“SEMI-RESISTANCE” OF BACTERIA TO A BACTERIOPHAGE

SECOND REPORT ON HOST-CONTROLLED VARIATION

TOMIE NOJIMA AND HIDEO FUKUMI

The National Institute of Health, Tokyo, Japan

(Received: March 19th, 1954)

INTRODUCTION

Wahl has already published several papers on the bacteria “semi-resistant” to a bacteriophage⁽¹⁻⁷⁾. According to him, in case a microorganism is semi-resistant to a certain bacteriophage, it indicates that the population produced by this host microorganism consists of two kinds of individual cells showing different reactivities to the bacteriophage, namely sensitive and resistant individuals. This concept seems, however, incompatible to the experimental findings obtained not only by us, but also by himself, and furthermore it appears to contain some self-contradiction in itself. Recently we obtained several strains, whose behavior was very similar to those reported by Wahl et al concerning the “semi-resistance”, and conducted some experiments with them to elucidate an essential feature of this characteristic phenomenon. The present publication is concerned with it and according to it, the “semi-resistance” of bacteria to a bacteriophage has an intimate relationship with the host-controlled variation of the bacteriophage.

BACTERIAL AND VIRAL STRAINS EMPLOYED

The bacterial strains used: the strains of *Shigella* described in the previous report⁽⁸⁾ were employed throughout this work. Especially *Shigella* No. 9 was mostly used, and the others were employed only for determination of the host range of the bacteriophages.

The bacteriophages: *The strain NG:* This was obtained from faeces of a hen by passage through *Shigella* No. 9.

The strain R461: This was obtained from stool of a dysenteric patient by passage through *Shigella* No. 9.

These two phage strains were antigenically entirely different from each other serum neutralization test.

The ultraviolet irradiation sensitivity of these two phage strains is illustrated in Fig. 1 in comparison with the T3 bacteriophage. They were a little more sensitive to ultraviolet irradiation than T3. It is said that there are two kinds of phages in T-system, one of which shows an upward concavity of the doses-time curve and the other of which an downward concavity of that⁽⁹⁾. As seen in Fig. 1 the phages employed in this work belong to the former in this respect.

Fig. 1 Effect of ultraviolet irradiation

Adams⁽¹⁰⁾ applied occurrence or non-occurrence of multiplicity reaction as a criterion for the classification of bacterial viruses. Multiplicity reactivation is clearly demonstrated in the case of the phages NG and R461 (Table 1).

Table 1. Multiplicity reactivation of ultraviolet irradiated phage

Phage	Host	Assay of infective centers per ml		
		Before irradiation	After irradiation	
			Single infection	Multiple infection
NG [9]	Shigella No. 9	2.5×10^7	1.0×10^5	9.8×10^5
R461[9]	Shigella No. 9	3.5×10^5	$1.0 \times 10^2 >$	1.8×10^4

The host range of these two viruses is shown in Table 2. As previously reported⁽⁸⁾, the host range of phages in general is shifted more or less in the

result of host-controlled variation, as hosts, in which they multiply, change. Therefore, in Table 2, the host ranges of NG[9] and of R461[9] are shown. Both of the viruses are able to multiply mostly in the bacteria of the *Shigella flexneri* group, and the host ranges of them are somewhat different from each other.

Table 2. Host range of the phages NG (9), R461 (9) and R461 (ng45)

