

ISOLATION OF BACTERIOPHAGES FROM COMMERCIAL SERA

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SUMMARY

Commercially available sera contain bacterial viruses. The possible origin of serum bacteriophages and the implication of this observation for tissue culture studies are discussed.

Commercially prepared serum is one of several widely utilized and generally essential ingredients of tissue culture media. Variability in the composition of the serum and the presence of contaminating organisms have marked effects on the results obtained from cells in tissue culture. Therefore, the quality of serum is monitored by commercial suppliers for bacteria, fungi, adventitious agents of bovine origin, and mycoplasmas (1). Nevertheless, commercially prepared sera occasionally contain bovine viruses and mycoplasmas (2, 3). Bacterial contamination has also been frequently detected in fetal bovine serum (2). It has been suggested, therefore, that commercially available sera may contain bacteriophages (4). In our laboratory, we recently began screening serum for bacteriophages before utilization in tissue culture and observed that many lots of serum from major commercial sources contained viruses that formed plaques of various morphologies on *Escherichia coli* C (Table 1 and Fig. 1).

METHODS AND RESULTS

The bacteriophages in commercially available sera were detected by the agar layer method (5), utilizing 1 ml of serum to 2.5 ml of tryptone B₁ top agar containing a suspension of *E. coli* C. The mixture was then layered onto tryptone

plates and incubated overnight at 37°C. *E. coli* strain C was used as the indicator strain, since it has no known DNA modification or restriction systems (6). If serum samples con-

TABLE 1
BACTERIOPHAGES IN COMMERCIALY
AVAILABLE SERA*

Company	Description of Serum	Lot No.	PFU/ml†
I	Calf serum, filter sterilized	1	270/1.0
I	Calf serum, filter sterilized	2‡	1600/1.0
I	Fetal bovine serum screened for adventitious bovine agents and mycoplasmas	3	47/30.0
I	Fetal bovine serum screened for adventitious bovine agents and mycoplasmas	4	2270/1.0
II	Fetal calf serum	5‡	101/1.0
II	Fetal calf serum	6	70/30.0
II	Fetal calf serum, virus screened	7	63/1.0
III	Fetal calf serum	8	0/30.0
IV	Lamb serum	9	53/1.0
IV	Horse serum	10	0/1.0
IV	Chicken serum	11	35/1.0

* Summary of results obtained by analyzing commercially available serum lots. The names of four companies and eleven lots are coded.

† PFU/ml, plaque-forming units per milliliter of serum screened for bacteriophage.

‡ Dr. H. Nash (NIMH, Bethesda, Maryland) and Dr. M. Gottesman (NCI, Bethesda, Maryland), given sealed bottles of serum, detected bacteriophages in lots 2 and 5. Dr. J. M. Boyle (Patterson Laboratories, Manchester, England) also found bacteriophages in two different lots of fetal bovine serum purchased in Scotland.

