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CHARACTERIZATION OF *ESCHERICHIA COLI* BACTERIAL VIRUSES IN COMMERCIAL SERA

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Bacteriophages have been shown to be present in sera from a variety of commercial sources (1). These studies may have grossly underestimated the extent of bacteriophage contamination in these sera. This is due to the fact that Merril and co-workers only screened for the presence of coliphages that were capable of growing on *Escherichia coli* C. Since this initial study, bacteriophages that have hosts other than *E. coli* have also been detected in commercial fetal calf sera (2, 3). Commercially available sera also occasionally contain bovine viruses and mycoplasmas (4, 5). This contamination occurs even though commercial suppliers monitor their sera for bacteria, fungi, adventitious agents of bovine origin, and mycoplasmas. Bacteriophages have been found in serum lots that are labelled "virus screened."

Investigation was undertaken of the bacteriophages that were found by plating on *E. coli* C, and of their distribution in various types of sera, with special emphasis on typing of the phage by use of resistant strains of bacteria.

METHODS AND RESULTS

Commercially available fetal calf serum was plated against *E. coli* C, a restriction- and modification-deficient strain. Various clear and turbid plaque types were observed. Four plaques with clear centers were selected and designated CS1, CS2, CS3, and CS4. The plaques were purified by repeated passage on *E. coli* C. A sterile plate stock, containing more than 10^8 plaque-forming units per ml, was prepared from each plaque.

E. coli C mutants were selected for their ability to resist infection by one of the four fetal calf serum viruses: CS1, CS2, CS3, and CS4. This was done by plating phages and bacteria with a multiplicity of infection of five phages per bacterium on tryptone agar media. Viral-resistant colonies were selected and colony-purified. Most of the bacterial mutants selected in this way can be expected to be resistant because they have lost the ability to absorb the phages to which they are resistant. This, however, is not the only way in which bacteria can become resistant (6). The bacterial strains that result from such selection are called indicator strains. An indicator strain of bacteria is resistant to one class of phage but susceptible to another. CS1, CS2, CS3, and CS4 phages were found, by spot testing against *E. coli* C mutant strains (Table 1), to fall into two classes (I and II). By subjecting bacteria that were resistant to phage class I to phage from class II, a strain of *E. coli* C resistant to both classes I and II phage was prepared. This strain failed to plate any of the four calf serum viruses.

Bacterial viruses from various commercial sera were tested for their ability to form plaques on the doubly resistant strain, each of the two singly resistant strains, and the nonresistant parent strain. The data from these tests are shown in Table 2. These data indicate that a significant proportion of the bacterial viruses found in commercially available sera from various sources could fit into the two classes we had defined. The percentage of phages that did not belong to either of the resistance classes I or II ranged from 0% for serum 13, to 57% for serum 11. On the whole, for the 13 sera batches tested, 72.5 ± 18.9% of the *E. coli* phages present belonged to resistance classes I or II.

DISCUSSION

Our data indicate that three-fourths of the *E. coli* phage found in the batches of serum tested were of the same type with respect to

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TABLE 1
SERUM BACTERIOPHAGE RESISTANCE OF THE *E. coli* C STRAINS USED

Bacterial Strain No.	Selected Bacterial Resistance	Serum Bacteriophages				Resistance Class of Bacteria
		CS1	CS2	CS3	CS4	
1	None	+	+	+	+	
2	CS1	−	+	+	+	I
3	CS2	+	−	−	+	II
4	CS3	+	−	−	+	II
5	CS4	−	+	+	−	I
6	CS2 + CS4	−	−	−	−	I + II

Table 1 shows the results of spot testing the four serum bacteriophage isolates on lawns of each of the bacterial strains used in this study. (+) indicates lytic action of the phage on the bacterial strain, (−) indicates no effect of the phage on the bacteria. The bacterial strains were isolated by subjecting *E. coli* C to a high multiplicity of infection of the indicated bacteriophage (CS1–4). Bacterial strain 1 is the parent *E. coli* C which has not undergone any selection for serum phage resistance. Strains 2 to 5 were selected for their ability to resist the indicated serum phage. Strain 6 was selected first for its resistance to serum phage CS2, and then for its resistance to phage CS4. This doubly resistant strain of *E. coli* C is resistant to all four of the serum phages. The lytic action of phage on the various strains of *E. coli* C tested are presented as Resistance Class I and II, as indicated in the table.

their host range, as defined by the resistance classes we have described.

A series of phages, all active against the original bacterial strain, can be grouped into types by examining the way a mutant bacterial strain, selected for its resistance to one phage, behaves with respect to its ability to be destroyed by the other phage isolates (7). Selection of bacterial mutants that are resistant to a particular bacteriophage is known to be specific, in that each change observed usually involves resistance to only a few of the bacteriophage strains capable of attaching to the original strain of bacteria (8). An example of the use of this technique is the separation of seven T coli-dysentery phages into six resistance classes (6). The technique is not completely reliable in determining whether or not phages are related. For example, the coli-dysentery phage T_1 and the coli phage phi-80 are known to share the same attachment site, although they are not closely related (9). However, on the whole, this technique is superior to electron microscopy, evaluation of plaque morphology, and other methods for rapidly evaluating how closely a phage in one sample is related to a phage in another sample.

