AD 677831

TRANSLATION NO. 2048

DATE: 31 October 1967

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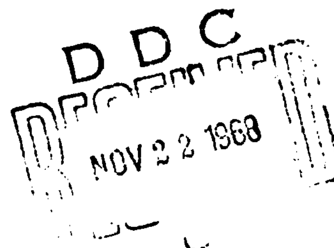

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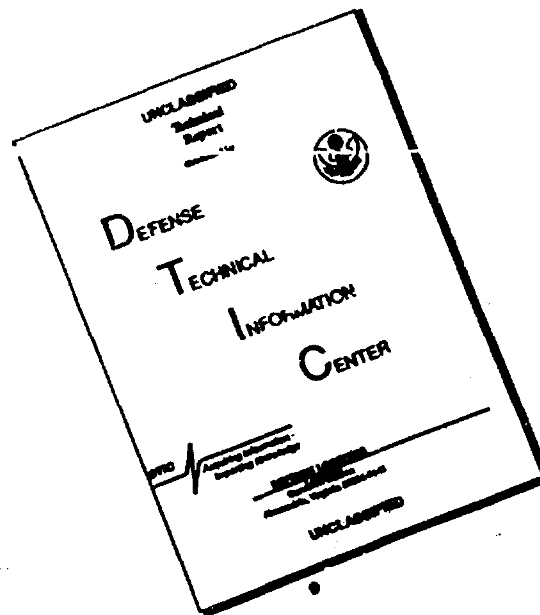

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SOME CRITERIA FOR JUDGING THE RELATIONSHIP BETWEEN BACTERIA AND VIRUSES

I. E. COLI BACTERIA AS ADSORBENTS OF ENTEROVIRUSES

Zh. Mikrobiol., Epid. i Immunobiol. (J. of Microbiol., Epidemiol., and Immunobiol.)
Vol. 43, No. 11, 1966, pages 69-73

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The relationship between bacteria and viruses is still an unresolved problem, despite the interest shown in it in the 1930s (Zil'ber et al., 1932, 1933, 1937; Timakov, 1936; Fal'kovich and Yanushevich, 1936; others). Imperfection of the available techniques and insufficient knowledge of viruses were the main reasons. Recent advances in virology have made it possible to study the problem on a higher technical level. Studies on models of hay bacillus and smallpox virus (Abel and Trautner, 1964), E. coli and encephalomyocarditis virus (Ben-Gurion and Ginzburg-Tietz, 1965) show that whole virus particles can be formed in bacteria exposed to viral nucleic acid.

Since viruses and bacteria under natural conditions may be in accidental associations or associations produced by evolution, it is first necessary to establish the character of the viral-bacterial association. This is important if the role of viral-bacterial associations in the pathology of man is to be elucidated etiologically, pathogenetically, clinically, and epidemiologically.

The adsorption of viruses on bacteria was carefully studied as a major factor in the possibility of viral-bacteria associations under conditions of indirect contact.

Study of the reaction of agglutination by viruses of bacteria, the AVB reaction (Roberts and Jones, 1941; Sergiyev et al., 1945;

Vaynberg et al., 1949, 1951; Gimmel'farb, et al., 1956; others), revealed the adsorption of vertain viruses on typhus bacteria and Bacillus prodigiosus. In recent years, certain strains of the viruses of influenza, ECHO, and vaccine were found to be capable of agglutinating suspensions of individual pathogenic and nonpathogenic strains of E. coli (Berger, 1963; Berger and Sauer, 1962).

However, the quantitative aspect of virus adsorption on bacteria has not yet been investigated. Study of the adsorption of small viruses in the AVB has been unsuccessful. We therefore set out to ascertain whether enteral viruses can be adsorbed on E. coli and to make an objective quantitative determination of the adsorbed virus. The choice of these bacteria and viruses was dictated by the fact that they often are found together in the normal intestinal tract where they probably form associations.

For this purpose we titrated virus on an adsorbent and in the wash fluid of the tissue culture exhibiting the cytopathic effect. We also used plaque assays, the AVB reaction, and the luminescent-serological method.

