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Studies on the Additivity of Action of Genes Affecting Host Range in Coliphage T2[1]

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Adsorption and heat inactivation studies were made on a series of host range, turbid (*ht*) mutants of coliphage T2H to determine the mechanisms of phenotypic expression of these genes and of the additivity of gene effects. The *ht* mutants adsorb to the resistant cell B/2H at a slower rate than the clear *h* mutant; double and triple *ht* mutants adsorb with increased efficiency. Some mutants show increased heat sensitivity compared with the wild type. The kinetics of heat inactivation of the progeny of crosses show discontinuity in rate with time. Phenotypic mixing occurs with respect to both adsorption and heat sensitivity. The evidence supports the conclusion that many *ht* mutations are in genes controlling the formation of separate protein components and that these proteins are incorporated in different positions in the external phage coat.

INTRODUCTION

The host range phenotype of coliphage has been used in studying the genetic map of phage and the relationship between the genes and their phenotypic products (Streisinger and Franklin, 1956; Streisinger *et al.*, 1957; Streisinger, 1956; Brenner, 1957). In coliphage T2H many scattered mutants control the host range by determining the specificity of adsorption to the host bacteria (Hershey and Davidson, 1951; Baylor *et al.*, 1957; Tanami, 1957). Baylor *et al.* (1957) mapped the thirteen host range turbid (*ht*) mutants used in this study and found that their loci are scattered over 190 map units, interspersed with the *c* (cofactor), *m* (minute), *s* (star), and *r* (rapid lysis) loci. Clearly, the *ht* mutants are not all affected by the same gene. It is the purpose of this paper to consider the manner in which cooperation among the *ht* genes is effected.

The adsorptive character and heat sensitivity are properties of the protein portion of the phage (Hershey and Chase, 1952; Herriott, 1951; Streisinger, 1956; Streisinger and Franklin, 1956; Pollard and Reaume, 1951), which is a superstructure made up of a number of separable components (Van Vunakis *et al.*, 1958; Brenner *et al.*, 1959). The present experiments using heat inactivation and adsorption have led us to the conclusion that each host range gene, *h* or *ht*, controls a separate protein component and that the components controlled by the different genes are incorporated into different positions in the protein superstructure; that is, there is no competition for position among the various components. The evidence presented here eliminates an alternative hypothesis (Brenner, 1959) that a single host range protein is determined by a single gene (*h*) and, secondarily, modified by the products of other genes (*ht*).

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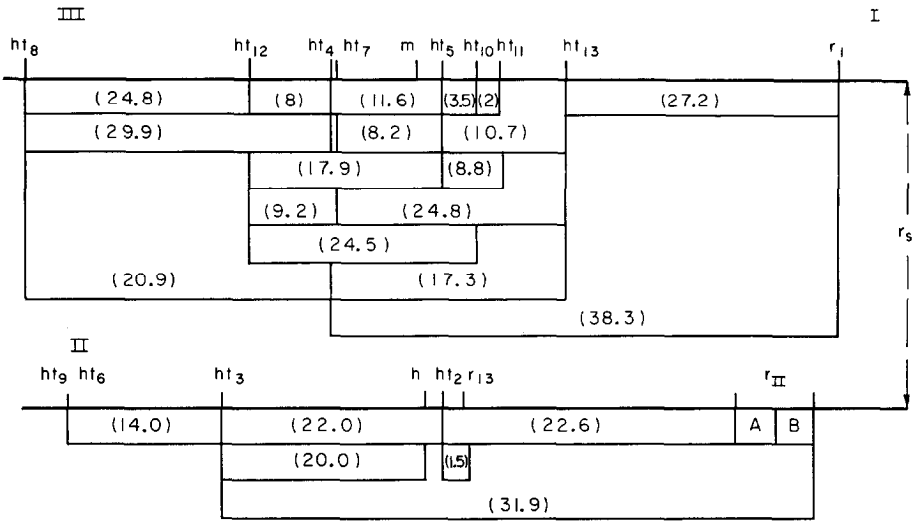

FIG. 1. Linkage may of T2H, showing the Hershey markers r_1, r_{13}, m, and the r_{II} and r_s regions in relation to the *ht* markers. Largely from Baylor *et al.* (1957) and Brenner (1959).

