GENETIC RECOMBINATION BETWEEN HOST-RANGE AND PLAQUE-TYPE MUTANTS OF BACTERIOPHAGE IN SINGLE BACTERIAL CELLS[1, 2]

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WE HAVE previously shown that any two of several independently arising plaque-type (*r*) mutants of the bacterial virus *T2H* interact with each other, in bacterial cells infected with both, to give rise to wild type and double mutant genetic recombinants (HERSHEY and ROTMAN 1948). In this paper we describe comparable interactions between host-range and *r* mutants of the same virus. The experiments furnish new information because it has proved possible to count the numbers of all four types of virus found in yields from the mixedly infected bacteria.

MATERIALS AND METHODS

The types of viral mutant to which we shall refer in this paper may be summarized in terms of the mutational pattern illustrated in fig. 1. In this diagram, *h* refers to a host-range mutant, *r* to any one of the rapidly lysing mutants (HERSHEY and ROTMAN 1948), and *m* ("minute") to a mutant not previously described which is characterized by a very small haloless plaque. The *h* mutant is one which forms plaques identical in appearance and number on typically sensitive strains of *Escherichia coli*, and on an indicator strain (No. 2 B/2H, 2K) resistant to h^+ forms of the virus (HERSHEY 1946a). All the steps indicated in the diagram by arrows can be observed either as spontaneous mutations, or by making the appropriate crosses. Only one example of the mutant *m*, obtained by crossing wild type with an *rm* arising in a stock of the mutant *r13*, has been studied. The plaques of *m* and *rm* are different, but are not easily distinguishable, as shown in the photograph (fig. 2).

In principle, the experimental technique we have to describe is very similar to that of genetic crossing, and will be referred to in this paper in genetic terms. One starts with a pair of mutants, each corresponding to a mutant haploid germ cell differing from wild type by a different unit change. Bacterial cells are infected with both members of the pair, and during viral growth the pair interact to produce viral progeny corresponding to germ cells of a new

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generation, but now including some individuals differing from wild type by both unit changes, and other individuals differing from wild type not at all.

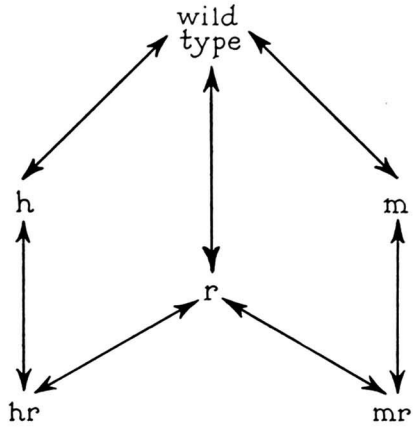

FIGURE 1.—Mutational pattern of the bacterial virus *T2H*.

The analogy to other genetic recombinations is obvious, and it is natural to look for a common mechanism.

The procedure of making a cross consists essentially in infecting a measured number of growing bacteria with larger measured numbers of two kinds of virus, diluting the culture before lysis begins to prevent readsorption of viral progeny to bacteria not yet lysed, and plating samples of the total yield of

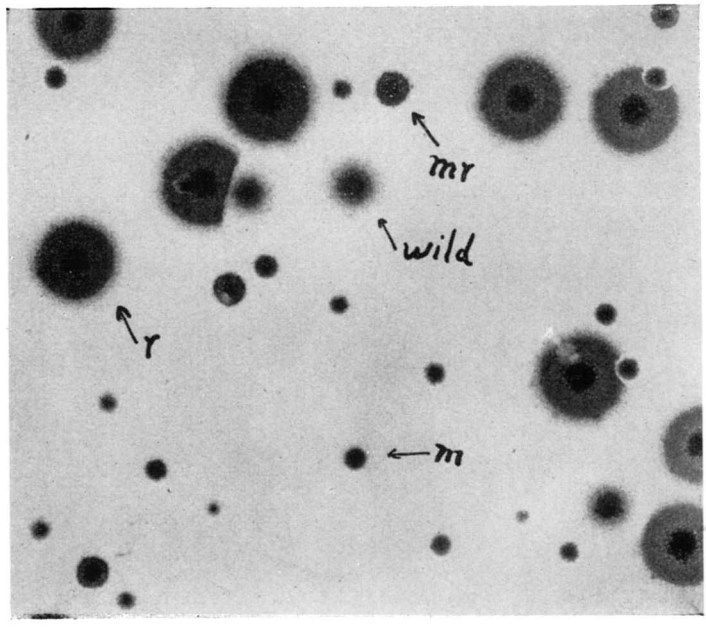

FIGURE 2.—Progeny of the cross $m \times rI$.

virus for a differential count of its component types. This procedure was first used, for another purpose, by DELBRÜCK and LURIA (1942).

The advantage of the $h \times r$ cross is that all the genetic types of virus to which it gives rise can be recognized in a single plating on a mixture of bacterial strains (fig. 3). This makes possible the analysis of viral yields from single bacterial cells. For this purpose the procedure already described is modified

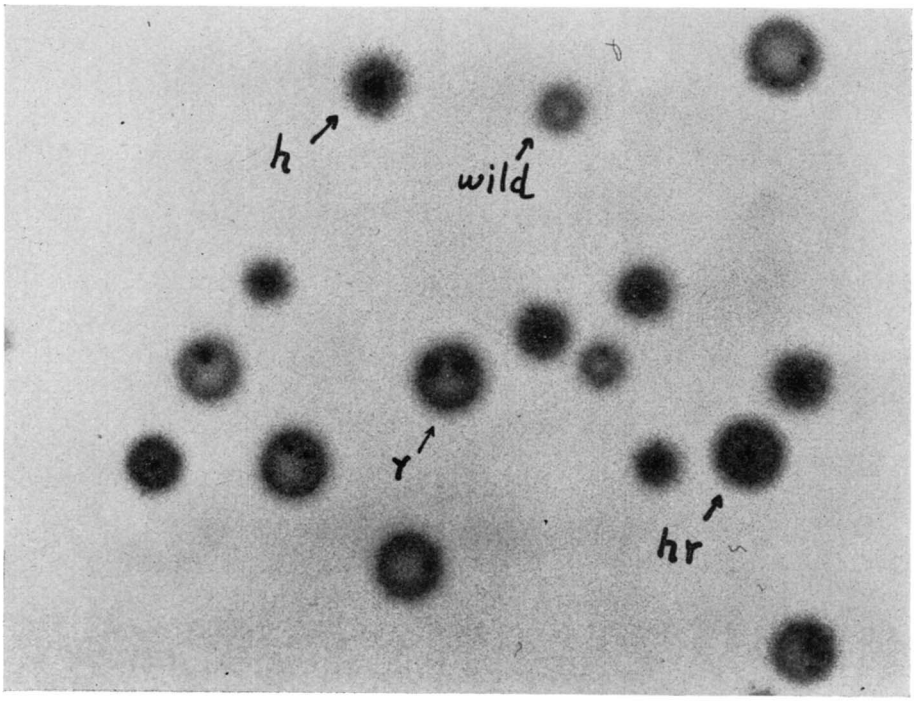

FIGURE 3.—Progeny of the cross $h \times r1$ plated on mixed indicator. The acentric clearings in the h^+r plaques result from secondary h mutations.

by increasing the factor of dilution to obtain only one infected bacterium to about three ml of nutrient broth. The culture is then divided up into samples of one ml, most of which will contain either no bacteria at all, or a single one. A large number of these samples are plated out after the bacteria lyse, and the elementary yields, averaging about 500 particles of virus in our experiments, are analyzed in toto. Both the mixed indicator method for differential counting of viral mixtures, and the single burst technique as here employed, were first used by DELBRÜCK (1945a, b).