Experiments were performed with Coxsackie B3 (Nancy 272/431) and B5 (Faulkner 331/501) viruses and with the Ovchinnikov strain of poliomyelitis virus. A culture of HEp-2 cells was used to accumulate the virus. As a cultural medium we used 0.5% lactalbumin hydrolyzate and medium 199 (1:1) with 10% untreated calf serum. Medium 199 served as the support medium.

The method of virus adsorption on the bacteria was as follows. A suspension containing 20 to 25 billion microbial cells in 1 ml obtained by washing a day-old agar culture was mixed in equal parts with liquid containing the virus and kept for 30 min at room temperature and then for 18 hours in a refrigerator at 4 to 5°. The bacterial suspension was allowed to settle and then washed three times during centrifugation (825 g) in 50 to 100 times the volume of physiological saline in such a way as to obtain a suspension with a concentration equal to the initial. As a control we used an adsorbent not loaded with virus but treated with the lysate of a noninfected cell culture.

The experiments were run with pathogenic and nonpathogenic coliform bacteria, live and killed by heating at 60° for an hour. Motile and nonmotile strains, strains fermenting and not fermenting sucrose were used.

A comparison of the adsorption capacity of the live and killed bacteria showed that the latter were sometimes less active adsorbents, and in some experiments there was no adsorption at all (Table 1).

Table 1. Adsorption of Coxsackie B3 Virus and Poliovirus on Live and Killed E. coli

Адсорбируемый вирус[1] (1)	Адсорбент E. coli (2)	(3) Состояние адсорбента	(4) Титр адсорбированного вируса (в lg ТЦД$_{50}$)			
			$\bar{X}$	σ	$\sigma_{\bar{x}}$	V%
Коксаки B3 (5)	1	7 Живой	3,5	0,92	0,41	26,3
		8 Убитый	2,7	1,34	0,67	49,6
	1a	7 Живой	4,2	1,03	0,51	24,5
		8 Убитый	0	—	—	—
Вирус полиомиелита, штамм Овчинников (6)	1	7 Живой	3,5	0,70	0,50	20,0
		8 Убитый	3,7	0,99	0,70	26,7
	1a	7 Живой	4,0	0,70	0,50	17,5
		8 Убитый	4,2	1,06	0,76	33,1

(9) [1] Титр исходного вируса Коксаки B3 6.3, полиовируса 6,5.

(10) Обозначения: $\bar{X}$—средняя арифметическая, σ—среднее квадратическое отклонение, $\sigma_{\bar{x}}$—средняя ошибка средней арифметической, V%—коэффициент вариации.

Key: 1 - Virus adsorbed[1]; 2 - E. coli adsorbent; 3 - State of adsorbent; 4 - Titer of adsorbed virus (in lg TCD_{50}); 5 - Coxsackie B3; 6 - Poliomyelitis virus, Ovchinnikov strain; 7 - Live; 8 - Killed; 9 - Titer of original Coxsackie B3 virus, poliovirus 6.3 and 6.5, respectively; 10 - Symbols: $\bar{X}$ - arithmetic mean; σ - standard deviation; $\sigma_{\bar{x}}$ - mean error of arithmetic mean; V% - variation factor.

The titer of the adsorbed virus was much greater than the original value. The smaller variation factors in the experiments with the suspension of live bacteria are noteworthy. The reason seems to be that the adsorption conditions in the experiments with live bacteria were more uniform and stable than in the experiments with the suspensions of the killed bacteria. The virus titer decreased after it was treated with a filtrate of the corresponding killed bacterial culture. When the bacteria in the physiological solution were heated, products lethal to the virus apparently were formed in the medium. Consequently, we used only live bacteria in the rest of our experiments.

Experiments with two nonpathogenic (1, 1a) and two pathogenic strains (026, 086; H7; B34) of E. coli and Coxsackie B3 (Nancy 272/431) virus showed adsorbed virus on both types of bacteria (Table 2). The differences in adsorption of the virus on the nonpathogenic and pathogenic strains of the bacteria were statistically insignificant.