MATERIALS AND METHODS

The bacteria and phage used in this study were derived from *Escherichia coli* strains S, H (B), and 2bc (B/2H) and from the coliphages T2 and T2*h* obtained originally from Dr. A. D. Hershey. The *ht* mutants, numbered consecutively from one to thirteen by subscript, were described by Baylor *et al.* (1957). They give a very low efficiency of plating (0.01) on the indicator strain B/2H, and produce very turbid plaques when transferred by the velvet replica method to plates seeded with B/2H. Mutant T2*h* gives a high efficiency of plating (1.0) and clear velvet transfer plaques. A map of the mutants is shown in Fig. 1. The wild phage genotype is designated as h^+ in this report, indicating that all loci concerned with host range are present as the wild alleles. The *r* (rapid lysis) mutations are used as additional markers in these crosses. The genotypes of all the double and triple recombinant stocks have been checked by the appropriate backcrosses.

Only those techniques concerned with the present work are given here. Other details have been published (Baylor *et al.*, 1957). Free phage was assayed after adsorption in buffer on freshly grown sensitive cells. Preadsorption increases the size and uniformity of the plaques.

In the adsorption experiments, the adsorbing cells were grown in the synthetic medium M9 ($Na_2HPO_4 \cdot 7H_2O$, 11 g; KH_2PO_4, 3 g; NaCl, 5 g; NH_4Cl, 1 g; $MgSO_4$, 0.12 g; glucose, 4 g; H_2O, 1 liter) to a concentration of 2–4 $\times$ 10^8 bacteria per milliliter. Chloramphenicol, 10 μg/ml, was added to the bacterial suspension to prevent phage multiplication. Phage was added at a concentration of 2–4 $\times$ 10^7 particles per milliliter. Adsorption was measured by two methods: (1) determination of free phage after killing infective centers by chloroform, and (2) determination of infective centers after treatment with enough antiserum to give $<10^{-4}$ survival of free phage. Except where rates were measured, the adsorption was allowed to proceed for 20 minutes at 37°. Then, after treatment with antiserum or chloroform, samples were plated with an excess of cells of strain B. Free phage was assayed after preadsorption on B cells. In most experiments a mixture of two phages was used: the test phage in the *r* form and a standard control phage in the r^+ form. The control phage used with single mutants was r^+ht_2; for double and triple mutants, r^+h.

The heat inactivation experiments were carried out at 65° in broth. Small samples of test phage (0.1 ml) with the *r* phenotype

were added to 10 ml of preheated broth, and the suspension was sampled at intervals to determine the proportion of survivors. The suspension contained an r^+ phage as a standard control (ht_2 or h for the sensitive forms, h^+ for the resistant forms). The r character did not affect the rate of inactivation.

The progeny from mixed bursts were obtained by multiple infection of bacteria with two parent phage types. To eliminate phenotypic mixing, in certain experiments the first-cycle burst was added to new host cells at low multiplicity (0.1) for a second cycle of growth. The progeny phage from mixed bursts involving *ht* markers was classified by the velvet transfer technique. The term "transfer phenotype" refers to the plaque type formed after replication from the sensitive to the indicator strain. Adsorption measurements on progeny phage were made on fresh lysates because the adsorptive properties of dilute phage suspensions (but not of high titer stocks) changed on storage.

The largest variations occurred among experiments run on different days. The standard deviations given in Table 4 relate only to different experiments, as the variation within experiments was within the range of sampling errors.