The remaining portion of this discussion of materials and methods is of technical interest only.

Viral stocks are prepared by seeding nutrient broth cultures of *E. coli* strain S with material taken directly from a single plaque. Stocks of wild type and h mutant usually have titers exceeding 10^{10}/ml; r mutant titers are always a

little less. Many of the stocks, particularly of the *r* mutants, contain unknown substances inhibiting adsorption of the virus if the dilution into bacterial suspension is less than 1:100 or so. There is no difference in the adsorption of different stocks at high dilutions of the virus. If a mixed infection is attempted with an *r* stock which contains inhibitor, and an *h* or wild type stock which does not, the adsorption of both viruses in the mixture is prevented equally. No undesirable disturbance of the relative multiplicity of infection in mixtures is therefore encountered. To obtain satisfactory levels of adsorption, all *r* mutant stocks are sedimented in a refrigerated International centrifuge with Multispeed head, and resuspended in a solution containing 1 percent Bactopeptone and 0.5 percent NaCl. Very little loss of virus occurs during sedimentation, and probably no permanent aggregation, since sedimented mixtures of *r* and wild type virus do not yield mixed plaques. The resuspended virus is stable for at least several weeks.

To make a cross, a two hour culture of *E. coli* strain H in nutrient broth, containing 2×10^7 bacteria per ml, is infected at 37°C in an aerated culture tube (the "adsorption tube") with $\frac{1}{10}$ volume of a mixture of diluted viral stocks containing 2×10^9 plaque forming particles of each kind per ml. After five minutes, during which equal numbers (about 50 percent) of each virus are adsorbed, a 10^4 dilution is made into broth (the "growth tube") for further incubation, and a second diluted sample is spun for the assay of unadsorbed virus. Sixty minutes after infection, an assay from the growth tube gives the average yield of virus from about 40,000 mixedly infected bacteria.

For the single burst experiments, an additional dilution from the adsorption tube into antiserum to neutralize the unadsorbed virus (DELBRÜCK 1945c), is made at the end of the adsorption period. Five minutes later, a further dilution from the antiserum tube is made into broth to contain about one infected bacterium per three ml. Before the 20th minute after infection, samples measuring one ml are distributed into a series of small tubes. The virus yields in these tubes are assayed by plating 0.3 ml 60 minutes or more after infection. The remainder of each sample, excepting those containing no virus or unmixed yields, is plated on two additional plates the next day. About 10 percent of each sample is mechanically lost. An important feature of these experiments is the guard against contamination of materials provided by the fact that about $\frac{3}{4}$ of the samples contain no virus, whereas the remainder contain more than 100 particles.

Viral yields from the growth tube are plated on sensitive bacteria (strain S), on the indicator strain (No. 2 B/2H, 2K), and on a mixture containing one volume S and two volumes indicator (day-old broth cultures). On the mixed indicator all four types of virus can be recognized (fig. 3), and their sum equals the count on S. Mixed indicators plates always show a few doubtful plaques which can be identified only by sampling and retesting, but their number is too small to be of importance, and mixtures of pure stocks can be counted with satisfactory accuracy. The counts on the single indicator, giving only the *h* mutants, are also satisfactory with mixtures of pure stocks. These counts tend to be low, however, for mixed yields of *h* and h^+ virus. The cause

of this "mixed indicator effect," similar to that observed by DELBRÜCK and BAILEY (1946), remains obscure. It does not appear to be the result of segregation of multiple *h* factors, because no intermediate genetic types can be found in crosses between *h* and wild type. In *h*×*r* crosses, it affects equally counts of parental and recombinant virus.

The validity of the mixed indicator count itself rests on three lines of evidence. First, plaques sampled and retested always conform to the genetic type deduced from inspection. Second, the ratio of *h* to h^+ virus in the yield following mixed infection, measured by the mixed indicator count, is the same as the corresponding ratio of infecting viruses, with minor exceptions to be mentioned below. Third, the yield of the two recombinants in either *h*×*r* or *hr* × wild type crosses is very nearly equal, and the slight bias (actually of doubtful significance) correlates not with the *h*, but with the *r* pair of alleles.

For these reasons, and because of its statistical efficiency, only the mixed indicator plating is used for counting single bursts. The reproducibility of the counts so obtained may be judged from the examples shown in table 1.

TABLE 1

Mixed Indicator Counts of Viral Types in the First Eight Bursts Examined from the Cross h×r7.

The counts shown are for 3 aliquots of 0.3 ml from each tube. The volumes are not measured very accurately, owing to the effort made to plate the entire sample. In computing results, it was assumed that totals of counts for each tube represented 90 percent of the actual virus content, 10 percent of the fluid being lost mechanically.

TUBE	h^+r^+	hr^+	h^+r	hr	TUBE	h^+r^+	hr^+	h^+r	hr
2	9	57	46	19	35	21	80	71	23
	9	77	56	20		24	74	65	18
	11	50	47	18		14	68	39	15
10	18	17	95	13	36	9	136	30	9
	10	14	73	8		7	100	22	7
	10	22	118	15		14	120	18	9
26	9	84	51	8	38	9	139	30	9
	10	124	53	5		9	130	19	13
	8	78	34	7		4	102	31	4
34	2	20	58	17	40	1	24	54	1
	6	26	78	32		3	38	83	10
	6	33	55	16		3	24	66	7

The nutrient broth referred to above is composed of Bacto-peptone 10 g, Bacto-beef extract 3 g, NaCl 5 g, glucose 1 g, per liter distilled water. The pH (unadjusted) is about 6.8. An occasional batch of broth prepared according to this formula proves unfavorable to the adsorption of the virus.

Nutrient agar plates are poured with a minimum of 35 ml per 9 cm Petri dish of Bacto-agar 10 g, Bacto-Tryptose 10 g, NaCl 8 g, sodium citrate crystals 2 g, glucose 1 g, per liter distilled water. The pH of the agar is adjusted to 6.8 to 7.0. Contrary to an early experience, we have recently found that Bacto-Tryptone can be substituted for Tryptose. Poured plates are stored in

the refrigerator. Agar for layer plating has the same nutrient composition, but contains only 0.5 to 0.6 percent agar, the optimal concentration depending on the age of the plates and on other variables.

All platings of virus are made by the agar layer method, by adding an aliquot of virus measuring 0.1 to 0.5 ml and about 0.1 ml of a day old unaerated broth culture of bacteria, to 2 ml of melted soft agar at 45°C, and pouring the mixture over the surface of an agar plate at room temperature. The plates are incubated 18 to 24 hours at 37°C without inverting, during which time fluid collects on the agar surface without undesirable effects if conditions are optimal.

Bacterial counts are made by spreading 0.1 ml aliquots on the surface of dried agar plates.

