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1 **Title:** The forgotten tale of Brazilian phage therapy

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11
12 **Summary** (179/200 words)

13 The use of bacteriophages to treat bacterial infections (phage therapy) is considered a possible solution to the
14 antimicrobial resistance crisis. However, phage therapy is not a new concept. The discovery of phages in the
15 early twentieth century was closely tied to clinical practice, and phage therapy quickly spread around the world.
16 The use of phage therapy in twentieth century South America is still shrouded in mystery and has been
17 mentioned only briefly in recent scientific literature. Research on Brazilian reference collections of medical
18 texts revealed that Brazil was an important, but so far little-known, player of phage therapy, uncovers interesting
19 priority claims and missing pieces of phage therapy history. Of note, there is the widespread use of phages
20 against bacillary dysentery and staphylococcal infections, with Dr José da Costa Cruz from the Institute
21 Oswaldo Cruz as Brazil's leading expert and pioneer. This review about historical phage use in a South
22 American country fills gaps in our knowledge about the "golden years" of phage therapy, providing information
23 about successful experiences that can be useful against dangerous pathogens in our time.

24
25 **Key points**

- 26
- 27 • Brazil had an important role in the golden years of phage therapy, which lasted until the mid- twentieth
28 century.
 - 29 • Dr José da Costa Cruz from the Institute Oswaldo Cruz in Rio de Janeiro was the leading expert.
 - 30 • The first phage therapy cases in Brazil took place in 1921 during an outbreak of dysentery in
31 Barbacena.
 - 32 • The mass testing of the Institute Oswaldo Cruz phage product during the Paulista revolution of 1924
33 preceded Dr d'Herelle's tests in India and the Soviet Union's tests on military troops.
 - 34 • Successful phage use in Brazil was against bacillary dysentery and staphylococcal infections.
 - 35 • The available information about phage therapy clinical cases and phage products was retrieved from
the literature investigated and presented in this publication.
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44 **Research in context**

45 **Evidence before this study**

46 Reports summarising the use of phage therapy in Brazil were scarce before this review. Only three recent
47 publications mentions, briefly and without details, that Brazilian doctors were involved with phage therapy in
48 the 1920s. Sixteen sources of Brazilian medical publications were checked for phage-related information,
49 looking at the period from 1915 (when Dr Frederik Twort published his phage discovery) to 1952. Any phage or
50 phage therapy related words in titles and abstracts were used as inclusion criteria. The risk of bias associated
51 with data inclusion is minimal, because the objective was to have all published material concerning phages.
52 However, since routine phage therapy cases were not always published, the data presented here are probably
53 only a fraction of the actual phage use at the time.

54 **Added value of this study**

55 With all the information at hand, it was possible to reconstruct phage therapy practices in Brazil during the first
56 half of the twentieth century, covering the beginning of Brazilian phage therapy to its demise. Interesting claims
57 of priority, acknowledgement of important researchers and Institutes, and successful phage use against bacillary
58 dysentery and staphylococcal infections are described. This review fills missing gaps in phage therapy history,
59 describes practices and potential international collaborations of the past and constitutes the most complete
60 description of phage therapy use in the history of a South American country to date.

61 **Implications of all the available evidence**

62 From a historical point of view, the recollection in this review has importance for the history of medical science
63 and consolidates the important, yet forgotten, role of Brazil in phage therapy. The current article may serve as
64 inspiration for similar publications about the history of other nations, filling gaps and improving our current
65 knowledge with experiences from the past. From practical and applied perspectives, this review provides
66 clinical data and medical practices that can help against important bacterial pathogens from our time. Phage
67 therapy in Brazil was focused against bacillary dysenteries and staphylococcal infections. Both still cause
68 problems for humans, the first being the second major cause of diarrheal-associated deaths in the world and the
69 second being an important infection with many clinical manifestations that is becoming increasingly more
70 dangerous as resistance to antibiotics spreads. The experience and clinical data described in this review can be
71 used to shape modern phage use and clinical trials.

72

73 **Search strategy and selection criteria**

74 Sixteen sources of Brazilian medical publications were checked for phage-related information. The name of
75 these journals, and the time periods verified, were: *Archivos Brasileiros de Medicina* (1920-1940, 1942-1945),
76 *Arquivos de Higiene* (1927-1945), *O Bisturi* (1930, 1933-1945), *Boletim da academia nacional de medicina*
77 (1920-1937, 1939-1940), *Brasil-Medico* (1915-1952), *Brasil Medico Cirurgico* (1941-1942, 1945), *Folha*
78 *medica* (1920-1932, 1934-1945), *Gazeta Clinica* (1920-1931, 1933-1943, 1945), *O Hospital* (1924, 1931-1945),
79 *Revista Brasileira de Medicina* (1944-1950), *Revista Medica Brasileira* (1939-1945), *Revista Medico Cirurgica*
80 *do Brasil* (1920-1921, 1923-1945), *Revista de Medicina e Higiene Militar* (1921-1927, 1929-1930, 1939-1945),
81 *Memorias do Instituto Oswaldo Cruz* (1915-1952), *Tribuna Medica* (1920-1930) and *Vida Medica* (1931-
82 1947). Temporal gaps are due to missing editions in the archives. None of these journals were focused on
83 microbiology alone, so space was used for all facets of medical practice at the time. Whole volumes were
84 checked for any phage-related publication, page by page whenever possible or through its summaries. Search
85 criteria was focused on phage therapy related words, searched in the summaries and publication titles of the
86 available journals. Examples of keywords used were “fago, bacteriofago, fagoterapia, principio litico de
87 d’Herelle” and their variations. Although a few cases might have slipped unnoticed, most if not all phage work
88 published at these journals at the verified timeframes were found and are mentioned here. Since most of these
89 publications have no other source besides the physical collections in which they are kept, this review presents a
90 rich and important retelling of Brazilian phage therapy to the modern world.

91

92

93 **Main text** (4462/4500 words)

94 Since the 1980s, the use of bacteriophages to treat bacterial infections (phage therapy) has been considered a
95 viable solution for the antimicrobial resistance crisis¹. Many research groups² and modern clinical trials³⁻⁵ have
96 focused on this subject of growing importance, with Belgium recently pioneering with alternative regulatory
97 pathways for the clinical use of phages⁶. However, phage therapy is not a new concept. The discovery of phages
98 in the early twentieth century was closely tied to clinical practice and, in the early 1920s, phage therapy spread
99 from France to the rest of Europe and the Americas⁷⁻⁸. Heralded as a significant advance in medical care at the
100 time, phage therapy was used as a routine treatment in several countries until, in the 1940s, a shift to the use of
101 antibiotics occurred, which made it disappear from most of the world. Although the Georgian, Russian and
102 Polish experiences with phage therapy are well documented, much of what has been achieved in other countries
103 remains under-reported.

104 One interesting missing piece of phage therapy history lies within South America. Mentioned only three times in
105 recent scientific literature, the role of Brazil in using phages as a treatment is still shrouded in mystery. First,
106 “*the epic of phage therapy*” (text based on a presentation given by Dr Alain Dublanquet in Oxford) mentions
107 that in the 1920s, bacteriologists from Germany, USA and Brazil tried to verify Dr Felix d’Herelle’s phage
108 therapy results, but failed⁸. Second, a report published in Portuguese by the Paraense^A Academy of Sciences⁹
109 highly praises Dr José da Costa Cruz^B career. He is said to have used “*bacteriophage therapy*” to treat diarrhoea
110 in children and created a phage-based product for clinical use. The third mention appears in a chapter focusing
111 on the history of phage therapy, written by Dr Nina Chanishvili from the Eliava Institute¹⁰. It states that the
112 Institute Oswaldo Cruz in Rio de Janeiro started to produce anti-dysentery bacteriophages to be used in Latin
113 America in 1924. From these publications, it is clear that Brazilian researchers were involved with phage
114 therapy, moving from failure in a clinical trial to mass production of phages within only a few years.

115 Additional information could be obtained from Dr Cruz’s obituary published in 1941¹¹, which also shows a
116 picture of him (**Figure 1A**) The text confirms Dr Cruz’s interest in “*bacteriophagy*” in his studies of child
117 diarrhoea at the Institute Oswaldo Cruz. He is said to have had spectacular success in treating dysentery cases,
118 and in a short time the Institute started to produce a commercial phage preparation. Its large scale production
119 and application, as well as the rational therapeutic use of phages to treat a number of infectious diseases at the
120 time, were attributed to him. From the obituary, it was possible to track his publications, and the name of the
121 journals in which he published. These were investigated, along with other sources of medical-related
122 information from Dr Cruz’s time.

123

124 **From failed clinical cases to mass production of phages (1921 to 1924)**

125 By the 1920s Brazil was a republic with Rio de Janeiro as its capital, which is where the Institute Oswaldo Cruz
126 was located. The Institute was founded in 1900 and became important for sanitary campaigns, the production of
127 biological products and medical research. The first publication mentioning phages in the Brazilian literature was
128 a study about their nature, co-authored by Dr Cruz in 1921¹². For more information on this publication and other
129 historical Brazilian phage research papers, see the *Supplementary Material*. The first mention of the clinical use
130 of phages in Brazil appeared in a publication in 1923 describing the results of two sets of clinical tests against
131 dysentery¹³. In this publication, Dr Cruz affirms that anyone who sees the *in vitro* activity of a phage is “*filled*
132 *with hope that a new therapeutic against infectious diseases will immediately appear*”, and states that he was
133 oblivious to Dr d’Herelle’s clinical observations at the time. The first phage therapy cases took place at the
134 Mantiqueira Mountains^C, near the city of Barbacena. Despite Shiga bacillus being isolated from two patients,
135 phage treatment was not successful in this early attempt. The second testing of phage therapy took place in
136 1923, when cases of dysentery started to appear around the Institute Oswaldo Cruz in Rio de Janeiro, and
137 phages were tested in “*better working conditions*”. Twenty-four clinical cases were reported, with most patients
138 reporting improvement of their symptoms four to five hours after the start of therapy. It was concluded that
139 phages were recommendable for dysenteries for being easy to administer, innocuous for the patients and
140 efficient in cases in which other treatments have failed.