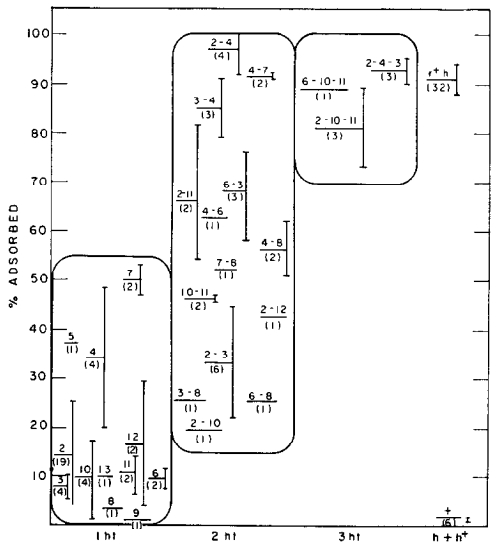

FIG. 2. Adsorption on B/2H after 20 minutes at 37° for various single, double, and triple *ht* mutants and recombinants. The markers involved are designated by the numbers above the bars. The number of determinations is given in parentheses below the bars. The length of the vertical bars corresponds to 2σ.

RESULTS

Adsorption of Mutant Phage

The adsorptive capacity of mutant strains was determined by measuring the extent of adsorption on B/2H after 20 minutes. Although there is a large day-to-day variation in adsorption (Fig. 2) it is clear that the *ht* markers affect adsorption (see Hershey and Davidson, 1951). The single-mutant strains adsorb very poorly; double-mutant strains adsorb significantly better; and triple-mutant strains adsorb as well as *h*, from which they are indistinguishable by velvet transfer and efficiency of plating. The dosage effect of the *ht*'s on adsorption explains, in part, the velvet transfer phenotypes by which the mutants and recombinants were first identified (Baylor *et al.*, 1957).

Adsorption measurements (Table 1) reveal individual differences among the *ht* mutants. Some mutants adsorb to B/2H almost as poorly as wild type (0.1–2% residual phage after antibody treatment of the adsorption mixture). Others adsorb as well as many double recombinants. No differences in burst size (25–50 phage per cell) or latent period (about 25 minutes) have been detected among the mutants growing on B/2H.

The adsorption of the double *ht* mutants (Table 2) depends on the *ht* markers involved. The double recombinant of two good adsorbers adsorbs better than the recombinant of two poor adsorbers. The adsorptive capacities are additive for closely linked (ht_4ht_7, $ht_{10}ht_{11}$, and hht_2) as well as for distantly linked markers.

The double recombinant ht_2ht_4 adsorbs almost as well as the triple recombinant $ht_2ht_3ht_4$ and *h*. This double, however, gives a turbid velvet transfer whereas the other two give clear transfers. Secondary factors on the agar plate may affect the adsorption and thus the degree of turbidity.

TABLE 1

ADSORPTION OF SINGLE-MUTANT PHAGE TO B/2H/BACTERIA[a]

Genotype	ht/ht_2	Genotype	ht/ht_2
h^+	0.17	ht_7	1.54
			1.35
ht_3	0.33		
	0.45	ht_8	0.65
	0.11		
	0.50	ht_9	0.50
ht_4	2.90	ht_{10}	0.42
	1.23		0.64
	3.86		0.60
	2.65		
	1.00	ht_{11}	1.08
			1.60
ht_5	5.90		
		ht_{12}	0.80
ht_6	0.20		0.90
	0.30		
		ht_{13}	1.60

[a] Ratios of fraction of *ht* mutant phage adsorbed to the resistant cell B/2H after 20 minutes at 37° to the fraction adsorbed of the internal standard ht_2. The internal standard was marked with the r^+ marker which did not affect adsorption. After 20 minutes, antiphage serum was added and the ratio of r/r^+ plaques was measured from the surviving infective centers.

Kinetics of Adsorption

Adsorption curves for several mutant phage strains on B/2H are shown in Fig. 3. Initial rapid adsorption is followed by a change to a much reduced rate. The change of rate occurs at nearly the same time for all phage strains, independently of the extent to which adsorption has proceeded. The measurements characterizing the genotypes were taken after the onset of the slow rate.

The discontinuity in the rate of adsorption is not due to genetic inhomogeneity. After prolonged exposure to B/2H, all plaques, both in the adsorbed and the unadsorbed fractions, give the same velvet transfer phenotype. Furthermore, ten plaques of genotype $r_1ht_2ht_3$, taken from the free phage sample gave phage that adsorbed about as well (66 ± 9%) as phage from four plaques from the adsorbed sample (53 ± 13%) and as the original virus stock (44%).