THE LINKAGE SYSTEM

The mutants *m* and *h* (*minute* plaque and *host-range* modification, respectively) have been crossed with wild type, with the mutants called *r1*, *r7*, and *r13*, and with each other. The crosses with wild type yield only the parental types of virus, confirming that the mutants are unit modifications. The crosses with *r* mutants, and the intercross, extend the linkage system previously described (HERSHEY and ROTMAN 1948) as shown in fig. 4. The *h* locus is closely linked to the locus *r13*; the *m* locus belongs in a third linkage group C.

It should be understood that the diagram of fig. 4 is only a convenient representation of linkage relations whose structural basis remains to be elucidated (HERSHEY and ROTMAN 1948). The question of linear structure will be returned to in the discussion of this paper. At this point we insert an experiment which supports the general interpretation in terms of linkage.

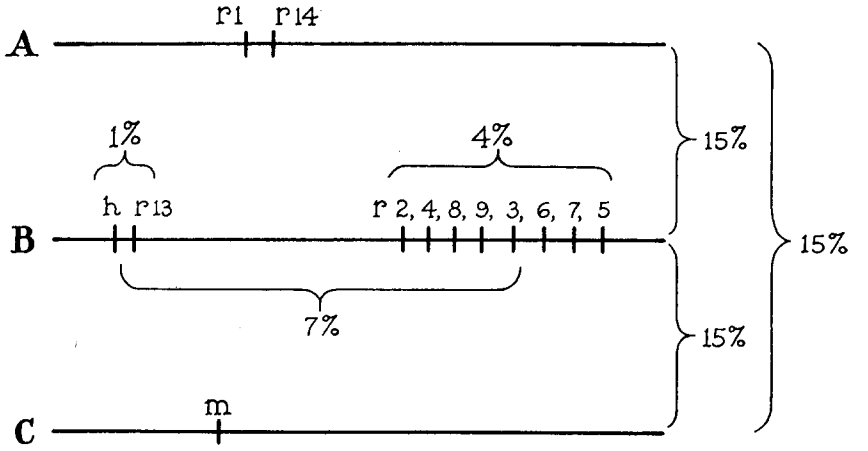

FIGURE 4.—Linkage relations among mutants of *T2H*. The percentages indicate yields of wild type in two factor crosses.

The double mutants *h r7* and *h r13* were isolated by making the respective crosses *h*×*r7* and *h*×*r13*. The three crosses *r7*×*r13*, *h r7*×*r13*, and *h r13*×*r7* were then compared with respect to the yield of r^+ virus, classified without

respect to the host-range character. In each case the yield was the same within experimental error, seven percent of the total virus. This experiment shows that the small yield of recombinants (one percent of wild type) in the cross *h*×*r13* cannot be attributed to suppression of a hypothetical conjugation, which would affect the interaction of *h r7* with *r13* as well, but indicates some kind of linkage between the genetic factors concerned.

AVERAGE YIELDS OF VIRUS IN CROSSES BETWEEN HOST-RANGE AND *r* MUTANTS

Six crosses have been studied by the single burst technique; namely, *h*×*r1*, *h*×*r7*, *h*×*r13* and the corresponding reverse crosses, *h r*× wild type. Each cross was made three to five times for the collection of the single burst data, and each time the viral yield was examined also from a culture tube containing about 40,000 mixedly infected bacteria. The average yields from the culture tubes are summarized in table 2. They show that the *h* locus is very closely linked to *r13* (less than one percent of wild type), and that the linkage relations to *r1* and *r7* are approximately what would have been predicted from this fact, respectively 12 and 6 percent of wild type. According to arguments previously given (HERSHEY and ROTMAN 1948), the factors *h*, *r7*, and *r13* are

TABLE 2

Average Percent Distribution of Viral Types in Yields from about 40,000 Mixedly Infected Bacteria

The results shown are from the same experiments for which single burst data are also reported, except that one growth tube was lost among the crosses *hr7* by wild type. The total multiplicity of infection is about five of each type per bacterium; the adsorption period is five minutes; the total incubation period one hour. The distribution of viral types is computed from the results of mixed indicator platings. The column headed eop(h) gives the efficiency of plating of *h* virus on single indicator as compared with mixed indicator, and illustrates the mixed indicator effect mentioned in Methods. The column headed p(h) gives the percent of virus containing the *h* allele, and shows the effect of selection during growth.

CROSS	NO. OF EXPTS.		h^+r^+	hr^+	h^+r	hr	BURST SIZE	eop(h)	p(h)
h×*r1*	5	input	0	53	47	0		1.0	53
		yield	12	42	34	12	630	0.8	54
hr1×++	3	input	57	0	0	43		1.0	43
		yield	44	14	13	29	680	0.7	43
h×*r7*	4	input	0	49	51	0		1.1	49
		yield	5.9	56	32	6.4	650	0.8	62
hr7×++	2	input	49	0	0	51		1.0	51
		yield	42	7.8	7.1	43	690	0.8	51
h×*r13*	3	input	0	49	51	0		1.1	49
		yield	0.74	59	39	0.94	510	0.8	60
hr13×++	4	input	52	0	0	48		0.9	48
		yield	50	0.83	0.76	48	590	0.7	49

linked to each other, but *r1* is probably situated on an independently exchanging structure.

The results of table 2 show further that the two recombinants appear in equal numbers in any one cross, and that pairs of reverse crosses yield equal numbers of recombinants. It is these relations, which increase the resemblance to simple types of Mendelian segregation, that we wish to examine by the single burst technique. In the remainder of the experimental part of this paper we describe the results of this examination, but limit our comments chiefly to the technical problems encountered. The general implications of the data will be considered in the discussion.

VARIATIONS IN YIELDS OF PARENTAL TYPES OF VIRUS AMONG INDIVIDUAL BACTERIA

Yields of virus from single bacteria show large fluctuations in size (DELBRÜCK 1945b) and, in our experiments, variations in relative yields of the two infecting viruses. The variations in total yield are shown in fig. 5 which includes the complete data for six experiments in which the proportion of multiple bursts is small (100 bursts out of 484 tubes, or 11 probable multiples). The burst sizes range from 150 to several thousand, with a mean of 520, or 470 corrected for probable multiples. The distribution is the same for the mixed bursts, and for the bursts containing only one viral type. Owing to these variations, it is convenient to describe the individual bursts in terms of the fractional yield of the several viral types.

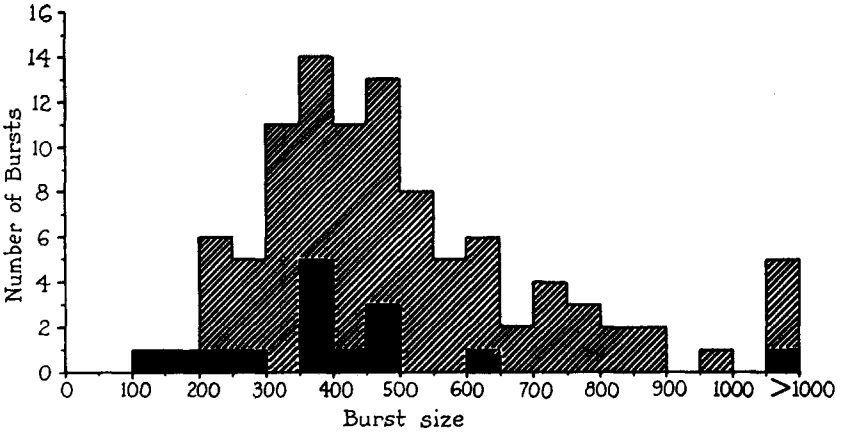

FIGURE 5.—Distribution of total viral yields among 100 single bursts from mixedly infected bacteria. The solidly shaded areas refer to unmixed yields.