host	Phage	NG[9]	R461[9]	R461[ng45]
No. 1	(<i>Sh. dysenteriae</i> 1)	—	—	(±)
No. 2	(" " 2)	—	—	—
No. 3	(" " 3)	—	—	—
No. 4	(" " 4)	—	—	—
No. 5	(" " 5)	+	+	+
No. 6	(" " 6)	—	+	+
No. 7	(" " 7)	—	+	+
No. 8	(<i>Sh. flexneri</i> 1a)	††	††	††
No. 9	(" " 1b)	††	††	††
No. 10	(" " 2a)	††	††	††
No. 11	(" " 2b)	††	††	††
No. 12	(" " var. X)	††	††	††
No. 13	(" " var. Y)	††	††	††
No. 14	(" " 3a)	††	††	††
No. 17	(" " 4a)	††	††	††
No. 18	(" " 4d)	+	††	††
No. 19	(" " 4c)	††	††	††
No. 21	(" " 5)	††	††	††
No. 22	(" " 6)	±	—	—
No. 26	(<i>Sh. boydii</i> 1)	±	—	—
No. 27	(" " 2)	±	—	—
No. 28	(" " 3)	±	+	(—)
No. 29	(" " 4)	—	—	—
No. 30	(" " 5)	††	††	††
No. 31	(" " 6)	±	—	—
No. 32	(" " 7)	—	+	—
No. 33	(<i>Sh. sonnei</i> I)	—	—	(+)
No. 34	(" " II)	—	††	††

Virulence of the bacteriophages: When excess number of bacteriophage particles and a culture of its host are mixed together and plated on agar medium, there appear usually a certain number of colonies showing refractoriness to the infection of the bacteriophage. Some of these colonies are real resistant colonies, but the others are apparently resistant because of their acquirement of lysogenity. The proportion of the lysogenic colonies to the total appearing colonies is said to be approximately constant in a given virus though there are some variations corresponding to experimental conditions. In the case of the phage R461, this proportion is about 50% while about 5% in the phage NG. The experimental data are shown in Table 3.

Table 3. Rate of lysogenic colonies among total resistant colonies

Phage	% of lysogenic colonies among total resistant colonies		
	Experiment 1	Experiment 2	Experiment 3
NG [9]	2.0	4.2	7.0
R461[9]	/	57	30

EXPERIMENTAL RESULTS

1. *Relationship between mutant strains resistant to either the phage NG or the phage R461:* A broth culture of *Shigella* No. 9 was divided into two parts. One part was spread on nutrient agar medium after mixed with an equal amount of concentrated NG bacteriophage, and the other part was spread in the same way with R461 bacteriophage. When phage-resistant colonies developed themselves on the agar plates after overnight incubation, they were counted. Two experiments were conducted in such a way. The results are shown in Table 4. In the first experiment, the ratio of the resistant colonies on phage NG to those on phage R461 was about 2:1, while it was about 4:1 in the second experiment.

Table 4. Comparison of the number of resistant colonies on NG plate to that on R461 plate

Phage	Number of resistant colonies	
	Culture 1	Culture 2
NG [9]	$150 \times 10/\text{ml}$	$175 \times 10/\text{ml}$
R461[9]	$78 \times 10/\text{ml}$	$40 \times 10/\text{ml}$

Among these resistant colonies either on phage NG or on phage R461, a certain number of lysogenic colonies must be included, and as already mentioned (Table 3), percentage of lysogenic ones to total resistant colonies is approximately fixed, namely about 5% for that on Phage NG and about 50% for phage R461. The colonies lysogenic for NG were not only resistant to the lysis by NG, but also so to the lysis by R461, and vice versa.

The resistant colonies on R461 plates thus obtained were all found also resistant against phage NG as far as our experiences were concerned, but the reverse does not hold true. Some of the resistant colonies on NG phage plates showed usual resistance against R461, namely no plaques were produced on these resistant strains by the phage R461, however, there were found some colonies on the same NG phage plates which showed no full resistance against the phage R461. The titers of R461[9] obtained on these organisms were significantly more or less lower than the titer of R461[9] on *Shigella* No. 9 (original strain). Total nine strains of this kind was so far obtained in this way from NG phage plates, naemely ng45, ng13, ng33, ng17, ng25, ng43, ng44, ng19 and ng32. The behavior of these strains to the phage R461 seemed to me very similar to those described by Wahl and Blum-Emerique as "semi-resistant" bacteria to bacteriophages, and therefore we describe several experimental results along this line by taking the strain ng45 as an representative of them.