Upon addition of fresh B/2H cells to the supernatant of an adsorbing mixture, or upon dilution into a suspension of B/2H cells, the adsorption does not increase. The free phage seems to be different from original phage in adsorption to B/2H but not in adsorption to cells of strain B. On dilution into a suspension of B, adsorption proceeds at a normal rate. The change of rate is not due to the onset of an equilibrium between adsorption and desorption (Garen, 1954): if, after a 20-minute adsorption period, the mixture is diluted or the bacteria are collected by centrifugation and resuspended in fresh buffer, no desorption is observed.

The change in adsorption rate occurs at the same time irrespective of the concentration of the input phage, between 10^4 and 10^7 per milliliter. Also, a second input of phage adsorbs normally to fresh B/2H either in the presence of already infected bacteria or in the medium in which adsorption has previously occurred. Thus, no ma-

TABLE 2

ADSORPTION OF DOUBLE AND TRIPLE MUTANTS[a]

Genotype *ht* components	Per cent adsorbed			
	Double mutants (ht_xht_y) or triple mutants ($ht_xht_yht_z$)	Single mutants		
		ht_x	ht_y	ht_z
2, 3	34	15	8	
2, 4	97	15	34	
2, 10	19	15	10	
2, 11	66	15	10	
2, 12	43	15	16	
3, 4	85	8	34	
4, 7	92	34	50	
4, 8	57	34	4	
4, 6	63	34	10	
3, 8	25	8	4	
6, 8	25	10	4	
7, 8	52	50	4	
6, 13	67	10	10	
10, 11	46	10	10	
2, 3, 4	93	15	8	34
2, 10, 11	81	15	10	10
6, 10, 11	89	10	10	10

[a] Comparison of the adsorption of double and triple mutants with that of the constituent single mutants. Data from the average percentages adsorbed on B/2H after 20 minutes are shown also in Fig. 2.

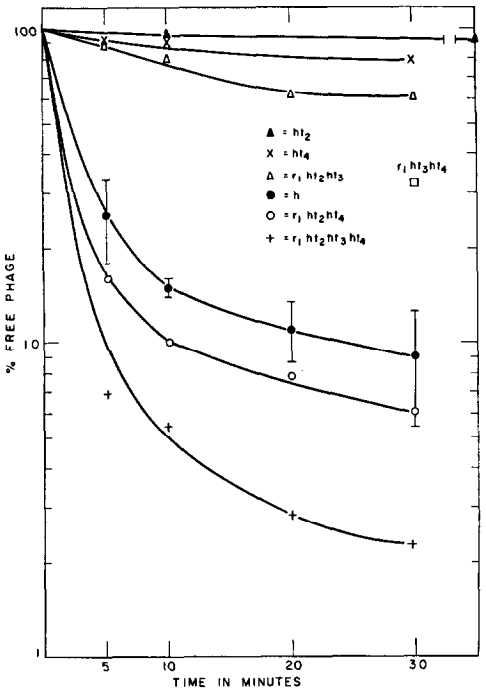

FIG. 3. Adsorption curves of mutants. The genotypes are given on the respective curves. The curve for *h* shows the average of three experiments; the length of the vertical bars is 2σ.

terials are released upon infection which inhibit further adsorption. Adsorption onto the sensitive strain shows that more than 95% of the phage have collided with cells in the first 3–5 minutes. The act of collision, if not followed by a successful adsorption, may alter the phage particle so that a future collision no longer results in adsorption to B/2H, but can still result in adsorption to B. Alternatively, the initial phage population may be phenotypically inhomogeneous in adsorption capacity.

Heat Inactivation of Mutants

There are differences in rates of heat inactivation of the *ht* mutants. Some are more sensitive than the wild type (Table 3 and Fig. 3); others have a heat stability similar to or greater than wild type. There is no correlation between adsorptive capacity and heat sensitivity. The indications are that each mutant is phenotypically distinct from all others.