Each cross yields four types of virus, wild type, the single mutants *h* and *r*, and the double mutant *h r*, of which two are parental and two are recombinant types. In order to examine the variations in relative yields of parental types independently of variations in yields of recombinants, it is convenient to express the former in terms of the proportion of virus containing a specified allele, viz:

$$p(h) = \frac{n(hr^+)+n(hr)}{n\ (total)} \quad (1)$$

$$p(r) = \frac{n(h^+r)+n(hr)}{n\ (total)} \quad (2)$$

where n indicates the number of the specified viral types, p(h) is the proportion of the yield containing the h allele, and $1-p(h)$, p(r), and $1-p(r)$ are respectively the proportions containing the h^+, r, and r^+ alleles. The fraction p(h) is equal to $1-p(r)$ if the numbers of the two recombinant types are the same, or approximately equal if these numbers are small. A fair idea of the complete distribution of alleles is therefore given by the distribution of p(h) alone. Two examples of this distribution, showing the proportions of the h allele in different single bursts for the crosses $h \times r1$ and $h \times r7$, are given in fig. 6.

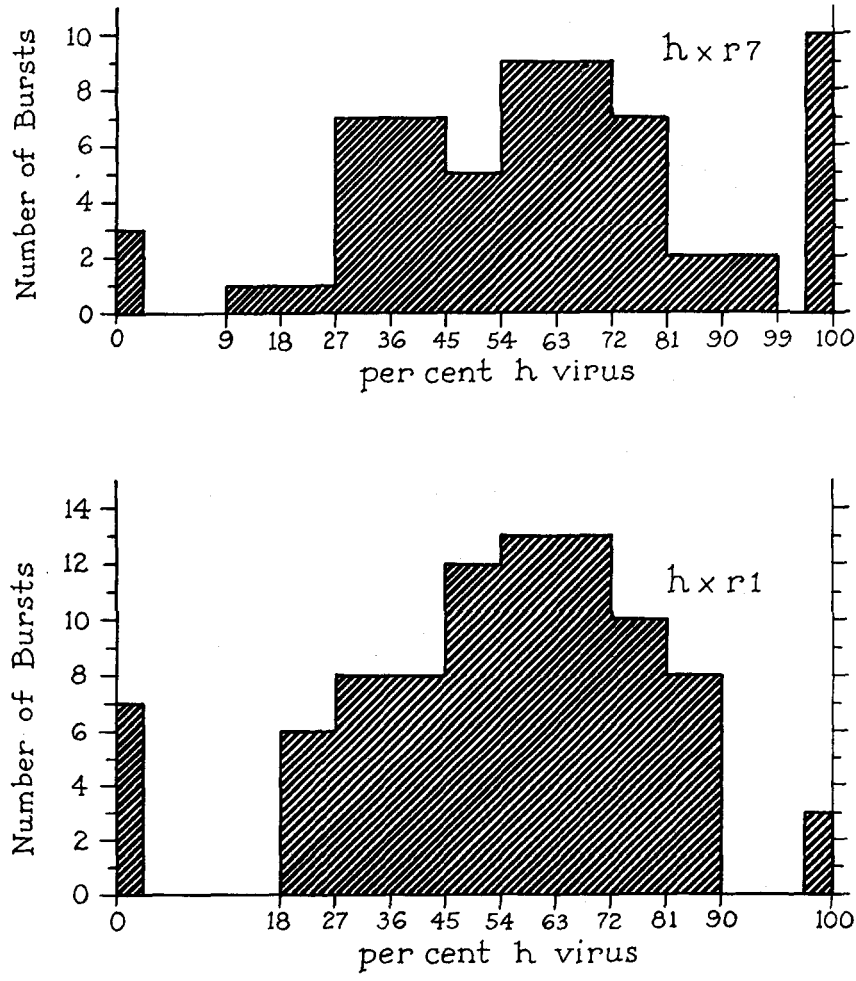

FIGURE 6.—Distribution of proportions of h virus in yields from single bacteria.

The variations shown in fig. 6 are evidently due in part to variations in relative numbers of the two kinds of virus adsorbed, and in part to variations in viral growth. For purposes of comparison, the variation in relative multiplicity has been computed on the assumption of a random distribution of two types of virus over a population of bacteria receiving on the average five particles of each kind. The distribution for this case is approximately that shown in table 3.

TABLE 3

Ideal Distribution of Multiplicities in Mixed Infection

The proportions of bacteria falling into the specified groups classified with respect to relative multiplicity of infection with two kinds of virus have been calculated for random adsorption with average multiplicity of five each of the two kinds.

NUMBERS OF FIRST KIND AS PERCENT OF TOTAL VIRUS ADSORBED	PERCENT OF BACTERIA
0 or 100	0.7 (exact)
1–9 or 91–99	0.2 (approximate)
10–18 or 82–90	2.0 "
19–27 or 73–81	5.1 "
28–36 or 64–72	10.8 "
37–45, 46–54, or 55–63	18.2 "
total	92.2

The distributions of yields actually found (fig. 6) differ from the theoretical distribution of multiplicities in showing a considerably broader spread, and a significant excess of yields containing only one kind of virus. These effects could be due in part to an inhomogeneity of the bacteria with respect to adsorbing power for virus; otherwise they suggest that a bacterium infected with two viruses is somewhat less likely to liberate a given one, than a bacterium infected with that one alone. DULBECCO (1949) has shown that the latter is, in fact, the case.

Another possible contribution to the variations described is connected with the relatively long period (five minutes) allowed for adsorption of virus, which permits some bacteria to be infected with one or more particles of one type of virus considerably in advance of infection by the second. Owing to the slow adsorption of the virus *T2H* and its mutants, the adsorption time cannot be much reduced without reducing the total multiplicity of infection, or introducing excessive amounts of virus. This contribution to the variation in composition of viral yields has not, therefore, been assessed.

COMPETITION BETWEEN VIRAL MUTANTS

The competition between viral mutants expresses itself in two ways; first, by the complete suppression of one virus or the other in mixedly infected bacteria, and second, by excessive growth of one of the two types in bacteria liberating both. These effects are slight among mutants of *T2H* so far examined,

and probably do not influence appreciably the yields of recombinants in genetic crosses, as the following discussion will show.

The two distributions shown in fig. 6 illustrate the competitive relations encountered. In the cross *h*×*r7*, most of the unmixed yields contain the *h* rather than the *r* parental type. Corresponding to this, there is a tendency for the mixed yields to contain an excess of *h* virus. The combined effect is to cause a definite increase in yield of *h* virus at the expense of *r*, as compared with the input proportions. The cross *h*×*r13* also shows these characteristics, the effects being evident in table 2.

