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Bacteriophages in Bacterial Cultures.

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In carrying out examinations of fecal specimens for the presence of bacteriophage, the 11 strains of *Eberthella typhi* from the American Type Culture Museum were utilized, together with 14 cultures of other organisms from our own laboratory. It was noted that most of the strains of *Eberthella typhi* were sensitive to bacteriophage action, but that two cultures appeared to be very resistant. The relative susceptibility of these cultures is shown by results from 39 fecal samples which contained bacteriophage active against one or more typhoid strains. The findings of the third generation against the 11 typhoid strains and 2 strains of *Escherichia coli* are shown in Table I.

TABLE I.

Relative Susceptibility to Bacteriophage Action. 39 fecal specimens. 3rd generation.

No.	Strain	Source	No. times susceptible
1	168	A. T. C. Museum	38
2	169	"	38
3	191	"	6
4	192	"	27
5	193	"	28
6	194	"	25
7	195	"	24
8	196	"	0
9	197	"	28
10	564	"	32
11	Thompson	"	22
12	B 70 A	Minn. Museum	6
13	B 71 A	"	0

Of the *Eb. typhi* cultures it is seen that strains 191 and 196 were very resistant to bacteriophage action. As no lysis of *Es. coli* B71A occurred while B70A was lysed 6 times, it would appear that B71A was relatively resistant.

The resistance of these cultures might be due to the presence of a bacteriophage in the cultures. In testing for the presence of a bacteriophage 12 more cultures were added to the group, making a total of 25. The additional cultures were: 3 species of *salmonella,* 1 strain *Eb. dysenteriae,* 1 strain *Eb. paradysenteriae,* 1 strain *Proteus vulgaris,* 3 species of *staphylococcus,* 2 species of *Aerobacter.*

These organisms were inoculated into plain broth and incubated for 24 hours. At the end of that time each culture was filtered through separate Berkfelt candles and the filtrates of each tested for activity against all 25 organisms. The test was performed by adding to a tube of plain broth 3/10 cc. of a 6 hour growth of the organism, and adding immediately following, 3/10 cc. of the filtrate to be tested. The culture was incubated 24 hours and read by comparing with a 24 hour growth used as a control. This reading represented the 1st generation. The broth was then filtered and the filtrate tested in an identical manner, the 2nd reading being the 2nd generation.

Of the 25 filtrates tested, filtrates from cultures 191, 196, and B71A were the only ones showing bacteriophage activity and their action was confined to the organisms listed in Table I. The activity of these filtrates in the 1st and 2nd generation is shown in Table II. In representing the comparative activity of the filtrates, the following definitions are used:

4+ Maximum bacteriophage action, no demonstrable growth, tube as clear as plain broth.

3+ Marked bacteriophage action, medium appears clear, growth apparent only by agitation.

2+ Definite bacteriophage action, marked clearing compared with control.

1+ Indefinite bacteriophage action, definite inhibition of growth when compared with control.

— No bacteriophage activity evident. Tube about as turbid as control.

TABLE II.
Bacteriophage Activity of Culture Filtrates.

			Cultures of Bacteria.														
		Gen.	168	169	191	192	193	194	195	196	197	564	Thompson	564	B70A	B71A
Culture Filtrates	191	1st	3+	3+	—	2+	—	2+	2+	—	—	2+	2+	—	—	—
		2nd	3+	3+	—	2+	—	—	2+	—	—	2+	—	—	—	—
	196	1st	3+	2+	—	2+	—	—	2+	—	—	1+	3+	—	—	—
		2nd	4+	3+	—	2+	—	—	2+	—	—	1+	3+	—	—	—
	B71A	1st	—	—	—	—	—	—	—	—	—	—	—	—	2+	—
		2nd	—	—	—	—	—	—	—	—	—	—	—	—	2+	—

The culture filtrates in each case were inactive against the culture from which they were derived. The active filtrates were most active

against cultures 168 and 169 which were also the most susceptible to the filtrates from fecal specimens.

The American Type Culture Museum's *Eb. typhi* cultures Nos. 191 and 196 apparently carry with them a bacteriophage. These bacteriophages are almost identical in their activity on the other museum strains. The resistance of these cultures to the bacteriophage action of feces filtrates is explained by the presence in the cultures of a bacteriophage. The cultures consist almost entirely of resistant cells, but there is probably produced in the growing culture enough susceptible cells to allow the bacteriophage to continue to multiply and maintain its presence.

Conclusion. 1. Cultures No. 168 and No. 169 are the most susceptible to bacteriophage action and are the most dependable to demonstrate typhoid bacteriophages in feces filtrates.

2. Cultures No. 191 and No. 196 are very resistant to bacteriophage action and give little indication of the presence of a bacteriophage when used as test organisms.

3. Cultures No. 191 and No. 196 carry with them a bacteriophage exhibiting activity against most other strains, but showing no reciprocal or homologous activity.

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Biological Study of Mushroom Extract and Effect of Sodium Ricinoleate on its Toxicity.

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A watery extract of the poisonous mushroom, *Amanita-phalloides,* was prepared according to the method of Ford.[1, 2] The extract was concentrated and sterilized by filtration. Using 1 cc. of a 5 per cent suspension of red blood corpuscles, 0.02 cc. of the extract produced complete hemolysis of guinea pig corpuscles. When injected into guinea pigs 1 cc. of the extract was sufficient to kill a 300 gm. guinea pig in 5 days. Upon the addition of sodium ricinoleate a precipitate was formed. A modified extract was prepared by the addition of sodium phosphate (Na_3PO_4), the precipitate being removed by filtration. Modified extract gave a clear, dark brown solution with sodium ricinoleate. 0.10 of this extract was necessary to hemolyze 1 cc. of a 5 per cent suspension of guinea pig erythro-