The presence of bacteriophages in commercial sera indicates that the sera were contaminated

TABLE 2
ABILITY OF BACTERIOPHAGES IN COMMERCIAL SERA TO FORM PLAQUES ON STRAINS OF *E. coli* C

Code Number	Type of Serum	Source	Lot No.	*E. coli* C Strains				Comments
				1	6	5	3	
1	Fetal calf	GIBCO	A2201N	17	5	1	12	
2	Fetal calf	GIBCO	R7173R	12	4	8	10	
3	Calf	Flow	421243	481	240	287	560	
4	Fetal calf	GIBCO	E014515/2	39	5	13	42	Virus screened
5	Fetal calf	GIBCO	C41470	16	7	12	7	
6	Lamb	Microbiological Associates	77336	84	12	54	59	
7	Fetal calf	Flow	455534	8	4	2	4	Screened for bovine viruses and mycoplasm
8	Calf	Microbiological Associates	80374	345	105	215	225	" "
9	Fetal calf	GIBCO	C3202K	45	2	13	36	
10	Fetal calf	Microbiological Associates	79168	13	4	11	4	
11	Fetal calf	GIBCO	C6132G	14	8	6	14	Dialysed
12	Fetal calf	GIBCO	E0145J	39	3	8	18	
13	Chicken	Microbiological Associates	80612	4	0	0	7	
14	Horse	Microbiological Associates	77928	0	0	0	0	

Table 2 shows the number of plaques formed when 1 ml of each of 14 lots of commercial serum was plated against strains of *E. coli* C with various resistances to fetal calf sera viruses. The selection and phage resistance properties of the bacterial strains are summarized in Table 1.

with bacteria before filter sterilization. A study of fetal calf sera before filter sterilization detected bacterial contamination in nine of nine samples from two distributors (10). Inasmuch as filter sterilization of the sera failed to remove bacteriophages, other molecules of prokaryotic origin probably also pass through the filter. Thus, the sera may contain prokaryotic proteins, nucleic acids, etc. The presence of bacterial molecules in sera might well affect a wide range of tissue culture experiments. For example, studies involving antibiotics and drug metabolism in tissue culture could be confused by the presence of penicillinase or other bacterial molecules capable of breaking down antibiotics. On the other hand, certain bacterial or viral products might themselves have antibiotic activities. A specific antiviral, peptide-like substance has been isolated from lambda phage. The substance has been shown to have activity, both in vitro and in vivo, against vaccinia and Herpes simplex viruses (11). Furthermore, electron microscopic studies of low level virus production by various cell lines could possibly be confused by the presence of bacteriophages in the serum. Similarities between electron micrographs of bacteriophage particles and "C" and other virus-like particles may be seen, by comparing examples in the literature (12, 13).

Furthermore, investigations which have been performed in many laboratories indicate that bacteriophage DNA can undergo transcription and translation in eukaryotic animals and plants (14–18).

The presence of bacteriophages in vaccines was predicted by Merril and co-workers and confirmed by Petricciani and co-workers (19). Vaccines containing related bacteriophages and possibly other bacterial components, present a potentially very serious medical problem, in need of further investigation (20). The presence of related bacteriophages, bacterial toxins and certain other bacterial molecules might help to explain many of the adverse reactions to vaccination that are encountered.

The possible importance of the presence of bacteriophages in commercially available sera has been emphasized by this study. The data (Tables 2 and 3) show that the distribution of these viruses is systematic rather than random. This means that any effects which the viruses have in eukaryotic systems may be observed wherever these sera are used. Furthermore, the influence of phages in tissue culture experiments cannot be ruled out by simply repeating the study with a different serum lot.

The relatedness of the serum viruses capable of plating on *E. coli* C may indicate that the workers who prepare the sera contaminate it with the same type of viruses; or, as appears more likely, properties of factors in serum itself are selected for the growth of certain types of phages. A more remote possibility is that the viruses are indeed intrinsic and that their relatedness is due to progenitors of the organisms from which they are derived.

Attempts to destroy phages in serum without harming its quality include differential centrifugation and chemical treatment of the sera. Ultrafiltration (21) and immunoabsorbent columns (22), which have been shown capable of removing bacteriophages from sera, may prove unsatisfactory because they may not remove toxins and other undesirable bacterial molecules from the sera. A better solution is to prevent serum from being exposed to prokaryotic organisms in the first place, by handling the serum in a sterile

TABLE 3

PERCENTAGE OF BACTERIOPHAGES IN COMMERCIAL SERA THAT DO NOT BELONG TO THE DEFINED RESISTANCE CLASSES

Code No.	Percentage of Phages Capable of Forming Plaques on Bacterial Strain		
	6	5	3
1	29	5	70
2	33	66	83
3	49	59	116
4	12	33	107
5	43	75	43
6	14	64	70
7	50	25	50
8	30	62	65
9	4	28	80
10	30	84	30
11	57	42	100
12	7	20	46
13	0	0	175

Table 3 shows the percentage of phage in various sera that are capable of making plaques on each of the resistance strains described above and summarized in Table 1. The code numbers designate serum lots described in Table 2.

manner. When serum has been successfully made free of prokaryotic contaminants, tissue culture systems may become cleaner and more reliable.

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