SOME OBSERVATIONS ON THE COLI/COLIPHAGE RELATIONSHIP IN SEWAGE

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It has been observed that during the treatment of sewage by biological filtration the numbers of coliform bacteria are considerably reduced (Allen, Tomlinson & Norton, 1944; Allen, Brooks & Williams, 1949). As it was thought that this reduction might be due to the action of bacteriophage known to be present in sewage, a short investigation to obtain evidence on this point was made at Stevenage sewage works.

At this works sewage from the residential and industrial areas of the town is mixed and screened, one-third of the flow being then treated in the old section of the works and two-thirds in a modern extension. In each section the sewage is settled in continuous flow tanks, treated biologically in percolating filters, and settled again in humus tanks before it flows to a sump, common to the two sections, from which it is pumped intermittently.

METHODS

Enumeration and isolation of coliform organisms

Counts of coliform bacteria were made from sewage taken at different stages of treatment by spreading 0·1 ml. of a suitable dilution in saline over the surface of Bacto eosin-methylene-blue agar and counting typical coliform colonies after incubation for 24 hr. at 37° C. This medium was almost completely specific for coliform organisms.

Over a period of several weeks pure cultures of forty-nine strains of coliform organism were obtained from the influent crude sewage and a further fifty from the effluent in the final sump by plating on double strength Bacto eosin-methylene-blue agar and subculturing typical *Bacterium coli* colonies twice on single strength eosin-methylene-blue medium; the resulting cultures were maintained on nutrient agar. All the organisms were subsequently confirmed biochemically to be either *Bact. coli*, *Bact. aerogenes*, or intermediates.

Isolation and enrichment of specific bacteriophage

Ten ml. of each of the sewage samples, of which a portion had been used for inoculation on to eosin-methylene-blue agar, was immediately treated with 3 drops of toluene at 37° C. for 2 hr. This procedure killed the majority of the bacteria in the sewage without affecting the bacteriophage count. The purified bacteriophage suspensions were stored in a refrigerator until the pure culture of the coliform isolated from the same sample was available. Fifteen ml. of nutrient broth was then mixed with 5 ml. of toluene-treated suspension, drops of free toluene being

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carefully excluded, and two drops of a logarithmic phase broth culture of the coliform organism, isolated from the same sewage sample, were added. The culture was incubated overnight at 37° C. On the morrow a portion was centrifuged to remove the majority of the bacteria and 1 ml. of the supernatant enriched bacteriophage suspension was mixed with 1 ml. of a logarithmic phase broth culture of the homologous coliform organism and 8 ml. of cooled molten 0·8 % nutrient agar. This mixture was layered on a 2 % nutrient agar plate. After incubation for 24 hr. the cultures were examined. The presence of bacteriophage plaques irrespective of number indicated that the bacterium was susceptible to co-present bacteriophage.

Each organism was also similarly tested for susceptibility to bacteriophage present in a sample of sewage collected 3 days after the sample from which the organism was isolated.

Numbers of bacteriophage in sewage

To estimate the numbers of bacteriophage in the sewage, samples from different parts of the works were treated with toluene; 1 ml. of undiluted samples and of 1/10 dilutions in saline were then plated with 1 ml. of a logarithmic phase broth culture of bacteriophage-sensitive *Bact. coli* (strain B). The number of plaques present after incubation was counted.

Over a period of 96 hr. counts were also made, while 15 ml. portions of an aerated glucose broth culture containing about 500×10^6 *Bact. coli* (strain B) per ml. were added automatically to each 3000 gal. of sewage entering the new section of the works, giving a concentration of $2{\cdot}5 \times 10^3$ organisms per ml.; the sewage entering the old section of the works served as a control.

RESULTS AND CONCLUSIONS

Of forty-nine organisms isolated from the crude sewage, twenty-one were resistant to the action of bacteriophage present in the sewage and twenty-eight were susceptible; of those isolated from the final effluent nine were resistant and forty-one were susceptible. The ratio of resistant to sensitive organisms thus decreased from 1:1·3 in the influent to 1:4·5 in the effluent—an increase in sensitivity of 46 %. The numbers of coliform organisms in the sewage passing through the newer section of the works fell markedly after primary sedimentation and again after biological filtration, but a consistent slight increase was observed during retention in the humus tank (Table 1).

The numbers of bacteriophage rose slightly during primary sedimentation and fell during later stages of treatment (Table 2). Addition of *Bact. coli* (strain B) caused no significant change in the numbers of bacteriophage (Table 2).

If the reduction in numbers of coliform bacteria had been due to the lytic action of bacteriophage, a greater proportional removal of sensitive than resistant forms would have been expected. Since the reverse was the case, and since no substantial increase in the coliphage population was observed, it seems unlikely that much bacteriophage lysis of coliform bacteria occurs during treatment.

Table 1. *Average number of probable coliforms growing on eosin-methylene-blue agar at* 37° C. *from sewage at different stages of treatment*

Stage of treatment	Presumptive coliform organisms per ml.	Percentage overall reduction	Total period of retention before sampling (hr.)	Percentage reduction in preceding stage	Period of retention in preceding stage (hr.)
Crude sewage	134,600	—	0	—	0
Settled sewage	45,790	65	9	65	9
Filter effluent	4,291	97	10	91	1
Humus tank effluent	4,962	96	14	Increase 16	4

Table 2. *Average numbers of bacteriophage active against* Bacterium coli *B at various stages of treatment of sewage, and of sewage to which had been added* $2{\cdot}5 \times 10^3$ *Bact. coli* B/*ml.*

(Crude sewage contained 376 bacteriophage per ml.)

	Bacteriophage per ml. in	
	Settled sewage	Humus tank effluent
Old section of works	388	195
New section of works		
Bact. coli B not added	455	196
Bact. coli B added	464	127

SUMMARY

No evidence was obtained to support the hypothesis that the observed reduction in numbers of coliform bacteria during sewage treatment was due to the action of bacteriophage.

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