141 More information about Dr Cruz’s early success with phage therapy was published in French in 1924¹⁴ and in a
142 review written by himself in 1940¹⁵. After one year of experience in applying oral phage therapy against
143 dysentery, probably as a follow-up to the 1923 trial in Rio de Janeiro, the Institute Oswaldo Cruz prepared

10.000 phage vials and distributed them to numerous doctors in Brazil. Positive results against acute and chronic cases were reported from the states of Pará, Maranhão, Pernambuco, Paraná and São Paulo. It was concluded that phage therapy was the best treatment for dysentery, surpassing any other treatment by quickly suppressing symptoms in a few hours and resulting in cure after one or two days. The positive feedback led the Institute Oswaldo Cruz to recommend phage use, and it started to provide phages by request, under the name *Bacteriophagina disenterica*. This product was noted to be the first therapeutic phage preparation offered to the public based on thorough observation. It reached national and international reputation because of the product's quality and the "choosing of the cases sent for observation". By this affirmation, Dr Cruz meant that the success of the product could be attributed to cases in which the phage preparation was given in a timeframe close to the beginning of the disease, hence improving its success rate. The *Bacteriophagina disenterica* was largely employed during the 1924 revolution^D in São Paulo among government troops, but no specific details besides its successful use were reported. This was probably the first large scale human trial in history, preceding the ones made by Dr d'Herelle in India¹⁶ and the ones by the Soviet Union¹⁰.

157

158 **The earlier years (1923 to 1929)**

Dr Cruz's knowledge of phage therapy is clear in his 1923 publication, in which he discusses international research and issues concerning successful phage use¹³. Broad knowledge of phage therapy was probably widespread in the Brazilian medical community because three clinical cases against staphylococcal infections are described by Dr Nelson Barbosa on the same year¹⁷. The Brasil-Medico journal published comments on two Italian papers in 1924¹⁸⁻¹⁹. In one of these comments, the lytic principle of d'Herelle is acknowledged for slowly being established as a therapeutic agent in Brazil, with contributions by Dr Cruz and Dr Barbosa, confirming their roles as leading phage therapists of the time.

In 1924, phage therapy work from Brazil was presented at the Society of Medicine and Surgery of Rio de Janeiro. The first presentation was about "bacteriophagy" and immunity, discussing whether phages were alive or not and cited Dr Cruz's work²⁰. The second presentation concentrated on the therapeutic use of "bacteriophagy"²¹. After an introduction on Dr d'Herelle's work and a recollection of clinical phage use in other countries, the presenter cited Dr Cruz's results and praised the phage product provided by the Institute Oswaldo Cruz, affirming that he had used it to treat almost fifty dysentery cases with success. The third presenter discussed immune responses to injected phages, the choice of phages and isolation of bacteria, and presented a recollection of his laboratorial and clinical cases²². Although no details about the cases are given by the conference summaries, they demonstrate wide use of phage therapy.

During the *IV South American Conference of Hygiene, Microbiology and Pathology* held in 1929, Dr Cruz summarised the therapeutic use of phages worldwide and presented more details about the Brazilian phage therapy experience²³. He mentioned his own failed attempts to treat bacteraemia in cases of typhus and paratyphus and that his plans to test phage therapy against cholera in 1924 ended because he was not able to isolate specific phages. At the same conference Dr Oscar Pereira, a microbiology professor at the Medicine School of Porto Alegre, shared his own phage therapy clinical experience²⁴. He was using phages against bacillary dysentery since 1924, resulting in a complete cure for the patients. According to him, phage therapy was also important for abolishing carrier states, because after phage use the patient's stool would not contain any infectious agent. He stated that the mortality related to dysentery in two hospitals from Porto Alegre city decreased considerably after phage therapy started to be used. He also presented one successful clinical case of phage therapy against a coli bacillus infection on the urinary tract and mentioned nine cases of pyodermitis and thirty-two of furunculosis treated with anti-staphylococcal phages.

The widespread knowledge on phage therapy is also supported by the published syllabus of a course offered by the Institute Oswaldo Cruz, in which one of the topics covered is "bacteriophagy"²⁵. This shows that health workers in Brazil were being trained in phage therapy as part of their studies at the time. A picture of students approved in the 1928 course can be seen in **Figure 1B**.

191

192 **From novelty to routine (1930 to 1944)**

193 In 1930, the Brasil-Medico journal published a translated version of a German phage paper, with a note stating:
194 “among us the anti-dysenteric bacteriophagina is largely employed. As for its efficacy, a lot is discussed”²⁶. A
195 few years later, in 1934, Dr Cruz was one of the authors of a communication presented to the Brazilian National
196 Academy of Medicine²⁷. Arguing that publications concerning the use of phage therapy against staphylococcal
197 infections were rare and still did not attract the deserved attention, it describes the clinical case of a young
198 patient suffering from staphylococcal septicaemia preceded by a furuncle. After a month with no results using
199 other treatments, a phage from a pre-existing collection was adapted to the bacterial strain infecting the patient
200 and used. As a result, the clinical status of the patient progressively improved, and he was cured after six phage
201 injections. Phages were also used topically with success to treat an abscess that appeared near the original
202 furuncle.

203 Brazilian phage therapy cases continued to appear in the literature, with four therapeutic notes presented in
204 1935. The first describes a case of staphylococcal furunculosis in a child treated with *Estaphylofagina* ampoules
205 and cured²⁸. In the second note, the patient was an undernourished child with pyelitis presenting diarrhoea, urine
206 incontinence and fever. She was treated with *Colifagina from Dr Raul Leite Laboratory*. The fever disappeared
207 in three days, and she recovered completely²⁹. The third case was also a child with long-term pyelitis, again
208 successfully treated with *Colifagina*³⁰. This note ends with a suggestion that this case was “enough reason to
209 always recommend” the phage product. The fourth note describes the cure of a patient with osteomyelitis by
210 using injections of the *Estafilofagina* phage preparation³¹. The names of the phage preparations used as
211 treatments on these cases are of commercial phage products. They were advertised at the medical literature of
212 the time (advertisements of phage-based products in Brazil are shown in **Figure 2**), and prepared by the Raul
213 Leite Laboratory. For more details see the *Supplementary Material*.

214 In 1938, seventeen years after his first phage publication, Dr Cruz published a review about thirty-three cases of
215 septicaemia that he was involved with during treatment³². The cases were divided into gonococcal (one),
216 streptococcal (fourteen), staphylococcal (twelve) and caused by a coli bacillus (six). Phage therapy was used
217 only for seven of the staphylococcal cases and attempted but not continued in one^E. Considering the
218 staphylococcal cases, he concludes that the best treatments were blood transfusions and phage therapy,
219 suggesting that injected phage preparations could be a first order therapeutic agent for staphylococcal
220 septicaemia, curing even those cases that transfusion could not. Mortality was lower than predicted among
221 phage-treated patients, and all who reached the third phage dose had sterile haemocultures. Although phage
222 therapy cleared bacteraemia in all patients, the appearance of phage-resistant localised bacterial foci led to death
223 or to the need for surgery in some cases. Of the four patients treated with phages who passed away, one had an
224 acute infection and did not reach the second phage dose, one died because of surgery complications and two had
225 localised focal infections. Dr Cruz’s review also gives an interesting overview of the phage therapy practices in
226 Brazil. First, not all bacterial diseases were targeted by phage therapy, and even in cases in which phages could
227 be used, other treatments were also applied. For example: of the twelve staphylococcal cases, five were treated
228 with phage-therapy only, two were treated with phage therapy after other approaches failed, two had phage
229 preparations made but not used because the patients recovered before, and three did not use phages at all.
230 Second, consent for using phages was also important for deciding its use. Dr Cruz mentions that no other phage
231 preparations were used in the case in which the patient had a strong reaction to the lysate because the patient’s
232 family was against the use of phages since the beginning, and only reluctantly consented on the first dose.

233 Dr Genesis Pacheco, a co-worker of Dr Cruz from the Instituto Oswaldo Cruz and author of his obituary,
234 published a review on “bacteriophagetherapy” in 1939³³. Written in a personal and critical tone it denounces the
235 high number of pharmaceutical products in Brazil and associated their mass scale production and abusive
236 advertising to the search for high profit. The problem with most commercial phage preparations was attributed
237 to the fact that the technical requirements for a perfect product made them unattractive monetarily, and that
238 profit was often made from technical imperfections that affected product quality. Then the association of
239 different medications was said to be responsible for “a multitude of products cluttering pharmacy shelves and
240 the pockets of pharmaceuticals and producers”. A product combining phages to lactic ferments approved by
241 public health officials was cited as an example. An advertisement of this product from 1934 can be seen in
242 **Figure 2C**. As a conclusion, Dr Pacheco classifies phage production as an activity that should not be subjected
243 to commercial ends, being as important as justice, culture, education and health-related issues such as
244 preparation of medicine. Although his point of view is similar to that of Dr d’Herelle regarding commercial
245 phage products, his connection to phage manufacturers in Brazil (for more details, see the *Supplementary*
246 *Material*) might present an undisclosed conflict of interest.