In the cross *h*×*r1*, on the other hand, the unmixed yields of each kind are approximately equal in number, and the mean proportion of *h* virus in the mixed bursts, in the total yield, and in the input mixture of viruses is the same. The crosses between wild type and *h r1*, *h r7*, and *h r13* are like *h*×*r1* in this respect, as shown in table 2.

It might be supposed that the suppression of one virus by a second is favored by an excess of the second. This is true only in a special sense, as DULBECCO (1949) has shown, and we have confirmed. An excess of one virus tends to suppress a minority type completely in some bacteria, but there is a compensating excess of this type among the mixed bursts, so that the average proportion of the minority virus in the yield averaged over many bacteria is the same as in the input mixture. This identity has been established with considerable precision for proportions of *r1* between 7 and 50 percent in mixed infection with wild type. The nature of this relationship, which is at first sight perplexing in the case of unequal multiplicity, has been explained by DULBECCO (1949) in terms of a limitation to the number of viral particles which can participate in growth in a single bacterium. If all those viral particles in excess of a certain number attached to the same bacterium fail to grow, and if the excluded ones are chosen at random, the result will be precisely the one described, provided there is no selection during the growth of the successful particles.

It is apparent that with certain viral pairs, the excluding mechanism does not operate at random, or there is continuing selection during growth. Thus *h* mutant slightly suppresses *r7* or *r13*, but not *r1* or wild type. Wild type suppresses *r13*, but not *r1* or *r7* (HERSHEY and ROTMAN 1948). There is no selection with respect to either *h* or *r* factors when wild type is crossed with *h r1*, *h r7*, or *h r13* (table 2).

The competitive relations discussed above are of immediate interest only in the negative sense that they probably do not influence the yields of recombinant virus in crosses. The evidence for the latter conclusion, drawn from data presented elsewhere in this paper, may be summarized as follows: (1) the linkage relations deduced from average yields of virus are the same as those deduced from single bursts selected for equality of yields of the two infecting viruses; (2) in the reverse crosses *h*×*r7* and *h r7* × wild type, one gets within experimental error equal numbers of all four recombinants in spite of the fact that in one case the infecting pair, and in the other the recombinant pair, have unequal excluding power; (3) in all crosses, the distribution of yields of recombinants among single bursts does not show one peak at zero and another

above the mean, as is the case with the yields of a minority infecting type, but shows a single mode slightly less than the mean.

The last two lines of evidence cited seem to show that the principle of limited participation (DULBECCO 1949) referred to above, operates only during the initial stages of infection, or at any rate does not influence the yields of genetic recombinants arising within the mixedly infected bacteria. They suggest further that the *h* mutant is superior to *r7* or *r13* in excluding power only, not as a competititor during actual multiplication.

YIELDS OF GENETIC RECOMBINANTS FROM SINGLE MIXEDLY INFECTED BACTERIA

In order to study the variations in yields of recombinants intrinsic to the recombination process, one would like to exclude as many as possible of the accessory sources of variation. The most important of these are variations in burst size, and variations in the relative numbers of the two infecting viruses adsorbed to individual bacteria. It will be seen presently that effects of variations in burst size can be avoided by the simple expedient of computing proportionate yields of recombinants, these being independent of burst size. The effect of variations in relative multiplicity could be minimized either by going to very small or very large total multiplicities. Low multiplicities are uneconomical, because at multiplicities sufficiently small so that most of the mixedly infected bacteria receive only one viral particle of each type, very few of the test cultures will yield a mixed burst. High multiplicities also introduce difficulties (DULBECCO 1949). We have chosen to use total multiplicities between 10 and 20, within which range the yield of recombinants is constant.

As previously described, the elementary viral yields vary considerably in the relative numbers of the two parental types of virus and, as expected, these variations influence in turn the yields of recombinants. A correction for this source of variation was devised as follows. Assuming that the genetic interaction occurs between unlike viral pairs, and that the composition of the viral yield provides a direct measure of the composition of the intracellular viral population during growth, one computes an interaction coefficient

$$k = p(h)[1 - p(h)] \tag{3}$$

in which p(h) is given by (1), and k expresses the influence of the composition of the population on the number of unlike viral pairs present in the cell, neglecting effects of genetic recombination.

The coefficient k has a maximum of 0.25 when half the viral yield contains the *h* allele. Dividing the proportions of recombinants by 4k serves therefore as a correction for inequality of yields of the parental viruses. This correction is ambiguous only for bursts in which the yields of the two recombinants are large and unequal, and bursts from which either recombinant is absent.

A summary of the single burst data is given in table 4, which includes the mixed bursts only. The bursts have been separated into the classes $k \geqq 0.21$ and $k \leqq 0.20$, to show the effect of the correction described above. It will be seen that the uncorrected mean proportion of recombinants is larger for the

bursts with the larger k, and that the effect of dividing each proportion by 4k is to make the results homogenous. These facts justify the use of the correction. Its theoretical significance is clarified in the discussion.

TABLE 4

Single burst data for h×r crosses

k = a measure of disproportion between yields of parental types (Eq. 3).
$\bar{x}$ = average yield of the r^+ recombinant as percent of total virus.
$\bar{y}$ = average yield of the r recombinant as percent of total virus.
$\bar{n}$ = average burst size.
r(x, y) = coefficient of correlation between proportionate yields of the two recombinants.
r(n, x+y) = coefficient of correlation between burst size and sum of proportions of the two recombinants.
The variations of x, y and n/100 shown are standard deviations within the sample. The standard errors of the means are obtained by dividing these by the square root of the number of bursts.
The correction referred to is described in the text.

CROSS	NO. OF BURSTS	k	$4\bar{k}$		$\bar{x}$	$\bar{y}$	$\bar{n}/100$	r(x, y)	r(n, x+y)
h×r1	25	≦0.20	0.65	uncorr.	8.9±3.8	12.1±6.2	5.5±2.4		−0.25
				corr.	13.9±5.3	19.0±9.2		0.09	
	52	≧0.21	0.94	uncorr.	15.5±6.9	17.1±7.1	5.8±3.2		0.15
				corr.	16.6±6.3	18.1±7.2		−0.01	
h r1×++	19	≦0.20	0.67	uncorr.	13.6±5.9	13.2±4.4	5.1±1.9		−0.26
				corr.	20.1±7.4	20.8±8.5		−0.27	
	36	≧0.21	0.94	uncorr.	16.5±5.8	17.1±5.6	5.4±3.0		−0.11
				corr.	17.5±6.2	18.2±6.0		0.16	
h×r7	13	≦0.20	0.64	uncorr.	5.7±3.5	6.6±4.6	6.2±1.9		−0.43
				corr.	8.8±4.8	9.9±5.9		0.65	
	35	≧0.21	0.93	uncorr.	6.8±4.2	9.2±4.5	5.9±3.2		0.16
				corr.	7.2±4.3	9.8±4.8		0.53	
h r7×++	17	≦0.20	0.63	uncorr.	6.4±3.1	4.8±2.9	6.4±7.8		−0.45
				corr.	10.0±4.3	8.0±5.0		0.12	
	26	≧0.21	0.95	uncorr.	6.7±3.2	8.9±5.4	5.8±3.7		0.02
				corr.	7.1±3.6	9.3±5.6		0.47	
h×r13	29	≦0.20	0.61	uncorr.	0.5±0.5	0.8±0.8	5.2±2.1	0.07	0.35
	29	≧0.21	0.94	uncorr.	0.9±0.9	1.1±0.9	5.1±2.3	−0.05	−0.30
h r13×++	21	≦0.20	0.69	uncorr.	0.6±0.7	0.6±0.9	4.6±1.4	0.15	0.22
	46	≧0.21	0.94	uncorr.	0.8±0.7	0.7±0.6	4.9±2.3	0.21	0.04

The data of table 4 for mixed single bursts confirm fairly well the average data of table 2, except that the yields of recombinants are somewhat greater owing to the exclusion of the unmixed bursts, and that the yields corrected for unequal growth of parental viruses are higher still.