Some of the heat inactivation curves (Fig. 4) extrapolate above the origin to

TABLE 3

HEAT INACTIVATION OF SINGLE MUTANTS[a]

Genotype	Number of experiments	% Survival ± σ_m after 20 min	k
ht_2	13	0.08 ± 0.02	0.35
h	10	0.31 ± 0.04	0.29
ht_4	11	2.35 ± 0.33	0.19
ht_{13}	11	2.52 ± 0.52	0.18
ht_{10}	3	10.40 ± 1.27	0.11
h^+	25	15.0 ± 1.3	0.095
ht_8	3	15.5 ± 3.8	0.093
ht_5	1	22.0	0.075
ht_7	4	22.0 ± 3.95	0.075
ht_6	5	24.0 ± 1.1	0.072
ht_9	1	26.7	0.066
ht_3	8	27.9 ± 4.0	0.064
ht_{12}	1	50.0	0.035
ht_{11}	1	59.0	0.027

[a] Survival of different *ht* mutants after heating in broth at 65°C for 20 minutes. The value of k calculated from the equation $P_t/P_0 = e^{-kt}$ where P_t/P_0 is the proportion surviving after t minutes.

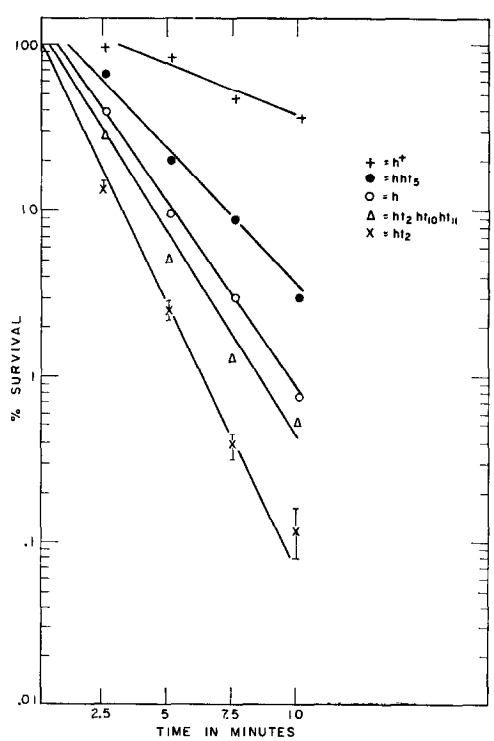

FIG. 4. Survival of mutant phage after incubation at 65° in broth. The genotypes are given on the respective curves.

TABLE 4

HEAT INACTIVATION RATES OF DOUBLE MUTANTS[a]

Genotype *ht* components	*k*, observed	*k*, calculated
2, 4	0.42	0.44
2, 13	0.38	0.44
2, 3	0.31	0.32
	0.44	
4, 8	0.26	0.19
6, 13	0.29	0.16
	0.22	
	0.21	
3, 4	0.20	0.16
3, 7	0.12	0.044
6, 8	0.11	0.070
3, 8	0.11	0.062
3, 10	0.11	0.083
6, 12	0.053	0.012

[a] Comparison of observed *k* values for double mutants to *k* values calculated as described in text. Calculated *k* values were obtained from the averages of Table 3, and were not determined in the experiments with the double and triple mutants.

about 1.5 times the initial titer. This phenomenon was noted by Adams (1949) and by Foster *et al.* (1949), who observed an actual rise in titer upon heating (see also Sagik, 1954).

As shown in Fig. 4, the inactivation proceeds as a first-order reaction, in some cases to less than 0.1% survival, for single, double, and triple mutants. Assuming the *ht* mutants to be in different genes, the homogeneity of both single mutants and double recombinants may be expected if the following "mosaic model" is postulated: (1) each gene determines a different product whose damage can result in inactivation; (2) each product is present in each phage particle; (3) there is no competition between the products of separate genes for sites on the phage particle. The single-hit nature of the curves shows that all *ht* components must be intact for a "live" phage.

If the components are independently inactivated, the rate of inactivation of a double recombinant should equal that of one of the constituent single mutants plus the difference between the rates of the other single and the wild type:

$$k_{(ht_x ht_y)} = k_{(ht_x)} + k_{(ht_y)} - k_{(\text{wild})} \quad (1)$$

Using this equation and the data of Table 3 the theoretical rate constants for the double mutants were calculated. The results (Table 4) agree roughly with the experimental finding. The discrepancies do not justify rejection of the mosaic model. Some degree of steric shielding of one element by another may be present.