247 In his 1940 review, Dr Cruz mentions his involvement in two Brazilian phage therapy clinical trials against
248 typhoid fever: one in 1924 in São Paulo and the other “recently” at the Hospital São Sebastião, both with
249 negative outcomes¹⁵. His experience with phage therapy against pyogenic infections, from dermal infections to
250 staphylococcal septicaemias, was positive as described previously. He considered phage therapy in these
251 infections extremely successful and the only solution to cases in which other treatments have failed, but he notes
252 that using phages adapted to the patient’s clinical isolates, as opposed to growing the phages in reference
253 bacterial strains, was critical for success. This was the last paper published by Dr Cruz, who passed away in the
254 same year.

255 In 1939, notes about international papers describing negative results for phage therapy appeared in the Brazilian
256 literature. These describe phages failing to protect rabbits that were experimentally infected with
257 *Staphylococcus*³⁴. In 1944, a review on phage therapy against staphylococcal infections in Brazil was
258 published³⁵. It mentions that phage treatments in these infections were rarely used, and usually done when other
259 treatments failed. The author reports good results using the oral route for treating furunculosis and acne, with
260 60% of furuncle cases being cured in eight days, and with recidive cases not cured by other means being cured
261 by phage therapy. Phages were considered to be a “heroic therapeutic”, and cases in which the bacteria became
262 resistant to the phage are noted to be rare. This publication was discussed on a questions and answers section of
263 the same journal, by a reader who only used phages against dysentery but became interested in phage therapy
264 against staphylococcal infections³⁶.

265

266 **Slowly fading away (1944 onwards)**

267 The first half of the 1940s was marked by an increase in publications about other antibacterial substances. For
268 example, a short note discussing an international publication on phage therapy use against staphylococcal
269 meningitis appeared in 1944³⁷. Although mentioning that the exclusive use of phages could cure patients, the
270 authors’ recommend using sulphonamides in association with phages and anti-toxic sera during the first week of
271 treatment. Then phage use could be continued for a longer time, to avoid complications such as cerebral
272 abscesses. A comment on publication by Jern and Meneley mentions that sulphonamides were not as efficient
273 against *Staphylococcus* as they were for *Streptococcus* infections, with the former being treated more efficiently
274 by phage therapy³⁸. Then an experiment using chicken eggs injected with *Staphylococcus* and many
275 antibacterial agents, including antibiotics and phages, was presented. This comparative approach revealed that
276 penicillin was the most efficient treatment. Another comment on an international paper by Boyd and Portnoy
277 was published in 1945, describing the negative results of human trials of phage therapy on war prisoners³⁹.

278 Phages are mentioned again in the questions and answers section in 1948⁴⁰. Even though noting that
279 sulphonamides were the current preference for treating enteric infections, a reader asks about the concomitant
280 use of phages and lactic ferments. This highlights that there was still some interest in phages, but that
281 sulphonamides/antibiotics were becoming the first choice of treatment and questionable phage products were
282 still on the market. A question about the use of phages to treat abdominal typhus also appears in the same year⁴¹.
283 In 1950, a doctor from the University of São Paulo published a review about the treatment of amoebal
284 dysentery⁴². Here, phages are indicated as auxiliary medication to treat cases in which secondary infections
285 might be present, together with antibiotics.

286 From these publications and comments, it is clear that there was still interest in phage therapy in the 1940s. The
287 associated use of phages and antibacterial medicine also became clear during this decade, as did the divulgation
288 of the international literature concerning the efficacy of antibiotics and failed phage clinical trials. Some
289 examples of advertisements for sulphonamides and penicillin found in the Brazilian medical literature are shown
290 in **Figure 3**.

291

292 **Why has phage therapy vanished?**

293 There is no clear delimitation for when phage therapy ended in Brazil. The literature used as a source for this
294 review does not contain any publications recommending that phages were not to be used, nor any mention to
295 laws or regulations on this matter. This was confirmed by a consultation made with the Brazilian Health
296 Regulatory Agency (contact protocol 2019084054), which determined that there are no current regulations

297 concerning the use of phages in Brazil. Dr Cruz's obituary, published in 1941, highlights his efforts in phage
298 therapy, with no mention of problems or any intentions to interrupting it¹¹. However, around the same time,
299 publications about phages and clinical phage therapy cases became rarer in the Brazilian literature.
300 Coincidentally, publications on antibiotic use increased in the early 1940s, culminating with a large focus on
301 penicillin in 1944. In this year, the *Brasil-Medico* journal published a translated version of a Dr Alexander
302 Flemming's paper, a translated transcript of a Winston Churchill speech praising the use of penicillin, and
303 several clinical cases of antibiotic use. In 1945 the *Memorias do Instituto Oswaldo Cruz* journal published a
304 series of penicillin trials, and a proposition about lowering penicillin taxes was being discussed by the
305 government in 1946. Also in 1946, Dr Flemming travelled to Rio de Janeiro to attend a medical conference and
306 visited the Institute Oswaldo Cruz. Furthermore, the 1940s marked the end of the generation trained during
307 phage discovery, with many health workers probably retiring or passing away during this decade. This, along
308 with the new and promising antibiotics, might have made phages an outdated therapy in the eyes of the new
309 medical generation. Health professionals might have seen antibiotics as an easier and more efficient novelty to
310 treat bacterial infections, including those not targeted by phage therapy. The flooding of the Brazilian market by
311 suboptimal commercial phage preparations, as implied by Dr Pacheco in 1939³³, could also have had a negative
312 impact on the reliability of phage therapy compared with antibiotics. Thus, the transition from phage therapy to
313 antibiotics appears to have been gradual, with the success of antibiotic use replacing the use of phages during
314 the 1940s. Years later, when bacterial resistance to antibiotics became a serious threat, much of the phage
315 collections and phage therapy expertise were already lost beyond repair and the search for new antibiotics
316 became more attractive than reviving phage treatments.

317

318 **Conclusions**

319 The Brazilian medical literature from the first half of the twentieth century reveals that Brazilian doctors and
320 researchers were active practitioners of phage therapy. Dr Cruz, from the Institute Oswaldo Cruz in Rio de
321 Janeiro, was the leading expert on the subject. He coordinated the first human cases in Brazil in 1921 and 1923,
322 resulting in a phage product distributed around the country for many years. Dr Cruz had some interesting claims
323 of priority. He mentioned that his 1921 tests were made before being aware of Dr d'Herelle's results with
324 human patients; claimed that his phage product was the first backed by strong trial data; and its mass testing on
325 soldiers during the 1924 revolution in São Paulo preceded Dr d'Herelle's mass test of phages in Calcutta¹⁶ and
326 the Russian tests on troops¹⁰ by a few years. A timeline showing an overview of major phage therapy
327 milestones, from Brazil and the rest of the world, is presented in **Figure 4**.

328 No evidence has been found to support a direct interaction between Brazilian researchers and Dr George Eliava
329 or Dr d'Herelle. The origin of the anti-dysentery phages used in Brazil remains a mystery, while at least part of
330 the anti-staphylococcal phages were obtained from the Pasteur Institute in Belgium. Clinical cases found often
331 lacked specific details, such as phage dosages, and all information available in the original references are
332 compiled in **Table 1**. No records of the destiny of Brazilian phage collections or large-scale phage production
333 and purification protocols could be retrieved. For more details about the origin of phages used in Brazil, their
334 production, administration protocols, and international collaborations of Brazilian phage researchers, see the
335 *Supplementary Material*.

336 Phage preparations were made by the Institute Oswaldo Cruz and distributed throughout the country, while
337 commercial phage products were also made and sold by private companies. Dr Cruz and Dr Pacheco believed
338 that phages were important enough to be made by state owned quality labs. However, both researchers were also
339 involved with a private laboratory. There is no clear milestone pointing to the stop of phage usage in Brazil.
340 What could be perceived is that the interest in phages decreased as the use of antibiotics increased, and this
341 serves as an indication that the same trend might have happened in other Western countries.

342 The use of phages in Brazil was mostly against bacillary dysentery and staphylococcal infections, with
343 successful outcomes reported for both. Phages were administered orally for dysenteries and injected for
344 staphylococcal infections, but local and oral use were also recorded for the latter. Details of past Brazilian
345 experiences in successfully treating these diseases are priceless to modern researchers and clinicians. In 2010
346 there were 188 million cases of bacillary dysentery caused by *Shigella* around the world, and these infections
347 are the second leading cause of diarrhoeal related deaths worldwide⁴⁸. Staphylococcal infections are still an
348 important threat with many clinical manifestations, and they are becoming increasingly more dangerous because

349 of the spread of antibiotic resistance⁴⁹⁻⁵⁰. The safe and efficient use of phages against these targets, as suggested
350 by the Brazilian experience shown in this review, can be taken as a guideline to develop modern phage therapy
351 trials and shape phage use in our time.

352 This review is also important for showing that Brazil has a strong past with phage use, bringing to light original
353 publications and clinical cases that were hidden from the international community by language barriers and for
354 being part of physical collections only. It fills some gaps in the past of phage therapy, with importance to the
355 history of medical science and to the phage research community. Despite its rich past with phage therapy, the
356 use of phages is not currently applied in modern Brazilian medical practice. The revival of phage therapy would
357 in theory fit well within the current Brazilian unified health system⁵¹, which with the support from Institutes and
358 Universities for phage collections and research, could provide efficient and cost-effective phage treatments for
359 the population. However, regulatory and political issues allied to increasing austerity measures would make it
360 hard to be achieved. For a country that after a brief respite is undergoing successive cuts in research funds⁵²⁻⁵⁵,
361 going through a loss of historical memory⁵⁶ and suffering from anthropogenic environmental catastrophes⁵⁷⁻⁵⁸,
362 this review might serve as an inspiration to look into a successful past and aim for a better future.