2. *Semi-resistance of the strain ng45 to the phage R461, and the host-controlled variation of the phage R461 by host-change between *Shigella* no. 9*

and the strain *ng45*: Wahl et al pointed out that each of the semi-resistant mutants obtained by them were capable of producing plaques only in a limited sense, namely in the way that the number of plaques produced on a semi-resistant mutant was more or less fewer than that of plaques produced on the original culture when a same dilution of the bacteriophage in question was employed, and furthermore, the ratio of the titer of the bacteriophage on a semi-resistant mutants to that on the original sensitive strain was approximately constant for each semi-resistant mutant.

The phage R461 was able to produce the mutant strain (*ng45*) of *Shigella* No. 9 much less than on the original strain. The titer ratio was usually about 1:500, and so it might be said that only one phage particle among 500 produced a plaque by chance on *ng45* if it could be assumed that every phage particle was capable of producing a plaque on the original strain. However, the phage particles produced by infecting the mutant *ng45* with the phage R461[9] were different from the original phage particles, namely R461[9]. In one of our experiments a sample of the phage R461[9] produced 3.5×10^9 plaques on *Shigella* No. 9, i.e. original culture, while 6.9×10^6 plaques on the mutant strain *ng45*, the titer ratio of about 500:1 (Table 5). The phenotype R461 [*ng45*] which was produced by infecting the mutant *ng45* with the phage R461[9], developed 5.7×10^9 plaques on *Shigella* No. 9, and 6.2×10^9 on the mutant *ng45*, namely titer ratio of about 1:1 (Table 5).

Table 5. Plaque counts of the phenotypes R461 (9) and R461 (*ng45*) on *Shigella* No. 9 and its mutant *ng 45*

Phenotype of phage R461	Plaque counts on	
	<i>Shigella</i> No. 9	<i>ng 45</i>
R461[9]	3.5×10^9	6.9×10^6
R461[<i>ng45</i>]	5.7×10^9	6.2×10^9
R461[<i>ng45</i>] _{/9}	8.5×10^8	1.5×10^8
R461[<i>ng45</i>] _{/9} ²	1.3×10^9	4.5×10^6

A suspension of the R461[*ng45*] was mixed with bacterial suspension of *Shigella* No. 9 and then washed three times with physiological saline by refrigerated centrifugation. The final bacterial deposit with adsorbed phages was resuspended in nutrient broth and incubated for two hour at 37°C. Centrifugation was made again, and the supernatant (R461[*ng45*]_{/9}) was titrated on *Shigella* No. 9 and mutant *ng45*. It developed 8.5×10^8 plaques on the mutant *ng45*. The titer ratio was approaching to that of R461[9] from that of R461 [*ng45*]. More or less contamination by R461[*ng45*] seems to prevent the titer ratio of the R461[*ng45*]_{/9} from approaching to that of R461[9]. Then, nutrient broth was inoculated with one ml of 24 hours' broth culture of *Shigella* No. 9

and one loopful of R461[ng45]/₉, and incubated overnight at 37°C. The broth culture was centrifuged and supernatant was designated R461[ng45]/₉². The titers of the R461[ng45]/₉² on Shigella No. 9 and the mutant ng45 were 1.3×10^9 and 4.5×10^6 respectively. The titer ratio became almost identical with that of R461[9] (Table 5).

From the above experimental results it has become clear that the R461[9] and the R461[ng45] are merely phenotypical variants of the R461 phage, and the variation is so-called host-controlled (Fig. 2).

Fig. 2 Schematic presentation of host-controlled variation of R461 to Shigella No. 9 and the mutant ng 45

3. *Criticism of Wahl's theory of semi-resistance:* Wahl postulates to interpret the phenomenon of semi-resistance that a semi-resistant mutant consists of two kinds of individuals, resistant and sensitive. His hypothesis is, however, very poor to explain the whole phenomenon of semi-resistance.