Inactivation tests were done also at 55°, 60°, and 70°. The rank of sensitivity of the various mutants was the same at all temperatures. By lowering the NaCl concentration or adding $CaCl_2$, the sensitivity of some of the more heat-sensitive mutants could be reduced to that of the wild type. No disproportionate effect on the inactivation rate of any particular mutant was found.

Growing of mutants in different host cells does not change their heat sensitivity. This was tested on phages h^+, h, ht_2, ht_3, and ht_2ht_3 grown on host *E. coli* B variants B (Luria), S(Hershey), and E(Berkeley). Previous experiments had shown that the transfer phenotype and linkage distances remained constant whether the mutants were grown on strains S, H, or 2bc.

Heat Inactivation of Progeny of Mixed Infection

Sensitive mutants (h, ht_2, ht_4, ht_{13}, and ht_2ht_3) were crossed with wild type, and the heat sensitivity of the first-cycle progeny was tested. The heat inactivation curves of the progeny phage (Figs. 5 and 6) reveal a mixed population of particles. An initial rapid inactivation rate, approaching the rate of the most sensitive parent, is replaced by a slower inactivation rate approaching that of the more resistant parent. These two distinctly different inactivation rates indicate that the proteins of both parents are formed. If the *ht* genes acted to modify a single "*h*" protein, either by altering the environment in which the protein was formed or by acting on the protein through enzymes, a homogeneous progeny might be expected. The heterogeneity of the progeny shows that the products of the separate *ht* genes are incorporated into the mature phage particle.

The second inactivation rate approaches, but rarely parallels, that of the wild parent; this suggests the presence of more than one copy of each *ht*-controlled protein (see Streisinger *et al.*, 1957). In Fig. 5 the progeny phage, containing 55% wild type, is compared to an artificial mixture containing 43% wild type. If only one copy of each protein were present in the intact phage, the inactivation curve of the progeny phage should parallel the wild-type curve and should remain above the curve of the artificial mixture. Extrapolation to determine the number of copies of h^+ protein is difficult, but the clear break in rate suggests a small number, perhaps two or three.

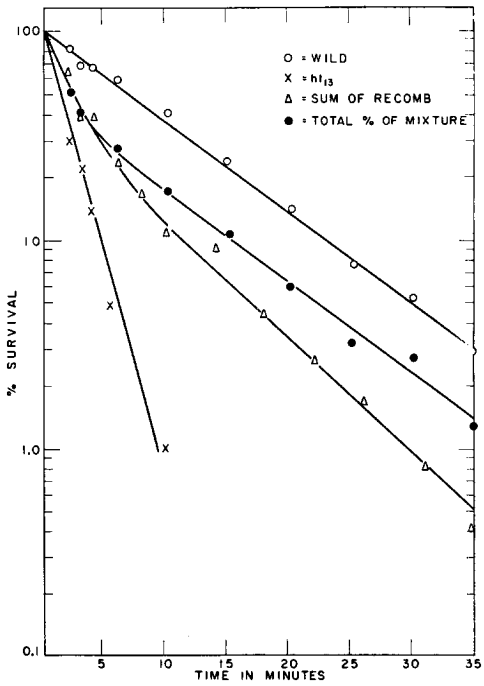

FIG. 5. Survival (at 65° in broth) of recombinant progeny phage from mixed bursts of h^+ and ht_{13} and of a control mixture of parental types.

Phenotypic Mixing

Phenotypic mixing indicates that the gene (DNA) and its product (protein) are assembled independently into the completed phage particle (Streisinger, 1956; Brenner, 1957). The *h* and *ht* markers in T2H show phenotypic mixing, in respect both to adsorption (Hershey *et al.*, 1951) and to heat inactivation. The progeny phage of crosses between phage differing in host range was adsorbed onto the resistant strain B/2H for 20 minutes, and the adsorbed fraction was analyzed for genotype by the velvet transfer method. The ratio of genotypes adsorbed to the B/2H cells was compared to the ratio adsorbed to B cells. The ratios did not change, as they should have if each phage particle had the adsorptive properties corresponding to its genotype (Table 5). Second-cycle progeny and mixtures of parent phages, both of which were used as controls, showed a striking dissimilarity in the ratios of genotypes adsorbed to the two bacterial strains: the resistant cells adsorbed selectively the phage of the highest host range.