363

364 **Footnotes**

365 ^A Paraense is an adjective used to designate someone born at or something originated from the Pará state,
366 northern Brazil.

367 ^B It is important not to mistake Dr Oswaldo Cruz (1872-1917), namesake of the Institute Oswaldo Cruz with Dr
368 José da Costa Cruz (1894-1940), the author responsible for many publications cited in this text. Dr José da
369 Costa Cruz worked at Institute Oswaldo Cruz and was responsible for much of the phage work in Brazil.

370 ^C Serra da Mantiqueira (Mantiqueira Mountains) is a mountain range in South-eastern Brazil, extending over the
371 states of São Paulo, Minas Gerais and Rio de Janeiro. The location of the outbreak was mentioned to be around
372 the city of Barbacena, Minas Gerais.

373 ^D The 1924 revolution in São Paulo mentioned is probably the Paulista Revolution, part of the Tenentist
374 movement. Army dissidents attempted to depose president Artur Bernardes, leading to a 23 days long
375 occupation of the city that ended after the city was bombarded by the Federal Government troops. The
376 estimative are of 1000 dead and 4000 injured.

377 ^E In this particular case the treatment was discontinued because a strong reaction to the phage started after only a
378 few drops of the medication. A footnote mentions that the reaction was probably not due to the phage itself,
379 explaining that although the phage preparation used was sterile, it was opaque and not transparent as it should
380 be. The opaqueness was attributed to a faulty candle used during the phage preparation, which released kaolin to
381 the filtrate.

382

383 **Contributors**

384 Gabriel MF Almeida made the literature search, collected the data, and prepared the first version of the
385 manuscript. Both authors contributed in writing the manuscript.

386

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399

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403

404 **Figures reproduction and references**

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407 reproduced/reprinted. The researched literature can be found at the collections mentioned in the
408 acknowledgments section, some freely available online and some by request only, or alternatively could be
409 requested from the corresponding author.

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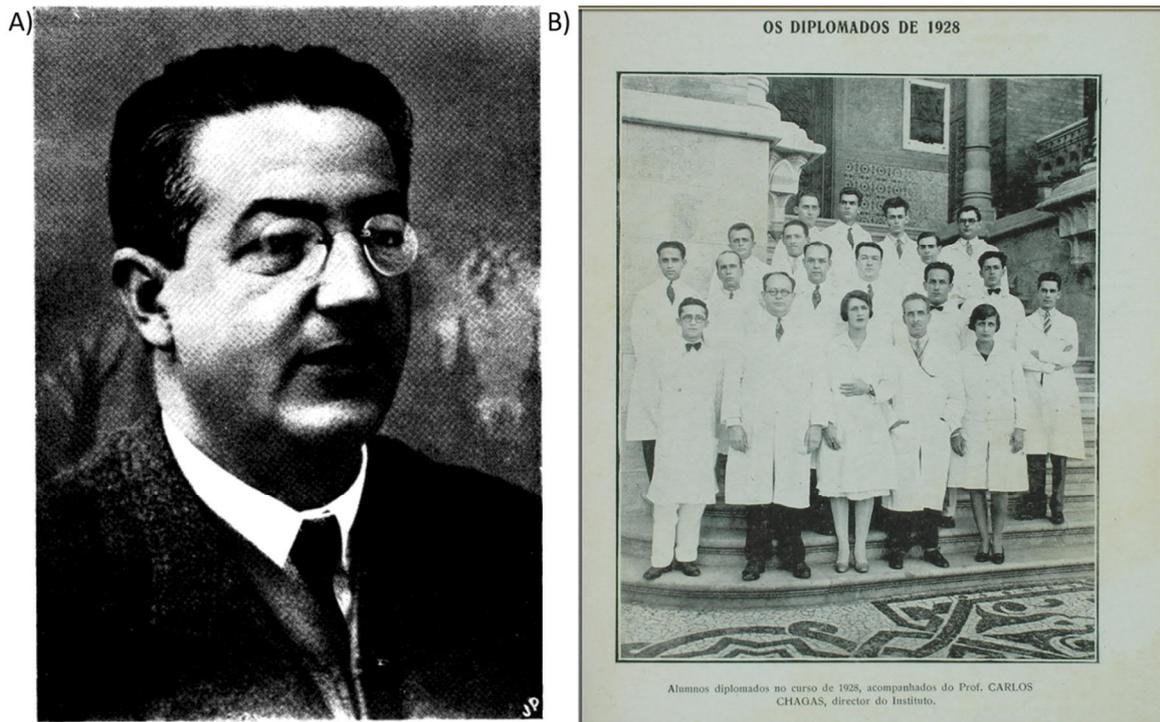
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517 Figure 1: Images related to Brazilian phage therapy.

518 A) Dr José da Costa Cruz, phage therapy pioneer in Brazil. Picture from his obituary¹¹. B) Students approved on
 519 an applied course offered by the Institute Oswaldo Cruz in 1928, in which "bacteriophagy" was included in the
 520 syllabus²⁵. These images are in public domain.

521



522

523 Figure 2: Advertisements for phage therapy products in Brazil.

524 A) Advertisement for the *Bacteriophagina dysenterica* phage preparation made by the Institute Oswaldo Cruz,
 525 printed at the Folha de São Paulo newspaper in 1928. B) Advertisement for the *Lisogenina-coli* product sold by
 526 the Raul Leite Laboratory, printed in the Brasil-Medico journal in 1935 (n.14, p. 328). C) Advertisement for the
 527 *Lactozym Alfa* product, which combined a polyvalent phage to a lactic probiotic, 1934. D) Advertisement of

528 many phage preparations made by the Raul Leite Laboratory, 1936. C and D were printed on the “O Bisturi”
 529 periodic, which contained general and medical related information for medicine students (1934, n.8, p.6 and
 530 1936, n.15, p.4 respectively). These images are in public domain.

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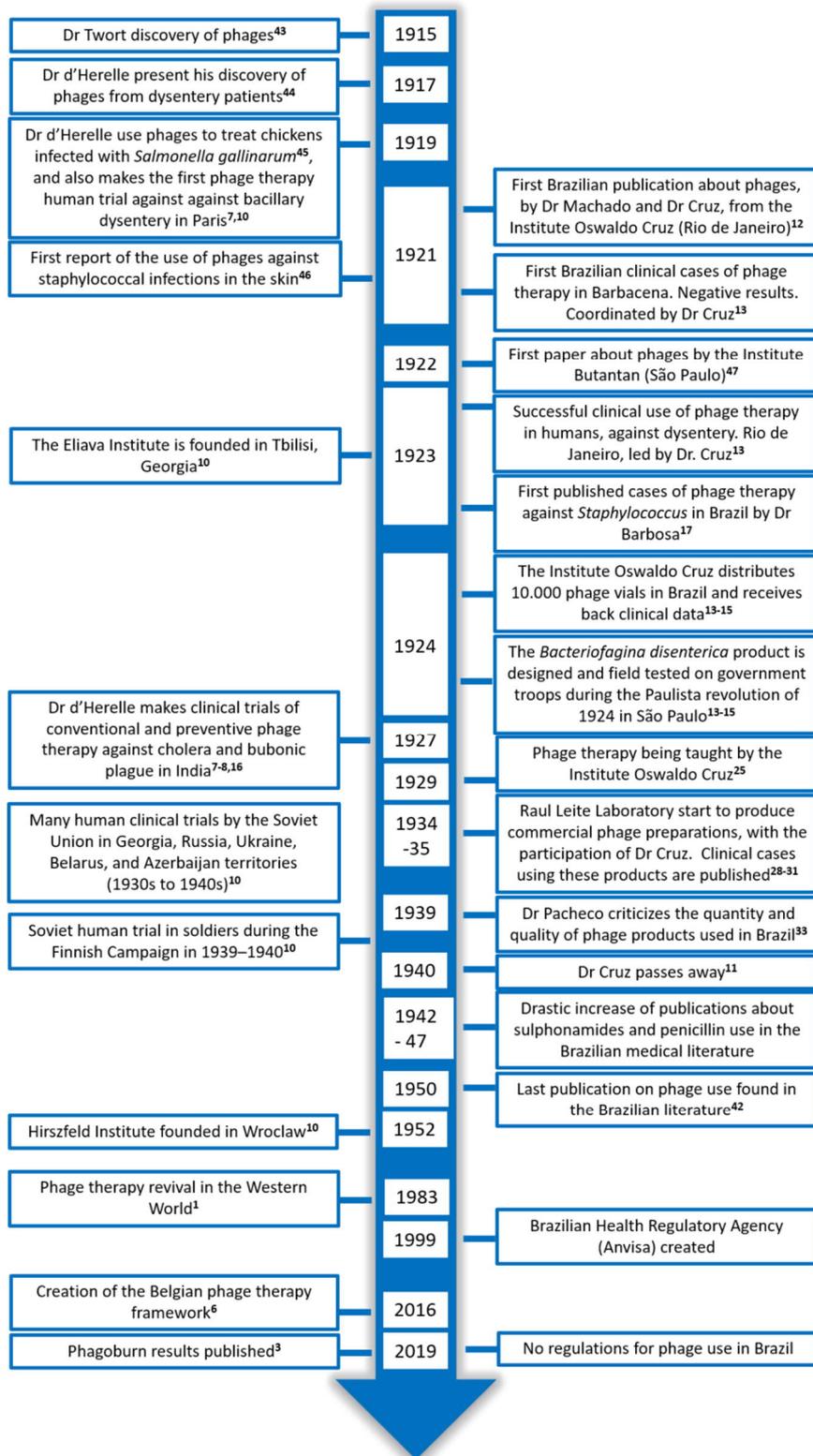


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533 **Figure 3: Advertisements for sulphonamides and penicillin in the Brazilian medical literature (1938-**
 534 **1947).**

535 A) One of the earliest advertisements for sulphonamides found (Brasil-Medico, n.18, 1938). B) Advertisement
 536 from an American sulphonamide preparation (Brasil-Medico, n. 16-17, p.26, 1945). C) Penicillin advertisement
 537 from a company in São Paulo (Brasil-Medico, n.42-43 , p.27, 1946). D) Penicillin advertisement from the Raul
 538 Leite Laboratory (Brasil-Medico, n.38-39, p.2, 1947). These products were imported from the United States of
 539 America and sold by Brazilian representatives. The images are in public domain.