Table 2. Adsorption of Coxsackie B3 Virus on Nonpathogenic and Pathogenic Strains of E. coli

Адсорбент E. coli (1)	(2) Титр адсорбированного вируса (в lg ТЦД$_{50}$[1])			
	$\bar{X}$	σ	$\sigma_{\bar{x}}$	V%
I	3,5	0,95	0,43	27,1
Ia	4,2	1,08	0,54	25,7
O26	3,2	0,65	0,33	20,3
O86	2,8	1,4	0,7	50

(3) [1] Титр исходного вируса 6,3. Обозначения те же, что в табл. 1.

Key: 1 - E. coli adsorbent; 2 - Titer of adsorbed virus (in lg TCD_{50}[1]); 3 - Titer of original virus 6.3. Symbols the same as in Table 1.

Since the quantity of adsorbed virus may vary both with the nature of the bacterial strain and with the viruses themselves, we thought it worthwhile to investigate different strains of viruses.

Nonpathogenic and pathogenic strains of E. coli adsorbed Coxsackie B3 (Nancy 272/431) and B5 (Faulkner 331/501) viruses and poliovirus (Table 3). The latter was adsorbed on bacteria in larger quantities than on Coxsackie virus. These differences were statistically more distinct in adsorption on the pathogenic O26 strain of E. coli. There were also statistically significant differences between Coxsackie B5 and poliomyelitis viruses on the nonpathogenic strains of E. coli.

It is evident from the foregoing data that the bacteria adsorbed not only the large viruses, as noted in the literature, but also the small ones (Coxsackie and poliomyelitis).

To determine the role of the adsorbent, experiments were performed with 8 nonpathogenic strains of E. coli differing in motility and action on sucrose. The bacteria included freshly isolated and stock strains isolated from adults and children. The Coxsackie B3 virus was used.

All the coliform bacteria adsorbed the virus (Table 4). The differences between the individual strains in this respect clearly appeared when the virus was titrated by the plaque assay method.

Table 3. Adsorption of Coxsackie B3, B5 and Poliomyelitis (Ovchannikov) Viruses on Pathogenic and Nonpathogenic Strains of E. coli

Адсорбируемый вирус[1] (1)	(2) Адсорбент E. coli	Титр адсорбированного вируса (в lg ТЦД$_{50}$) (3)			
		$\bar{X}$	σ	$\sigma_{\bar{x}}$	V%
Коксаки B3 (4)	1	3,9	1,02	0,51	26,2
	O26	3,1	0,27	0,14	8,7
Коксаки B5 (5)	1	3,0	0,08	0,05	2,7
	O26	2,9	0,81	0,47	27,6
Полиовирус (6)	1	4,3	0,27	0,14	6,3
	O26	4,2	0,09	0,06	2,1

(7) [1] Исходный титр B3 6,3; B5 6,5; полиовируса 6,5. Обозначения те же, что и в табл. 1.

Key: 1 - Adsorbed virus[1]; 2 - E. coli adsorbent; 3 - Titer of adsorbed virus (in lg TCD_{50}); 4 - Coxsackie B3; 5 - Coxsackie B5; 6 - Poliovirus; 7 - Original titer of B3, B5, and poliovirus 6.3, 6.5, 6.5, respectively. Symbols the same as in Table 1.

Table 4. Amount (Arithmetic Mean) of Adsorbed Coxsackie B5[1] Virus on Different E. coli Strains

Бактерия-адсорбент (1)			Титр адсорбированного вируса (6)	
штаммы E. coli (2)	(3) основные свойства			
	подвижность (4)	сахароза (5)	в lg ТЦД$_{50}$ (7)	в БОЕ на 1 мл взвеси адсорбата (8)
1	+	—	5,7	$4,2 \cdot 10^5$
2	+	—	5,2	$5,2 \cdot 10^5$
12	+	—	4,9	$2,9 \cdot 10^4$
B	+	—	4,7	$3,6 \cdot 10^4$
1a	—	—	5,4	$4,9 \cdot 10^4$
8	—	—	5,4	$1,4 \cdot 10^4$
M-17	+	+	5,5	$12 \cdot 10^5$
4	+	+	5,3	$2,5 \cdot 10^4$

(9) Титр исходного вируса в lg ТЦД$_{50}$ 6,5 и в БОЕ на 1 мл $5 \cdot 10^{6,5}$.