The chief point of interest is the question of the correlation between yields of the two recombinants in single bursts. This has been measured in terms of the correlation coefficient r (RIDER 1939). This measure varies between −1 and +1, a value near 0 indicating independence of variates, and values near unity indicating negative or positive correlation, respectively. The data of table 4 show clearly that there is no significant correlation between the proportions of the two recombinants in single bursts except for the crosses *h×r7* and *h r7*× wild type. Even for these crosses the correlation is weak and not entirely convincing, especially since the data are not completely unselected (see below).

The correlation between the uncorrected proportions of the two recombinants is shown in the form of scatter diagrams in fig. 7. These data might be expected to show some degree of spurious correlation owing to the fact that bursts with disproportionate yields of the two parental types tend to contain diminished numbers of both recombinants. This tendency can be seen in the

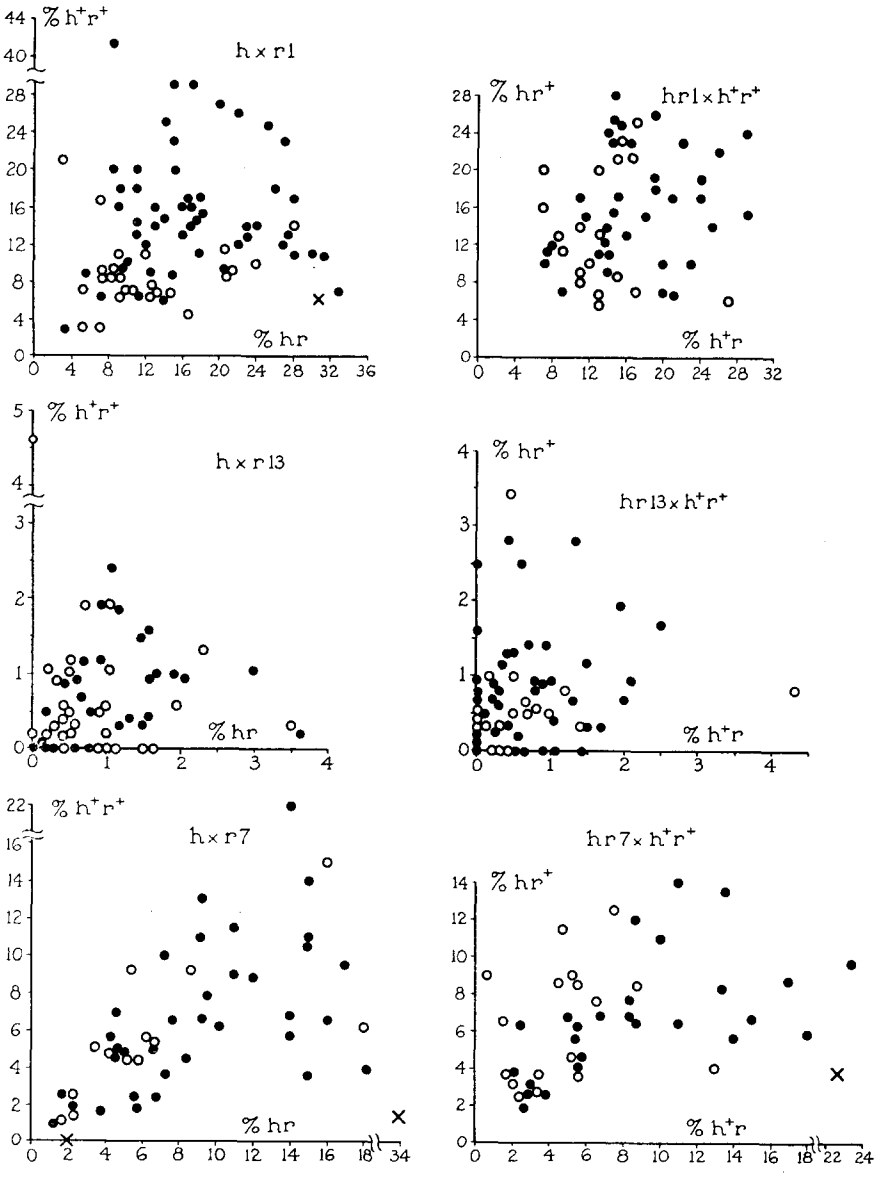

FIGURE 7.—Correlation between proportionate yields of the two recombinants in single bursts. The open circles indicate bursts with disproportionate yields of the two parental viruses. Crosses indicate yields omitted from the data of table 4.

diagrams in which the disproportionate yields ($k \leqq 0.20$) are indicated by open circles. Actually this effect is of minor importance, since the variations due to unknown causes are so much greater than those due to variations in k. Consequently, the uncorrected data lead to the same conclusions as the corrected data; namely, that the proportionate yields of the recombinants are uncorrelated in the crosses *h*×*r1*, *h*×*r13*, and the corresponding reverse crosses, but that there is a weak positive correlation for the crosses *h*×*r7* and *h r7*× wild type.

As mentioned earlier, the correlation data in table 4 are not unselected. The diagrams of fig. 7, however, show all the mixed bursts for the respective experiments, those omitted from the table being indicated by crosses in the diagram. It is plausible that some of the discrepant bursts came from bacteria infected with a spontaneous mutant present in one of the parental stocks of virus. In fact, two bursts from the cross *h*×*r7* were found to contain a large proportion of mutants, *m* in one case and weak inhibitor (HERSHEY 1946b) in the other, which almost certainly arose in this way. The *h* stock used in these crosses contained about 0.1 percent of *h r* virus, so that in crosses with *r* at least one bacterium in 200 was infected with three types of virus, and would be likely to yield an excess of one of the recombinant phenotypes. Unfortunately the recombinant progeny in the exceptional bursts were not checked by crossing with the parental stocks, which should be done in any further experiments of this type. For the present these bursts throw some doubt on the significance of the positive correlation between proportions of recombinants in the crosses *h*×*r7* and *h r7*× wild type.

The results of the cross *h*×*r13* are of special interest because of the small yield of recombinants. The distribution of the bursts with respect to absence of recombinants is shown in table 5. Nine of the 125 mixed bursts fail to show either recombinant, and 31 more lack one recombinant or the other. One can test the hypothesis that the two sister recombinants arise independently as follows. About 20 percent of the bursts fail to show a specified one of the two possible recombinants. If the absence of the one were independent of the absence of the other, about $(0.2)^2$ or four percent of the bursts should show neither recombinant. The number found, 9/125, is larger than this, but not significantly so. Moreover, bursts lacking one recombinant do not show less than the average proportion of the other (table 5). The data evidently fail to exclude the hypothesis of independent origin of the two recombinants, but do not, of course, rule out the hypothesis of reciprocal exchange.