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541

542 **Figure 4: Timeline showing major phage therapy milestones.**

543 On the left there are the well-documented international events, and on the right the events specific for Brazilian
 544 phage therapy. These were hidden in old publications and became known through this review. The starting time
 545 point is the original publication by Dr Twort describing phages in 1915, and the last time point is the publication
 546 of the results from the PhagoBurn European phage therapy clinical trial in 2019.

547 **Table 1:** Compilation of phage therapy clinical cases published in the Brazilian literature in the first half of the
 548 twentieth century. An empty entry means that the information was not mentioned on the case description.

Bacterial agent	Case description	Age	Sex	Survived	Number of phage doses	Details	Phage administration routine	Other treatments	Reference and year publication
Hiss Bacillus	6 days of stomach pain and diarrhoea	25	Male	Yes	2	Better few hours after dose 1, cured on next day	20 cc. , one dose per day		13 ; 1923
Hiss Bacillus	4 days of vomiting, fever, headaches and body pain followed by bloody diarrhoea	16	Male	Yes	2	Better few hours after dose 1, cured on next day	20 cc. , one dose per day		13 ; 1923
Hiss Bacillus	2 days of stomach pain and fever followed by bloody diarrhoea	17	Female	Yes	1	Cured after the first dose			13 ; 1923
Hiss Bacillus	3 days of bloody diarrhoea	11	Female	Yes	3	Better 2 hours after dose 1, cured 4 days later	One dose per day		13 ; 1923
Flexner Bacillus	Fever, headaches, diarrhoea	32	Male	Yes	3	First dose cured diarrhoea, two more to cure stomach pain	Two days interval between doses 1 and 2		13 ; 1923
Flexner Bacillus	Chronic dysentery for years with monthly crisis	36	Male	Not cured	at least one	The patient bacterial strain was shown to be phage resistant <i>in vitro</i>			13 ; 1923
Hiss Bacillus	Diarrhoea	16	Female	Yes	3	Cured 48 hours after dose 1	12 hours interval between doses		13 ; 1923
Hiss Bacillus	3 days of diarrhoea	40	Female	Yes	3	Cured	One dose per day		13 ; 1923
No bacillus isolated	Diarrhoea	42	Male	Yes	1	Felt cured 48 hours later			13 ; 1923
Flexner Bacillus	Diarrhoea	Widow [#]	Female	Yes	2	Cured after 2 days			13 ; 1923
Flexner Bacillus	Diarrhoea	23	Male	Yes	3	Cured after 2 days			13 ; 1923
Shiga Bacillus	2 months of diarrhoea	19	Male	Yes	many	Better after dose 1, cured after a few days	12 hours interval between doses	Many other treatments including emetine injections	13 ; 1923
Fecal exams not made	2 days of bloody diarrhoea	Married [#]	Male	Yes	3	Better after dose 1, cured after dose 3	12 hours interval between doses		13 ; 1923
Fecal exams not made	Bloody diarrhoea, stomach pain, fever	42	Female	Yes	at least one	Better after dose 1, cured after 2 days			13 ; 1923
Fecal exams not made	Diarrhoea and fever	6	Male	Yes	at least one	Cured in 24h			13 ; 1923
Fecal exams not made	Fever	4	Male	Yes	at least one	Cured in 24h			13 ; 1923
Fecal exams not made	5 days of dysentery	39	Female	Yes	2	Better after dose 1, cured after dose 2			13 ; 1923
Fecal exams not made	2 days of diarrhoea, fever, vomiting and stomach pain	5	Male	Yes	2	Better after dose 1, cured after dose 2			13 ; 1923
Fecal exams not made	6 days of diarrhoea, vomiting and stomach pain	3	Male	Yes	3	No recovery after dose 1, better after 2, cured after 3			13 ; 1923
Fecal exams not made	2 months of intestinal problems, with recent fever and diarrhoea	2	Female	Yes	1	Immediate recovery			13 ; 1923

Fecal exams not made	Diarrhoea, fever and stomach pain	4	Male	Yes	1	Immediate recovery			13 ; 1923
Fecal exams not made	Dysentery		Female	Yes	2	Cured after dose 2		Emetine	13 ; 1923
Fecal exams not made	Fever, stomach pain and bloody diarrhoea for 4 days		Male	Yes	2	Better after dose 1, cured after dose 2			13 ; 1923
Fecal exams not made	6 months of dysentery	16	Male	Yes	6	Better after dose 2, stomach pains cured after four more doses		Other medications (serum, emetine, others not specified)	13 ; 1923
Staphylococcal	Tibia osteomyelitis followed by pyelonephritis	Around 40	Female	Yes	1	Urine culture sterile 2 days after phage treatment	Injection of 1 cc.	23 days of "various therapeutic resources" before phages	17 ; 1923
Staphylococcal	Kidney pain and painful urination	Madam [#]	Female	Yes	1	Urine culture clearer after 24h later and sterile after 3 days	Injection of 2 cc.	Rotropine	17 ; 1923
Staphylococcal	Infected grenade wound during the "recent revolution"	Lady [#]	Female	Yes	Several	Pus almost gone after first dose	Injection of 40 cc. on the wound (daily at first, then every 2 days)	Removal of necrotic tissue, 15 days of common antiseptics	17 ; 1923
	Dysentery (around 50 cases)			Yes		Cure. Phage preparation provided by the Institute Oswaldo Cruz.			21 ; 1924
Coli bacillus	Urinary tract infection resistant to conventional treatments	6	Female	Yes	13	Cure with sterile urine and weight gain. No recurrent episodes.	5 intravesical injections, 3 subcutaneous injections and 5 oral doses	Other treatments before phage therapy (not mentioned)	24 ; 1929
Staphylococcal	Pyodermitis (9 cases)			Yes		Cure with quick reduction of pain and inflammation	Polyvalent anti-staphylococcal phage preparation made in Rio Grande do Sul		24 ; 1929
Staphylococcal	Furunculosis (32 cases)			Yes		Cure with quick reduction of pain and inflammation, no recurrent episodes	Polyvalent anti-staphylococcal phage preparation made in Rio Grande do Sul		24 ; 1929
Staphylococcal	Septicemia preceded by furunculosis	20	Male	Yes	6 injected + 1 topical	Better after dose 2, negative haemoculture after dose 3	5-7 days interval between doses	Many others (not specified)	27 ; 1934 32 ; 1938
Staphylococcal	Staphylococcal furunculosis for 1 year	4	Female	Yes	Several	Completely cured after a few doses	"Estaphylofagina" product used		28 ; 1935
	Pyelitis, dhiarrea, urine incontinence, daily fever, undernourished	2	Female	Yes		Fever disappeared in 3 days and she completely recovered	"Colifagina" product from Raul Leite laboratory used		29 ; 1935
Not mentioned	Long term pyelitis	3		Yes	6 ampoules	Better after dose 3, cured after dose 6	"Colifagina" product used		30 ; 1935
Not mentioned	Osteomyelitis (unknown cause)	41	Male	Yes	30 injections	Pain gone after dose 3. Suppuration stopped after dose 6. Cured after dose 30.	"Estafilofagina" from Raul Leite Laboratory used	Surgery and scraping of infected tissue	31 ; 1935
Staphylococcal	Septicemia preceded by knee furuncle	12	Male	No	Few drops	Phage therapy discontinued after reaction to	Only a few drops injected	Blood transfusion	32 ; 1938

						a contaminated injection			
Staphylococcal	Septicemia preceded by osteomyelitis	10	Male	Yes	5	Negative haemoculture after dose 2		Blood transfusions, trepanation, draining of infected tissue, carbon injections	32 ; 1938
Staphylococcal	Septicemia preceded by osteomyelitis	Adult [#]	Male	No	5	Negative haemoculture after dose 1		Blood transfusion, bone surgery, "Staphylococcal anatoxin" imported from the Pasteur Institute	32 ; 1938
Staphylococcal	Septicemia preceded by furunculosis and hemorrhoids abcess		Male	No	6	Negative haemoculture after dose 3		Only phage therapy	32 ; 1938
Staphylococcal	Septicemia preceded osteomyelitis	12	Male	No	2	Passed away from surgery complications		Only phage therapy	32 ; 1938
Staphylococcal	Septicemia preceded osteomyelitis	Adult [#]	Male	Yes	At least 2	Negative haemoculture after dose 2		Only phage therapy	32 ; 1938
Staphylococcal	Acute septicemia preceded by provoked abortion	around 30	Female	No	1	Did not survive for dose 2 (acute disease)		Only phage therapy	32 ; 1938

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550 # Although no precise age was recorded, these designations used to describe the patient gives an idea about the
551 age group.