First, if, as he postulates, a semi-resistant mutant consists of resistant and sensitive individuals and only sensitive ones produce bacteriophage particles by lysis, phage particles produced should be identical with those produced by the original sensitive strain, but on the contrary, as already mentioned above, R461[9] and R461[ng45] are proved phenotypically different. The fact that the titers of R461[9] on Shigella No. 9 and on the mutant ng45 are different, seems to be explain just as in the case of the host-controlled variation reported previously⁽⁸⁾, where we dealt with the difference of the titers of R461[9] on Shigella No. 9 and Shigella No. 6. If the plaque counts of R461[9] on Shigella No. 9 represent accurately the number of the particles contained in a sample of R461[9], only 1/1000 of the particles in the same sample of R461[9] produce

a plaque on *Shigella* No. 6. According to our consideration, it does not mean that the sample of R461[9] contains heterogenous particles at the rate of 1:1000, but merely indicates that one particle produces a plaques among 1000 by chance, just as ultraviolet irradiation inactivates phage particles at a certain rate in time-course, without indicating any inhomogeneity in phage particle population.

Wahl put an emphasis on the fact that numbers of plaques produced on semi-resistant culture were always unproportional to the dilution of the sample, and took this fact as indicating the inhomogeneity of a culture of the semi-resistant mutant. Our experiments with R461[9] and the semi-resistant mutant ng45, however, showed no irregularity of plaque counts to the dilution of the sample (Fig. 3).

Fig. 3 Relationship between plaque counts and the dilution

Thus, it is concluded that the interpretation of semi-resistance phenomenon by Wahl does not hold true, and that this phenomenon is very well interpreted by regarding it as a phenomenon closely related with host-controlled variation.

4. *Degree of "semi-resistance"*: As already indicated by Wahl and Blum-Emerique, there are several degrees of semi-resistance. Nine semi-resistant mutant strains were obtained by us from Shigella No. 9, as mentioned above. Degrees of semi-resistance of these mutant strains to R461[9] are shown in Table 6. At least four groups of the mutant bacteria were distinguishable according to their titer to R461[9]. Next, these semi-resistant mutants were examined for their plaque-developing capacity to R461[ng45], which had been produced by infecting the mutant ng45 with R461[9], and also to R461[ng45] produced by the mutant ng44 infected with R461[9] (Table 6).

Table 6. Plaque-developing capacity of the semi-resistant mutants to several phenotypes of R 461

mutants \ Phenotypes	R461[9]	R461[ng45]	R461[ng44]	R461[ng19]
ng 45	6.9×10^6	4.0×10^9	2.8×10^7	$1.0 \times 10^2 >$
„ 13	3.4×10^7	6.2×10^9	6.1×10^7	$1.0 \times 10^2 >$
„ 33	4.2×10^7	7.6×10^9	8.7×10^7	$1.0 \times 10^2 >$
„ 17	2.5×10^6	$1.0 \times 10^2 >$	—	$1.0 \times 10^2 >$
„ 25	8.4×10^6	1.0×10^7	1.0×10^7	$1.0 \times 10^2 >$
„ 43	8.2×10^6	6.6×10^6	$1.0 \times 10^2 >$	$1.0 \times 10^2 >$
„ 44	4.3×10^6	6.1×10^6	4.1×10^7	$1.0 \times 10^2 >$
„ 19	2.2×10^8	6.4×10^3	$1.0 \times 10^2 >$	8.3×10^6
„ 32	1.0×10^4	$1.0 \times 10^2 >$	$1.0 \times 10^2 >$	$1.0 \times 10^2 >$
Sh. No. 9 (original)	3.5×10^9	5.7×10^9	2.3×10^7	1.9×10^8