Progeny phage from cells mixedly infected with sensitive and resistant parents was tested by heat inactivation, and the ratios of the genotypes, as determined by the host range, were compared at different levels of inactivation. The ratios remained constant, although the break in the curves showed the populations to be heterogeneous (Table 6, Figs. 5 and 6). Second-cycle progeny was inactivated like the artificial mixtures of the parental types: that is, the host range corresponding to greater heat stability predominates in the surviving fraction.

Linkage of Heat Sensitivity to Host Range

Inactivation of second-cycle progeny (Table 6) shows that heat sensitivity and host range are closely linked in these crosses. The ratios of the survivals of wild type and mutant type are as to be expected if every wild type particle were heat stable and every host range mutant particle were sensitive. Any appreciable fraction of host range mutant, heat-stable progeny would have been detected. Since the *ht* mutants were originally selected as single-step mutants toward increased host range and were later found to differ from wild type in heat sensitivity, no segregation of the two phenotypes was to be expected.

Cis-trans Test

In the *ht* case the *cis-trans* test requires mixed infection with two closely linked,

TABLE 5

PHENOTYPIC MIXING BY ADSORPTION TESTS[a]

Cross	Adsorbing bacteria	Progeny of cross; % genotype among the adsorbed phage			% Adsorbed on B/2H after 20 minutes		
		Double mutant	*ht* recombinant	Wild type	Total 1st cycle progeny	Double-mutant control	Wild-type control
ht_4ht_7 × wild, m.o.i. 5.8	B	54.8	<1	45.2			
	B/2H	50.8	<1	49.2	19	40.0	1.7
	Expected on B/2H	96.5	<1	3.5			
ht_2ht_3 × wild, m.o.i. 6	B	59.2	6.2	34.9			
	B/2H	58.2	10.9	30.8	~50	27.5	2.2
	Expected on B/2H	92.0	3.2	4.8			
		ht or h^+		*h* or *hht*	ht_4 parent		*h* parent
ht_4 × $r_{13}h$, m.o.i. 9.8	B	41.8		58.2			
	B/2H	42.2		57.8	27		100
	Expected on B/2H	16.3		83.7			

[a] Samples from the progeny of crosses were adsorbed onto the sensitive strain B and the resistant strain B/2H. The genotypes of the adsorbed fractions were tested by the velvet transfer method. The expected values with B/2H are calculated from the amount of adsorption of parental controls. m.o.i = Total multiplicity of infection in the crosses.

heat-sensitive mutants. The combination ht_2 and *h* is the only one to fit these requirements. The heat inactivation of progeny from the cross ht_2 × *h* is shown in Fig. 6. The wild alleles of ht_2 and *h* were introduced by different phage particles. The inactivation curve was examined to detect the presence of wild-type protein. No heat stable phage were detected down to less than one in a hundred particles. This result may mean that ht_2 and *h* are part of the same cistron. However, if a protein mosaic consisted of a large number of sites, even if some wild-type protein were produced, the fraction of phenotypically wild-type phage might be too small to be measured. Streisinger and Franklin (1956), by similar experiments, showed that a series of closely linked, host range mutants of T2L belong to the same cistron. Inactivation of the progeny of the cross in the *cis* configuration, ht_2h × h^+, gives the same curve as shown in Fig. 6 for the progeny of ht_2 × h^+ and *h* × h^+.

DISCUSSION

The genetic map of T2H contains many *ht* genes scattered over most of the map. The phenotypic mixing experiments establish that the *ht* genes, like the *h* gene, determine products that are assembled into the mature phage independently of the genome. The heat sensitivity of the progeny of mixed bursts show that both alleles are expressed when present in the same cell. The one-hit nature of the heat inactivation kinetics of the mutants and the additivity of adsorptive capacity in multiple mutants establish that the product of each gene is present in the final coat of the phage. The evidence as a whole constitutes compelling support of the hypothesis that each *ht* gene determines a separate product and that each product has its place in the mature phage particle.