552 Grey lines represent several cases mentioned together in the publications, with no individual details.

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569 **Phage research in Brazil: investigating the nature of phages**

570 The first paper mentioning phages in the Brazilian literature was published by Dr Cruz and Dr A. Machado in
571 1921¹. The paper makes clear that the true nature of phages was still questionable, and that different points of
572 view existed at the time. Three theories are presented in the paper: a filterable virus capable of parasiting
573 bacteria as considered by Dr d'Herelle ; a mutation in the bacteria provoked by an immunised host which leads
574 to secretion of a bacteriolytic ferment as defended by Dr Bordet ; or by a precursor in the bacteria activated by
575 intestinal kinases as supposed by Dr Kabeshima. The presented results show that the addition of sera against the
576 bacterial host in cultures containing phages and hosts abolished the activity of the phage. Since addition of
577 specific antibacterial sera abolished phage activity, the conclusion was that phages could be a ferment released
578 by dead bacterial cells when lysed in a special way. This idea appears again in other publications by Dr Cruz. In
579 a paper published in 1922, seven phages were isolated from eight stool samples from patients with Shiga
580 bacillus infection². These were tested for host range, adaptation to other strains, growth conditions and resistant
581 hosts were isolated. Then a series of immunological tests using sera from patients and from animals injected
582 with bacteria or with the phages were presented, indicating that the phage adhered to the bacterium by the same
583 specific adsorption process used by antibodies. Although mentioning that most authors believed in Dr d'Herelle
584 opinions, Dr Cruz concludes the paper with the idea that the phage was a ferment, a sort of catalyser present in
585 the bacterium used for the disintegration of membranes that lysis cultures if released by cell death. It is even
586 stated that, "*without any pretension to affirm absolute truths*", that their point of view linked the phenomena of
587 lysis in series to humoral immunity. In a follow-up paper published in the same year he studies sera against the
588 Flexner phage made in rabbits³. The results show that specific sera inactivated the phage lytic activity, while
589 sera against the host protected it from phage lysis. He also selected, *in vitro*, a phage resistant to the sera.
590 Interestingly, this phage was not as efficient as the original one in infecting its host. In the end, Dr Cruz
591 mentions that the data could be interpreted from Dr Bordet's point of view, but that Dr d'Herelle's opinion on
592 the viral nature of phages offers an easier explanation. From these publications it is clear that Dr Cruz was
593 divided between opinions on the nature of phages, but his immunology expertise led him to interpret the
594 problem from an immunological point of view.

595 Dr Cruz's investigations of the nature of phages continued in the following years. One publication describes the
596 influence of electrolytes on phage-mediated lysis, proposing it to be a way to end the controversies regarding the
597 nature of phages⁴. The addition of NaCl, CaCl or NaSOMg improved phage lytic activity, taken as proof that
598 phages were closer to ferments and antibodies than to viruses. This was further investigated in another
599 publication in the same year⁵. The introduction cites many evidence that phages could be living viruses,
600 including the fact that they easily adapt to adverse conditions, but the publication concludes that their other
601 properties have made most researchers reject the idea. After showing that phages, as antibodies, were electrolyte
602 dependent, he compared the activity of phage solutions made in distilled water, passed through filters and the
603 filter eluate by physiological solutions. The conclusion is that electrolytes dispersed phages (as with antibodies),
604 keeping them in the right state to act, while tending to flocculate living beings. Saying that phages are not alive
605 and have the physical properties similar to antibodies, he speculates whether other viruses (chicken pest,
606 smallpox, rabies) would also behave the same. At the same year his connection to the international literature is
607 shown when he responds, with simple experiments and discussion, two other papers that had diverging results or
608 conclusions when compared to his own⁶. The first paper is related to the action of sera on phage activity, and the
609 second to phage-mediated lysis in distilled water. In the same publication a small test of phage resistance to
610 silver is presented, showing that lytic activity was abolished in the right conditions. In a follow-up paper he
611 tested whether acid conditions would make phages precipitate as with antibodies⁷. The conclusion is that they
612 did, strengthening his theory on the nature of phages. He also presented these results to the international
613 community via publications in French⁸⁻¹¹. In 1929, Dr Eduardo de Araujo (director of the Institute Oswaldo
614 Cruz from Bahia state) presented a note on the presence of phages in different water sources from his state,
615 demonstrating that other researchers from the Institute Oswaldo Cruz were also interested in phage biology¹².

616 Meanwhile, publications on the nature of phages from the Butantan Institute in São Paulo also appeared in the
617 Brazilian literature, with no clear connection to the work done at the Institute Oswaldo Cruz. The first
618 publication is a paper divided into three sections¹³. The first part describes that Dr d'Herelle's experiments on
619 phages were successfully repeated and confirmed by the isolation of bacteria and phage from human stool
620 samples. The second tests the idea that phages could originate from bacteria, and the third focuses on the nature

621 of the phage and whether it could lyse red blood cells and influence the bacterial fermentation of sugars. The
622 third section is further investigated by another publication of the same Institute, which concludes that the phage
623 did not act on a hemotoxin not on dysenteric toxins but had an influence on some sugars and on the fermentative
624 activity of a bacillus¹⁴. Another paper provides an introduction about Dr d’Herelle’s work on the microbial lysis
625 in series¹⁵. It focuses on finding lytic activity from liquid and solid cultures of different bacterial strains, finding
626 discreet lysis for many and hypothesising that lysins secreted by bacterial cells *in vitro* were less active than the
627 ones secreted by bacteria infecting an animal as in the case of Dr d’Herelle’s experiments. The fourth paper
628 mentions that the enthusiasm following the Twort-d’Herelle phenomenon resulted in many researchers claiming
629 to have found “*the bacteriophage*” in many organic liquids without taking proper care regarding the basic facts
630 of the “*bacteriophagy*”¹⁶. The bacteriolytic activity from the venom of different snake species was tested.
631 Although many had lytic activity, the destroyed cells did not result in the “*formation of a new lytic principle*”
632 and the conclusion states that the lysis mediated by venoms was unrelated to phages. In 1923, another paper on
633 the nature of phages was published, with an introduction mentioning the history behind phage discovery and
634 discussions of the nature of phages, acknowledging Dr Twort’s role¹⁷. Inspired by international authors who
635 described lytic activity from river water, such as Ernest Hankin’s pioneer study, Dr Monteiro shows that it also
636 happened with waters from the rivers Pinheiros and Tiete in São Paulo¹⁸. Years later, he investigated the
637 presence of phages in bovines used for the preparation of the Brazilian smallpox vaccine and in the vaccine
638 itself, linking his data to Dr Twort’s phage description¹⁹. The last publication mentioning phages from the
639 Butantan Institute is from 1942, in which phages were used as one of many variables to study the effect of an
640 electrical current in microorganisms²⁰. It is interesting to note that another Brazilian Institute related to public
641 health also showed an interest in phages in the past, although only these papers have been retrieved from the
642 literature. A contact with the Documentation Nucleus of the Butantan Institute revealed that there is a folder in
643 their collection named “*Notes and studies on the nature of bacteriophages*” among the documents from Dr
644 Lemos Monteiro, dated from 1920-1935. These documents are not available in digital format and can only be
645 accessed on site after a formal request. Until further information is found, the Butantan Institute did not studied
646 applied phage therapy nor made phage products.

647 Years later, two other papers investigating the nature of phages appeared, with no affiliation given to the author.
648 The first discusses “*bacteriophagy*”, mentioned to be a phenomenon more interesting from the biological than
649 from the therapeutical or microbiological point of view²¹. It states that understanding the mechanism of the lytic
650 phenomenon would result in solving several problems related to it, with profitable rewards for therapeutic use.
651 The author believed that phages came from the bacteria and acted by digesting the bacterial cells, resulting in
652 “*bacteriophagy*”. The difference among viruses, phages and toxins would be only the level of biochemical
653 complexity, with phages and viruses being at the same level. In the second paper, the nature of phages and
654 phage mediated lysis is studied from an interaction between liquids and solids perspective²². The hypothesis was
655 that mixing phages and bacteria would lead to three states: only bacteria after phage assimilation (minimum
656 hydration state), only phages after complete lysis (maximum hydration state), or mixed cultures (intermediate).
657 The “*bacteriophagic virulence*” is mentioned, phages are described as a proteinaceous substance, and Dr
658 d’Herelle is cited on many occasions, so the author could have been a defender of the viral hypothesis. He also
659 investigates the influence of water on phage activity and studies bacterial resistance to phages, correlating it to
660 cell morphology. The conclusion is that phages were a product of the hydrolytic disintegration of the bacterial
661 cell, resulting in a virus capable of reproducing in series (an analogy to the Rous sarcoma virus was made). The
662 paper presents a drawing depicting phages as spheres surrounded by water, and it may be one of the earliest
663 attempts to represent a phage structure in the literature (**supplementary Figure 1**). Also, in 1929, Dr Cruz
664 presented an extensive review on the nature and properties of phages in a South American medical conference²³.