From these experimental results (Table 6), it seems that the mutants ng45, ng13 and ng33 are identical to each other and different from the remaining mutants. The mutants ng25 and ng44 are also possible to be grouped. The mutants ng17, ng43, ng19 and ng32 seem to be somewhat different to each other and to the others. At any rate, it is very clear that there exist many kinds of "semi-resistant" mutants. If *p* is a gene of Shigella No. 9 controlling resistance to the phage R461, and *p*⁺ is one in the wild type, there are several *ps*, namely *p*1, *p*2, *p*3, and so on, each representing each degree of semi-resistance. Furthermore, a semi-resistant mutant also splits off a resistant mutant (*R*) which is unable to develop any plaque to R461 (Fig. 4). Further studies are to be done of whether or not the genes *p*⁺, *p*1, *p*2,, *R* make alleles. It is to be mentioned that each phenotype of R461, for example R461[ng45], R461[ng44], and so on, corresponds to each of the *p*-genes representing semi-resistance of several degrees.

Fig. 4 Genetic variation of the p-genes

DISCUSSION

There are as already reported several degrees of plaque-developing capacity to the phage R461 among the different types of *Shigella* and the host-controlled variants are produced by each type. This holds also true for some mutants of the original strain *Shigella* No. 9, namely there exists several mutants showing certain degrees of semi-resistance different from each other, and each group of mutants produces corresponding host-controlled variants of the phage R461.

The theory of semi-resistance proposed by Wahl does not explain the whole phenomenon of semi-resistance to phage and moreover, seems to include some self-contradictions in it. As for this, detailed discussions have been made in the above description. According to our opinion, the phenomenon "semi-resistance" is to be explained neither by the inhomogeneity of host cells, nor by that of phage particles.

SUMMARY

1. The semi-resistant mutants were isolated among the phage-host-system of R461-*Shigella* No. 9.

2. The essential feature of the phenomenon "semi-resistance" has been presented about this system.

REFERENCES

1. Wahl, R., et Blum-Emerique, L.: Les bactéries semi-résistantes au bactériophage, I. Techniques: souches et phage utilisés lyse sur milieu gélosé, *Ann. de l'Inst. Pasteur*, 82, 29-43, 1952.
2. Wahl, R., et Blum-Emerique, L.: Les bactéries semi-résistantes au bactériophage, II. Etude quantitative de l'adsorption des phages sur les bactéries semi-résistantes et de l'infection de ces bactéries, *Ann. de l'Inst. Pasteur*, 82, 44-49, 1952.

3. Wahl, R., et Blum-Emerique, L.: Les bactéries semi-résistantes au bactériophage. III. Etude quantitative de la production des phages par les bactéries semi-résistantes infectées, *Ann. de l'Inst. Pasteur*, 82, 194-205, 1952.
4. Wahl, R., et Blum-Emerique, L.: Les bactéries semi-résistantes au bactériophage. IV. Influence de la concentration de certains ions sur le comportement des bactéries semi-résistantes vis-a-vis du phage, Signification de ces bactéries. *Ann. de l'Inst. Pasteur*, 82, 266-276, 1952.
5. Wahl, R.: Les types réactionnels des souches vis-a-vis des phages et leur variantes, *Ann. de l'Inst. Pasteur*, 83, 464-476, 1952.
6. Wahl, R.: Les réactivités des souches bactériennes vis-a-vis des phages, méthodes d'évaluation. *Ann. de l'Inst. Pasteur*, 83, 653-665, 1952.
7. Wahl, R.: La semi-résistance aux bactériophages. *Ann. de l'Inst. Pasteur*, 84, 51-59, 1953.
8. Nojima, T., and Fukumi, H.: Host controlled variation of *Shigella* bacteriophage, *Jap. J. Med. Sci. Biol.*, in press.
9. Delbrück, M.: *Viruses* 1950, p. 112-113, California Institute of Technology, 1950.
10. Adams, M. H.: Classification of bacterial viruses: characteristics of the T5 species and of the T2, C16 species, *J. Bact.*, 64, 387-396, 1952.