years of organic evolution. Furthermore, the phage potential to self amplify, *in situ*, can obviate many pharmacokinetic issues that otherwise could negatively impact their potential to achieve minimally effective antibacterial densities in the vicinity of target bacteria. The upshot is an emergent property laden but nonetheless non-xenobiotic pharmaceutical that can be more predictable than traditional antibiotics at the 'higher levels' of antibacterial characterization, such as in terms of animal or clinical testing, even as lower-level, especially biochemical understanding of the phage impact on target bacteria can be vastly more difficult to achieve.

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