665 In 1940 Dr Cruz published a review on the properties, nature and therapeutic use of phages²⁴. The introduction
666 mentions Dr Twort and Dr d’Herelle, highlighting that the latter was responsible for the description of the
667 “*bacteriophagy*” phenomenon. Then he argues against Dr d’Herelle view on the viral nature of phages,
668 discussing that the “*serial transmission*” was not an inherent characteristic of living beings, pointing out flaws
669 in experiments made to adapt phages to different conditions, besides citing his own results with salts and
670 specific sera. He concludes that phages belonged to the group of ultra-microscopic viruses, which comprised
671 agents of varied nature, biotic and abiotic. Dr Cruz mentions that phytopatologists at the time considered that
672 some plant diseases, such as tobacco mosaic, could be caused by processes not parasitic in nature and discusses
673 literature on this subject. In 1943, a summary from a paper published in 1942 appeared describing seasonal
674 variations of typhoid-paratyphoid phages in rivers from the city of São Paulo²⁵. Phages were found all year long,
675 but in less abundance in colder months and during rainy periods. Phage load varied between the two rivers

676 sampled, and phage quantity from the rivers was directly proportional to the number of human disease cases in
677 the same period. This is the latest publication concerning the nature of phages on the literature and timeframe
678 searched.

679 The number of publications investigating the nature of phages published in the Brazilian literature can be seen
680 as proof of how this subject was intriguing at the time. Although many important observations were made, some
681 Brazilian researchers who worked on the subject missed the nature of phages as viruses, including Dr Cruz. In
682 hindsight, it is easy to detect flaws, but we must remember that at the time phages were a mystery for
683 microbiologists, and the confirmation of their viral nature only came in the 1940s with the advent of the electron
684 microscope²⁶.

685

686 **Origins of the phages used for phage therapy in Brazil**

687 Two independent publications, one from the Instituto Oswaldo Cruz in Rio de Janeiro and the other from the
688 Instituto Butantan in São Paulo, described the isolation of phages from Brazilian samples in 1922^{1,13}. However,
689 already in 1921, Dr Cruz made his first clinical tests against dysentery at the city of Barbacena. In addition, Dr
690 Monteiro mentioned that the Instituto Butantan had been working with phages for about a year at the time of his
691 1922 publication. Thus, phages were already present at both Institutes before their publications describing phage
692 isolation in 1922. Were these phages isolated from Brazilian samples in unpublished experiments or were they
693 obtained from abroad?

694 There is no clear evidence that Brazilian doctors had any direct collaboration with phage researchers from
695 abroad, such as Dr d'Herelle or Dr Eliava. However, it is clear that there was a certain degree of intellectual and
696 material exchange between Brazilian and foreign doctors at the time. Dr Cruz himself studied in Portugal
697 before starting his medicine course in Rio de Janeiro. Besides, it was stated that Dr Jules Bordet* once said that
698 Dr Cruz was the most brilliant student he ever had²⁷. However, when exactly Dr Cruz interacted with Dr Bordet
699 is not known. Dr Cruz's obituary mentions that he was in Europe in 1925 and 1931 for improvement studies, but
700 no details are given regarding which Institution or what the focus of the studies was²⁸. From the Instituto
701 Butantan side, one publication from 1922 reveals that one of the authors (Professor R. Kraus) studied phages in
702 Buenos Aires, Argentina¹³. There is also evidence of material exchange between Brazil and the Instituto Pasteur,
703 although the material in these documented cases was not phage related. For treating a difficult septicaemia case
704 in the 1930s, the Instituto Oswaldo Cruz received a sample of staphylococcal anatoxin by plane from the Pasteur
705 Institute²⁹. Brazilian samples were also sent to Paris by Dr Cruz²⁷.

706 Phage exchange was mentioned a few years later. In 1923 Dr Barbosa revealed that the phage used to treat
707 staphylococcal infections in Brazil was isolated by Dr Andre Gratia of the Instituto Pasteur of Brussels in 1921,
708 and obtained by Dr Madeira from Professor Bordet³⁰. Then it was adapted to white and golden staphylococci
709 isolated from Brazilian patients for phage therapy use. In his 1938 publication, Dr Cruz acknowledges that the
710 phage used against staphylococcal septicaemias was the "staphylococcal phage H isolated by Gratia"²⁹. This
711 phage was able to infect all isolates from the clinical cases treated, but how it was obtained by Dr Cruz (a
712 second exchange between Brazil and Europe, or given by Brazilian colleagues working with Dr Barbosa) is not
713 mentioned.

714 Although it is clear that the phages used in the first Brazilian phage therapy cases against staphylococcal
715 infections came from abroad, could the phages from an international collection, such as the one from Dr
716 d'Herelle from the Instituto Pasteur in France, be the ones used for the 1921 clinical cases in Barbacena? If the
717 phages used were not adapted to Brazilian host strains, this could explain the failure. Then, after isolating
718 phages himself, Dr Cruz would have used Brazilian phages for the successful 1923 testing. However, this is
719 only speculative. The differences between the 1921 and 1923 phage therapy testings are not clear. The origin of
720 the phages used in each is not mentioned, there are no details about the number of phage treated patients in
721 1921, and no information about the better working conditions of the 1923 cases is given. However, in a 1940
722 review, Dr Cruz revealed that, in 1921, he gave the phage ampoules to the medical staff responsible to treat the
723 outbreak, and only later received the report that the results were negative²⁴. The location of the 1921 cases,
724 which was far from the Instituto Oswaldo Cruz, could have posed a problem for keeping the phage preparations
725 stable between production in Rio de Janeiro and transport until its final use in Barbacena. A combination of Dr
726 Cruz not being directly involved with the treatments, the composition of the early prototype product and its

727 stability during transportation and storage could have influenced the clinical outcome. In 1923, the product
728 could have received improvements in its formulation, the outbreak location was close to the production site, and
729 Dr Cruz was directly involved in the cases. Regardless of the origins, the phages used for the successful trial in
730 1923 were tested for one year and in 1924 they were used as the base for the *Bacteriophage disenterica* product.

731

732 * Dr Jules Bordet mentioned is probably the Belgian immunologist and Nobel Prize winner in 1919 for his work
733 on antibodies and the complement system.

734

735 **International collaborations**

736 As mentioned above no direct connection between Brazilian doctors and international phage therapy excellence
737 centres, such as the Eliava and Pasteur Institutes, were found. The closest evidence of phage related exchanges
738 was the staphylococcal phage obtained from the Institute Pasteur of Brussels. However, indirect evidence of
739 international collaborations or at least exchange of intellectual information existed.

740 Part of Dr Cruz work was published in French and cited by phage researchers of the time, including Dr
741 d'Herelle, demonstrating that Brazilian phage therapy efforts were reaching Europe and other leading experts in
742 the field. Dr Eliava himself mentions Brazilian phage therapy practice as a successful example when requesting
743 funds from the Soviet authorities for expanding his own Institute in Georgia, in the 1930s³¹. Dr Kraus, from the
744 Institute Butantan in São Paulo, was for some time in Buenos Aires (Argentina) studying phages, but no details
745 are given regarding in which Institution¹³. In 1923, Argentinian phage work was acknowledged by Dr Cruz
746 when he mentioned Dr Pico's studies⁶. Phage use in Argentina is mentioned again by Brazilians in 1924, against
747 colites, nephrites, pielites, typho, dysentery, septicaemia, endocarditis and rheumatism³². This may indicate an
748 intense exchange of information and maybe collaborations between Brazil and Argentina in the early 1920s.
749 Also, it points that Argentina may have been another unacknowledged South American hotspot for phage
750 therapy and research in the past. During the *IV South American Conference of Hygiene, Microbiology and*
751 *Pathology* held in 1929, Dr Cruz (Institute Oswaldo Cruz) and Dr Pereira (Medicine School of Porto Alegre)
752 presented data about their own experiences with phage therapy^{23,33}. The conference had delegates from at least
753 Chile, Argentina and Uruguay, proving that the Brazilian experience with phage therapy was being shown to
754 doctors from other South American countries.

755 Much later, in 1950, an author from the Hygiene Municipal Institute in Warsaw published a therapeutic note in
756 the *Brasil-Medico* journal³⁴. Written in Portuguese as a recommendation to the Brazilian medical community, it
757 focuses on the prophylactic use of phages to protect new-borns against diarrhoea. The note starts with an
758 introduction about the colonisation of new-born's digestive systems, followed by a description of the Polish
759 experience in protecting children by giving phages as the first liquid after birth, a "simple and harmless"
760 prophylactic mean to protect against intestinal infections. Although this suggestion to use phages preventively
761 came from Poland in 1950, no indication exists that it was considered or followed by Brazilian doctors. Also, no
762 other evidence about collaborations between Brazil and Poland at the time was found.

763

764 **Producers of phage preparations in Brazil**

765 Based on the researched literature, the first Brazilian phage preparation designed for mass human use was
766 produced by the Institute Oswaldo Cruz. This preparation targeted bacterial dysenteries and started to be
767 produced on a large scale around 1923, under the auspices of Dr Cruz and was named *Bacteriophage*
768 *disenterica*. An advertisement from 1928 (**Figure 2A, main text**) shows that the production site was in
769 Manguinhos (Rio de Janeiro). Part of the Institute Oswaldo Cruz, it produces biological products for human use
770 to this date.