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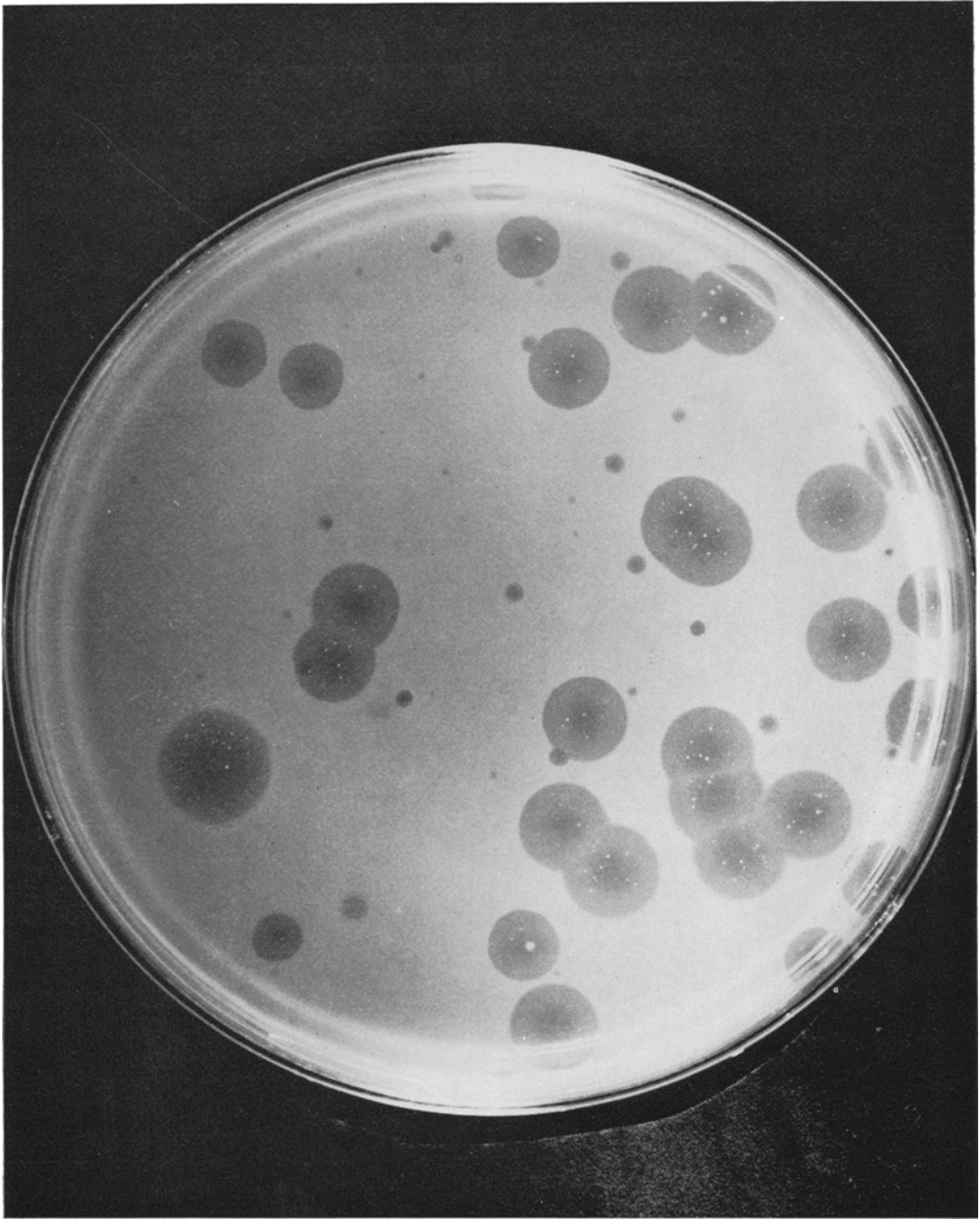


FIG. 1. Photograph of a tryptone B₁ plate with plaques formed by bacteriophage from company II lot 7. It is evident, from the heterogeneity of plaque morphology, that there are numerous strains of bacteriophages. Control experiments lacking serum showed no plaques. Several plaques were also picked and titered against *E. coli* C to demonstrate the presence of true breeding infectious particles.

tained less than 10 plaque-forming units per ml, 30 ml of serum were centrifuged over night at $105,650 \times g$, and the pellet was resuspended in 1 ml of phosphate-buffered saline containing 0.01 M $MgSO_4$ at pH 6.8. The resuspended pellet was titered to determine the number of bacteriophages present. The data in Table 1 indicate wide variability in the number of plaque-forming units per ml among suppliers and lot numbers. Our determination of the number of bacteriophages may be an underestimate, since we only screened for viruses which formed plaques on *E. coli* C.

DISCUSSION

If bacteriophages in serum stem from absorption through the gut, or if they arise through synthesis somewhere in the animal, interactions between these viruses and mammalian cells should be investigated. Lambda bacteriophage appears to be capable of influencing the metabolism of human fibroblasts in tissue culture (7, 8).

If the presence of bacteriophages in serum is indicative of bacteria inadvertently introduced during processing of the blood before filter sterilization, "bacterial-free" filtered serum may also contain macromolecules of bacterial origin, such as toxins, nucleic acids, and enzymes. Investiga-

tors should be aware of the possible presence of bacteriophages in commercially prepared serum which may be utilized for tissue culture and vaccine production.

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