Another question that arises in connection with the data is concerned with the number of genetic exchanges per bacterium. For explicitness, we consider separately the hypotheses of reciprocal and non-reciprocal exchange. If exchanges are reciprocal, the bursts lacking a single recombinant are the result of failure to recognize the few plaques of either type, of losses in the ten percent of each culture not examined, and of unspecified biological accidents. As previously computed, four percent or 5 of the 125 bursts fail to show either recombinant for one or another of these reasons, leaving only 4 without recombinants possibly owing to failure of exchange. This number is too small

if some of the single absences result from erroneous recognition of one recombinant, but is otherwise subject only to its sampling errors. Taken as a measure of failure of reciprocal exchange, the fraction 4/125 implies an average of 3.4 exchanges per bacterium. If, on the other hand, exchanges are not reciprocal, the fraction (20 percent) of the bursts lacking any given recombinant corresponds to 1.7 genetic transfers per bacterium.

These estimates may be too low if conditions vary from bacterium to bacterium in such a way that genetic exchange is suppressed in some bacteria.

TABLE 5

Distribution of Bursts with Respect to Absence of Recombinants

See legend table 4. The proportions of recombinants have not been corrected for disproportionate yields of parental types of virus.

CROSS	CLASS	NO. OF BURSTS	$\bar{x}$	$\bar{y}$	$\bar{n}$	$\bar{k}$
h×*r13*	both absent	2	0.0	0.0	390	0.20
	h^+r^+ absent	10	0.0	0.81	460	0.19
	h r absent	2	2.4	0.0	390	0.19
	total	58	0.72	0.97	520	0.20
h r13×++	both absent	7	0.0	0.0	340	0.21
	$h\,r^+$ absent	8	0.0	0.66	360	0.21
	h^+r absent	11	0.75	0.0	380	0.22
	total	67	0.73	0.64	480	0.22

Yields with small k and small burst size must have this effect, but are evidently not very important in the data of table 5, since the different classes are very similar with respect to k and burst size. In short, it is necessary to conclude that there are at least two or three genetic exchanges per bacterium, independently of the mechanism by which recombinants arise.

A different kind of estimate of the number of exchanges per bacterium is obtained from the number of recombinants actually found. The data are summarized in table 6 in the form of distributions of numbers of the several recombinant types. One finds on the average 3.4 recombinants of any one kind per bacterium. This evidently furnishes an upper limit to the number of exchanges per bacterium, insofar as exchanges yield viable and countable progeny. This result, taken in conjunction with the preceding estimates, leads to several remarkable conclusions.

First, since the two methods of estimation, one minimal and one maximal, yield about the same result, there must be in fact only two or three exchanges per bacterium in the crosses between closely linked factors.

Second, the recombinants must undergo little multiplication after they arise in the cell.

Third, the conditions of viral growth in different bacterial cells must be equally favorable to genetic recombination; otherwise a larger proportion of bursts would fail to show recombinants.

These conclusions are substantially confirmed by the distributions of numbers of recombinants shown in table 6, which are essentially of the Poisson type, with variance only moderately greater than the mean. In other words, the individual particles of any one recombinant must often arise independently of each other in the same bacterial cell, and with equal probability in different bacterial cells. The deviations from the Poisson distribution are nevertheless significant, and can be attributed to a moderate amount of growth of recombinants.

TABLE 6

Distributions of Numbers of Recombinants in Single Bursts from the Crosses h×r13 and h r13×wild type

The Poisson distributions show the numbers of tubes in the various classes expected if there were no growth of recombinants. The distribution with mean 3.4 is appropriate to the hypothesis of reciprocal exchange, and the distribution with mean 1.7 to the hypothesis of non-reciprocal genetic interaction.

NO. OF RECOMBINANTS PER TUBE	NO. OF TUBES FOUND				POISSON DISTRIBUTIONS	
	h^+r^+	$h\,r$	$h\,r^+$	h^+r		
0	12	4	15	18	2.1	11.5
1	11	11	11	14	7.2	19.6
2	9	8	8	6	12.2	16.7
3	6	9	11	9	13.8	9.5
4	3	7	6	4	11.7	4.0
5	7	3	4	2	8.0	1.4
6	1	2	3	6	4.5	0.4
7	3	6	0	2	2.2	0.1
8	2	2	3	3	0.9	0.0
9	1	2	2	1	0.3	0.0
10–18	3	2	3	2	0.1	0.0
19–27	0	1	1	0	0.0	0.0
28–36	0	1	0	0	0.0	0.0
No. of tubes	58	58	67	67	63	63
Mean per tube	3.1	4.5	3.3	2.8	3.4	1.7
Variance	9.2	27	16	10	3.4	1.7

The conclusion that genetic recombination is not suppressed in some of the bacteria is also supported by the results of the other crosses, in which no bursts yielding both parental viruses and lacking both recombinants were found. Only one burst, from the cross *h*×*r7*, failed to show one of the recombinants; it contained 95 percent of the *h* parent.

INDEPENDENCE BETWEEN PROPORTION OF RECOMBINANTS AND BURST SIZE

The data of table 4 do not show any significant correlation between burst size and proportion of recombinants, which means that the number of recombinants must be very nearly proportional to the total yield of virus in single bursts.

This conclusion must be qualified in view of the following considerations.

Some of the tubes contain one or more mixed bursts plus one or more unmixed bursts. Such tubes would tend to show less than average values of k and less than average yields of recombinants together with greater than average burst size. The number of such tubes is about five percent of the total in our experiments and these tend to be concentrated in the class $k \leqq 0.2$. This probably explains the negative correlation between proportions of recombinants and burst size among tubes selected for small k.

A larger proportion of the tubes, between 10 and 20 percent in our experiments, contain two or more mixed bursts. These tubes would tend to have greater than average k and greater than average burst size, but will not show exceptional proportions of recombinants. These tubes have the effect of weakening any correlation that may exist between burst size and proportion of recombinants, especially among the class $k \geqq 0.21$. The data previously considered do not, therefore, exclude the possibility of a weak positive correlation between proportion of recombinants and size of bursts.

Advantage was taken of the finding that high pH reduces size of bursts, to examine the relation between burst size and yield of recombinants in another

TABLE 7

Small Bursts from the Cross h×r7 in Broth of pH 9.0

See legend table 4. The proportions of recombinants have not been corrected for disproportionate yields of parental types of virus. The individual cultures contain an average of about 1.4 mixed bursts.

NO. OF BURSTS	k	$4\bar{k}$	$\bar{x}$	$\bar{y}$	$\bar{n}$	r(x, y)	r(n, x+y)
20	$\leqq 0.20$	0.61	6.3±3.8	10.7±6.2	99±50	0.53	−0.34
34	$\geqq 0.21$	0.93	7.4±3.5	10.5±4.6	123±73	0.61	0.35
54	all	0.81	7.0±3.7	10.6±5.2	114±66	0.56	0.12

way. Since increased pH was found also to cause some of the bacteria to fail to liberate virus, the single burst technique was chosen.