771 The success of this phage preparation probably raised interest in particular laboratories, which also started to
772 produce phage preparations with varying qualities, as noted by Dr Pacheco in 1939³⁵. One particular laboratory,
773 often mentioned in clinical case descriptions and advertisements, is the Raul Leite Laboratory. While checking
774 the *Brasil-Medico* journal archives, what might have been a flyer named "A visit to the *Raul Leite Laboratories*"
775 was found³⁶. Located in Rio de Janeiro, it was founded as a flour factory in 1921 and became an industry for

776 biological and pharmaceutical products twelve years later. It is highlighted that investments in advertising led to
777 increased revenue and the consequent diversification of products. The Raul Leite Laboratory was also known
778 for its product line named “medicine for the poor”, which consisted of cheap packages with smaller amounts of
779 the medicine inside. Product quality was said to be the same, but the price of the packing and smaller quantities
780 were more accessible to people with a lower income (who could buy the medicine in parts during the treatment,
781 instead of buying the whole dose at the same time). The text does not mention phages, but lists as products oral
782 and injectable medicine, vaccines, hormones, sera and as future product prospects medicinal plants plus
783 veterinary products. The text states that Dr Pacheco, from the Institute Oswaldo Cruz, would manage the
784 production of the veterinary products. In 1935, an advertisement of a Raul Leite Laboratory phage product
785 (**Figure 2B, main text**) mentions Dr Cruz’s role in its production. Another advertisement from 1936 reveals that
786 six phage products from the Raul Leite Laboratory microbiology section were being sold in São Paulo (**Figure**
787 **2D, main text**). Publications on the clinical use of the Raul Leite Laboratory phage preparations appeared in
788 1935³⁷⁻⁴⁰. Taken together, it is possible to speculate that the appointment of Dr Pacheco as manager of a section
789 of the Raul Leite Laboratory in 1934 quickly led to the production of phage products, with the participation of
790 his colleague Dr Cruz. These products were advertised in medical journals, used in clinical publications, and
791 sold outside Rio de Janeiro. An advertisement from 1947 (**Figure 3D, main text**) shows that the Raul Leite
792 Laboratory continued to expand and became a national distributor for the penicillin-G produced by the
793 Commercial Solvents Corporation (C.S.C., New York, USA).

794

795 **Protocols for phage production and administration in Brazil**

796 The recorded phage products in early twentieth century Brazil includes the *Bacteriophage disenterica* product
797 from the Institute Oswaldo Cruz, the Raul Leite Laboratory preparations (*Estafilofagina*, *Colifagina*,
798 *Disenterifagina*, *Tifofagina*, *Estreptofagina* and *Plurifagina*, for oral or injected use) and the association
799 between polyvalent phages and a lactic probiotic from the Vicente Amato Sobrinho Company (*Lactozym Alfa*).
800 The advertisements and trademarks suggest that these were readily made products, probably prepared using
801 reference strains and bought from pharmacies, and not tailor made for each patient bacterial pathogen. Despite
802 the information gathered on the existence and use of these products in Brazil, no specific details could be found
803 about the technology used for mass scale production and purification. However, some information on how these
804 products were used and also of patient-specific phage preparations can be found in the literature.

805 During the successful 1923 trial against dysentery in Rio de Janeiro, patients were treated using the gastric
806 route, with a hydrocarbonated diet recommended to promote phage-mediated lysis⁴¹. A subsequent publication
807 recommends that the phage ampoules were to be diluted in 200 millilitres of water and drank twice a day⁴². This
808 is probably how the *Bacteriophage disenterica* preparation was used around Brazil. In Porto Alegre, doctors
809 made their own polyvalent phage preparations containing a mixture of anti-dysentery phages and these were
810 given to patients mixed with water, with five-hour intervals between doses³³. It is noted that there were no
811 restrictions to phage use, but an adequate diet was important and milk was not recommended.

812 Staphylococcal infections were often treated by local or intravenous injections of the phage preparations, with
813 descriptions of topical use in certain cases. In some cases phage products not adapted to the patient pathogen
814 were used with success³⁰, but growing the phages on the patient bacterial isolate for making personalised phage
815 preparations was often considered key for curing the infections. Dr Cruz published detailed protocols for the
816 production of tailored-made staphylococcal phage preparations and their use²⁹. Each phage preparation was
817 adapted to the bacterial strain isolated from the patient who was to be treated. During routine diagnostics of
818 septicaemia cases, haemocultures were made. An aliquot of the bacterium was taken from the patient’s
819 haemoculture, grown overnight in inclined agar, resuspended in broth the next day and used to inoculate one
820 200ml and four 10ml flasks containing peptoned-water. A half millilitre of a stock phage was added to three of
821 the 10ml flasks while the fourth 10ml flask was left as control. On the next day, the 10ml flasks inoculated with
822 the phages were added to the 200ml flask containing a liquid culture of the bacterium. After incubation (often
823 48h), the lysate was filtered in Chamberland filters, aliquoted into 20ml ampoules and sealed for later use on
824 that patient. At the time of use, one ampoule was opened and 15ml of the phage lysate were diluted in 250ml of
825 physiological solution. This mixture was injected slowly (gotta-injector Seabra) into the patient’s bloodstream,
826 at forty drops per minute, with the whole volume taking two hours to be injected. Injections were repeated every
827 five days until the patient was cured, with clinical sign evaluation and negative haemocultures used to
828 demonstrate that the treatment was effective. Immune reactions to the phage injections often occurred around

829 the third dose and were taken as an indication that the treatment was working. Other doctors used similar
830 protocols⁴³. The topical use of staphylococcal phage preparations was also made in cases of localised infections,
831 and even oral doses of these phages were used⁴⁴. In the case of oral use, the recommendation was to take the
832 phages diluted in cold water in the morning, with an empty stomach, during a three to four week-long treatment.
833 Furthermore, one publication mentions, without many details, intra-uterine phage therapy and also hypodermic
834 treatments in cases of puerperal infections³².

835

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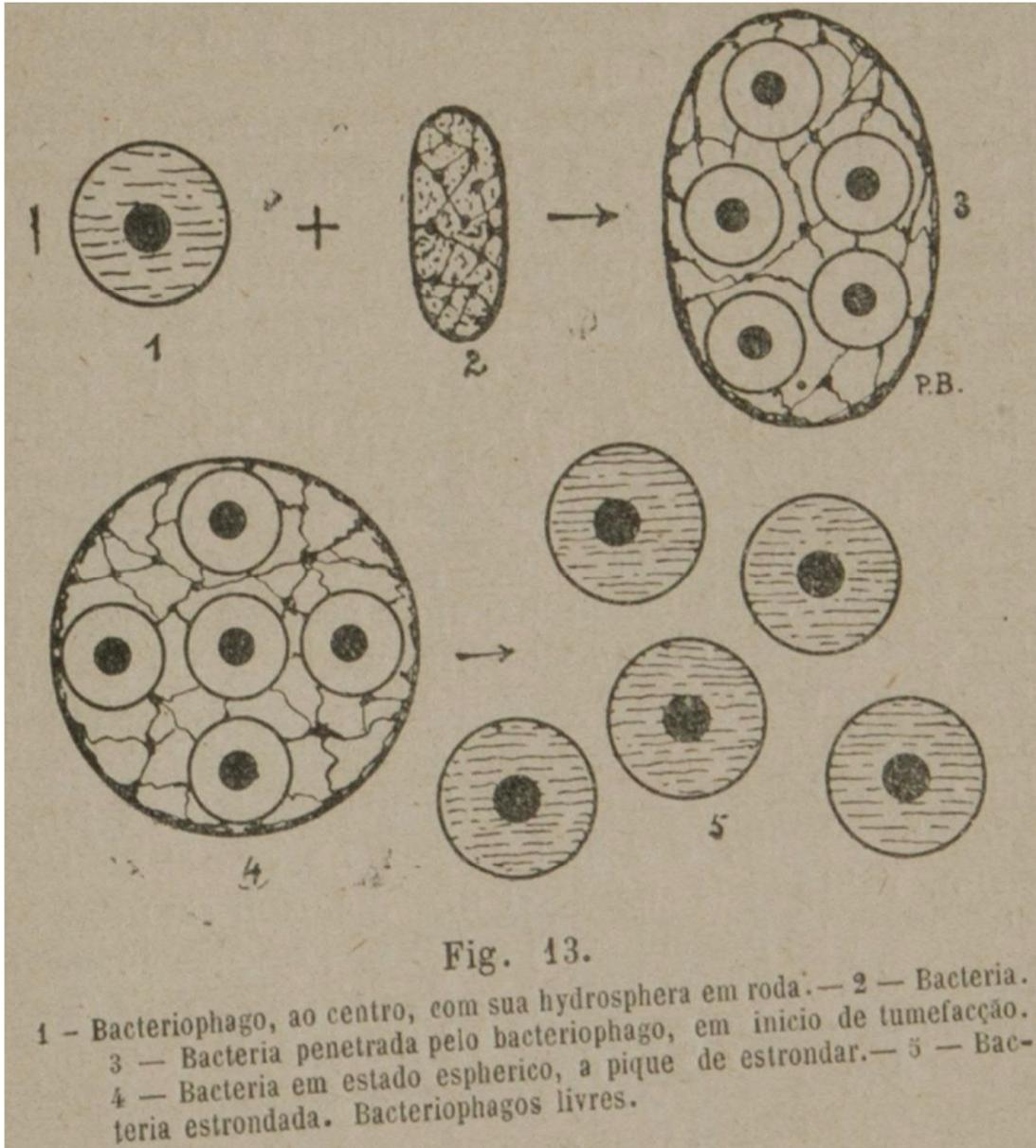
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913

914

915 **Supplementary figure legends**



916

917 **Supplementary figure 1:** An early attempt to represent phages published in 1929²². This image is in public
918 domain and thus can be reproduced here.

919