Key: 1 - Bacteria adsorbent; 2 - E. coli strain; 3 - Main properties; 4 - Motility; 5 - Sucrose; 6 - Titer of adsorbed virus; 7 - in 1 g TCD_{50}; 8 - in BOE (?) in 1 ml of suspension of adsorbate; 9 - Titer of original virus in lg TCD_{50} 6.5 and in BOE in 1 ml $5 \cdot 10^{6.5}$.

The stock strain M-17 was the most active adsorbent; the stock strain of E. coli B was the least active. Among the freshly isolated strains, strain 2 isolated from an 18-month-old child and strains 1 and 1a isolated from an adult were the most active.

No correlation was observed between the adsorption properties of the bacteria, their motility, and capacity to ferment sucrose. The variations in degree of adsorption were apparently due to still undetermined characteristics of the bacterial strains and to the selectivity of the viruses themselves for the adsorbent.

In some instances the data on adsorption correlated with the characteristics of the respiration of the bacteria and changes in electrophoretic motility. For example, in the E.coli strain No. 8, a somewhat less active adsorbent (cf. Table 4), respiratory activity decreased to a smaller extent when the virus was adsorbed on it and electrophoretic motility did not change in the case of adsorption of Sabin type II poliomyelitis virus. A definite relationship between virus (Coxsackie B3) and bacteria (E. coli strain No. 1) was indicated by the experiments on detecting the virus in three successive generations of bacteria loaded with virus. In the first culturing of the adsorbate on meat-peptone agar, virus was found on 1 colony; in the second generation, on 5 colonies; in the third generation, on 3 colonies of 10 tested.

The ABV reaction in the usual modification proved to be insufficiently sensitive and unsuitable for detecting the adsorption of enteroviruses on coliform bacteria. It produced a large number of nonspecific reactions caused by agglutination of the bacteria due to the presence of agglutinins in the serum. Depletion of the serum likewise failed to yield the desired results.

The luminescent-serological method was effective in detecting adsorbed virus. Acetone and alcohol were used to prepare the specimen by Coons' method. However, this method turned out to be ineffective. Better results were obtained by the following procedure. Virus was adsorbed on the bacteria at room temperature for 30 min and then at 4 to 5° for 18 hours. An equal amount of suitable luminescent serum was added to a bacterial suspension with adsorbed Coxsackie B3 virus. After 30 min of contact at 36°, the suspension was washed three times during centrifugation (825 g) in phosphate buffer.

In the preparations with virus adsorbed on the bacteria, the latter exhibited distinct luminescence, which was not the case with the control. Accumulations in the form of microagglutinations of bacteria loaded with virus were noteworthy.

The data presented above on the adsorption of enteroviruses on *E. coli* indicate that adsorbates can be formed under conditions of direct contact. This situation is possible not only for bacteria and large viruses, but also for bacteria and small viruses. This is particularly important with respect to the described bacteria (*E.coli*) and viruses (enteral) because they are found in natural association in the human intestine.

Conclusions

1. Different *E. coli* strains are capable of adsorbing Coxsackie B3, B5, and poliomyelitis viruses.

2. Virus adsorbed on bacteria can be detected from the cytopathic effect in tissue culture and by the plaque assay procedure (the amount of virus will be expressed in TCD_{50} on plaque-forming units, respectively).

3. Titration of adsorbed virus by the plaque assay procedure reveals the quantitative fluctuations in adsorption more distinctly.

4. The factors directly responsible for the adsorption of the different bacterial strains could not be determined.

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