Thus, the problem of phenotypic additivity between genes with apparently simi-

lar effects (Baylor *et al.*, 1957) is solved by showing that the genes produce different products. If the results of the *cis-trans* test are accepted as evidence that the ht_2 mutant is part of the *h* gene, additivity within a gene also occurs. The double mutant hht_2 is distinctly different from either *h* or ht_2. The double recombinant hht_2 gives a turbid velvet transfer on the very resistant strain B/2H/*h*, while neither *h* nor ht_2 give any transfer (Baylor *et al.*, 1957). It is possible that *h* and ht_2 affect different parts of the same protein. Such intragenic additivity can be found only when two phenotypically different mutants are available each of which is functionally altered but still active. It would be interesting to look for intragenic additivity in other systems.

TABLE 6

PHENOTYPIC MIXING BY HEAT INACTIVATION TESTS[a]

Cross	Minutes at 65°	1st-Cycle lysate	2nd-Cycle lysate
		h^*/total × 100	h^+/total × 100
$h \times h^+$	0	47	48
	5	49	69
	10	56	85
	20	64	97
	40	55	—
$ht_2 \times h^+$	0	45	55
	5	—	70
	10	48	—
	20	—	98
	30	51	—
	40	48	—
$ht_2 \times ht_3$	0	50	—
	10	56	—
	40	45	—
		rh^+ recomb./total × 100	h^+/total × 100
$rht_2ht_3 \times r^+h^+$	0	16	63
	5	22	86
	10	16	96
	20	13	99
	40	17	—

[a] Proportions of h^+ survivors in progeny of mixed bursts from crosses of heat-sensitive and heat-resistant parents after exposure in broth at 65°. Total multiplicities were 7–8. Progeny from second-cycle lysates (m.o.i. = 0.1) to remove phenotypic mixing served as controls.

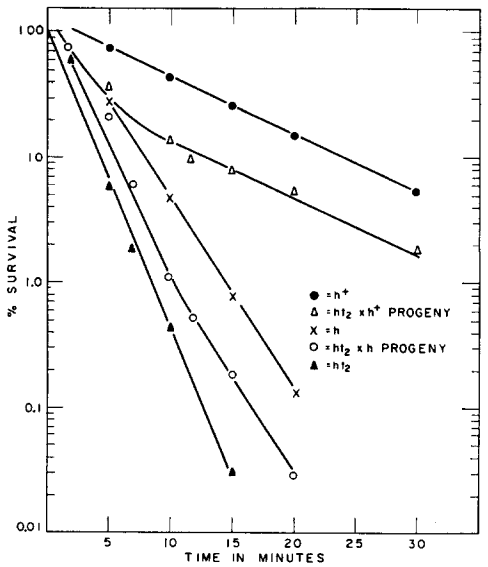

FIG. 6. Survival of progeny phage from mixed bursts of ht_2 and h^+, ht_2 and *h*, and of control parental types after incubation at 65° in broth.

The external phage protein consists of at least five components: head, sheath, core, plate, and tail fibers (Kellenberger *et al.*, 1959; Brenner *et al.*, 1959). The tail fibers appear to be specifically concerned with adsorption (Anderson, 1953; Williams and Fraser, 1956; N. Franklin, 1960, personal communication). However, all host range genes need not be concerned with tail fibers. Any change in surface charge of the particle may affect the approach of the phage to the cell and thereby affect adsorption. The amount of protein involved in the tail assembly (mol. wt. 15×10^6: Brenner *et al.*, 1959) could easily accommodate several hundred protein molecules. All the host range factors, including *h* (Streisinger, 1956; Tanami, 1960), *c* (Brenner, 1957), and the *ht's* might fit into the tail. The *ht* genes are so numerous and occupy such a proportion of the known linkage group, however, that it seems likely that some of these mutants are concerned with structures other than the tail fibers.

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