Bacteria were infected in the usual way with mutants *h* and *r7* in broth of pH 6.8. At the end of the five minute adsorption period, and without treatment with antiserum, single burst cultures were prepared after diluting in broth of pH 9.0. Preliminary experiments showed that the yield of virus was complete under these conditions within one hour, and than no inactivation occurred during two additional hours at 37°C, or overnight in the refrigerator. Entire samples were plated on single mixed indicator plates. One successful experiment yielded 66 bursts, of which 54 were mixed, among 104 tubes receiving on the average 1.4 bacteria per tube. Evidently most of the bacteria liberated some virus. The average burst size, after subtracting the virus carried over from the input (totalling 36 particles per tube), was only 114 per tube, or about 70 per bacterium corrected for the probable multiples. The average proportion of recombinants was nevertheless of normal size (table 7).

It will be noticed also that the correlation between the numbers of the two recombinants in these bursts is exceptionally good. The correlation is, however,

slightly exaggerated owing to the fact that the 54 tubes contain on the average mixed yields of virus from about 1.4 bacteria per tube.

THE EFFECT OF A SHORT PERIOD OF ADSORPTION AND LOW MULTIPLICITY OF INFECTION ON THE DISTRIBUTION OF RECOMBINANTS

The following experiment shows that when the multiplicity of infection in the cross $h \times r7$ is reduced from five to about one of each viral type per bacterium, the distribution of recombinants among single mixed bursts is little if any altered.

Six crosses were made in the usual way, except that the period allowed for adsorption was reduced to one minute, without reducing the total input of virus. The amount of virus adsorbed was too small to be measured, but the multiplicity of infection can be estimated from the data given below.

The single burst cultures collected from the six crosses are sufficiently similar to be considered together. The mean number of bacteria per tube for the six sets, determined by colony counts from the growth tubes immediately before adding virus, is 0.23. The mean number of infected bacteria per tube determined by plaque counts of samples taken before lysis is 0.20. The mean number of bursts per tube calculated from the proportion, 141 out of 720, of tubes containing virus is 0.22. The 141 tubes therefore contained about 157 infected bacteria.

From the distribution of viral types among the tubes, namely, 69 containing *h* only, 22 containing *r* only, 50 containing both, and 579 containing neither, one finds the probable distribution with respect to bacteria to be 80 infected with *h* only, 28 with *r* only, 49 both, and 46 neither. The multiplicity of infection is therefore about 1.0 with respect to *h*, and 0.48 with respect to *r*. One can estimate further that about seven of the tubes contained one or more mixed bursts plus one or more unmixed; and that about three contained both *h* and *r* bursts without any mixed bursts. Also, among the mixedly infected bacteria, 45 percent were infected with one particle only of each viral type.

In making the above computations we have neglected the probability that the *h* mutant suppresses the growth of *r* in some bacteria adsorbing both types of virus. The apparent inequality of infection is probably due in some part to this effect. However, in other experiments with low multiplicity of infection with *h* and *r7* designed to check this point, the split into *h*, *r*, and mixed yielders was nearly equal. It seems likely, therefore, that in the experiments reported here the two viral types were unequally adsorbed for unknown reasons.

The tubes containing only one viral type may be dismissed by saying that their average content of virus did not differ significantly from that of the mixed yields, and that the *h* and *r* yields per bacterium were the same. There was one exceptional burst containing only $h\,r^+$ and $h\,r$ phenotypes. The characteristics of the remaining 49 cultures containing *h* and *r* virus are summarized in table 8. The data show no unusual features excepting the small burst size, which is a direct effect of the low multiplicity of infection, and the somewhat

small proportion of recombinants, probably due to the appreciable number of superimposed unmixed bursts.

TABLE 8

Single Bursts from the Cross h×r7 with Low Multiplicity of Infection

See legend table 4. The proportion of recombinants have not been corrected for disproportionate yields of parental types of virus. Adsorption time one minute. Multiplicity 1.0 *h* and 0.5 *r* per bacterium.

NO. OF BURSTS	k	$4\bar{k}$	$\bar{x}$	$\bar{y}$	$\bar{n}/100$	r(x, y)	r(n, x+y)
17	≦0.20	0.59	5.3±4.0	4.5±3.7	2.9±1.6	0.38	−0.17
32	≧0.21	0.95	4.4±3.1	6.1±5.4	3.5±2.0	0.20	−0.33
49	all	0.84	4.7±3.5	5.7±4.9	3.2±1.9	0.24	−0.30

The principal point to be made here is that the variation in yields of recombinants from tube to tube is not exceptional. In this connection it must be mentioned that 5 of the 49 tubes contained *h* and *r* virus without any recombinants. Of these, one was exceptional in containing only 24 viral particles, and another for the extreme disproportion of parental types (88 percent *h*). The remaining three, each containing from 450 to 570 particles, are probably superimposed unmixed *h* and *r* bursts.

It should be noted also that the correlation between the yields of the two recombinants in this set is not significant, since the observed correlation is exaggerated by the tubes containing unmixed *h* and *r* bursts without recombinants. Whether the poor correlation is accidental, or an effect of the low multiplicity of infection, remains to be determined. The negative correlation between burst size and proportion of recombinants can, however, be ascribed to the superimposed mixed and unmixed bursts, as well as to the unmixed *h* and *r* bursts, in some of the tubes.

IDENTITY OF RECOMBINANTS WITH THE CORRESPONDING ANCESTRAL TYPES

According to any simple hypothesis of factorial recombination, one expects the recombinant virus arising in crosses not to differ genetically from the corresponding ancestral type. Two kinds of test indicate that this is so. In the first kind of test (HERSHEY and ROTMAN 1948) stocks of the phenotypic wild type arising from the cross between two different *r* mutants were backcrossed to authentic wild type. No *r* mutants appeared in such crosses, and it was concluded that the stocks were genetically identical.

The second kind of test is the following. The double mutant *h r7*, itself obtained by crossing the two single mutants, was crossed with wild type and the recombinants *h* and *r* were re-isolated. These were then tested by making the homologous (parental *h* by recombinant *h* and parental *r* by recombinant *r*) and heterologous (parental *h* by recombinant *r* and parental *r* by recombinant *h*) back crosses. In both cases, the homologous cross yielded only one type of

virus, and the heterologous cross yielded recombinants in the same proportion as found with the parental stocks. These tests show not only that the recombinants contain the same genetic markers as the corresponding progenitive types, but also that the region between the markers is unchanged.

MIXED YIELDS CONTAINING ONLY ONE PARENTAL TYPE OF VIRUS

Only four mixed bursts lacking one of the parental types of virus were found among the experiments reported in this paper. One, from the cross $h \times r1$, contained 86 percent h^+r and 14 percent $h\ r$. A second, from the cross $h \times r7$ contained 99.6 percent $h\ r^+$ and 0.4 per cent $h\ r$. A third, from the same cross with low multiplicity of infection, contained 83 percent $h\ r^+$, and 17 percent $h\ r$. The fourth, from the cross $h\ r7 \times$ wild type, contained 23 percent $h\ r^+$ and 77 percent $h\ r$. In this case the yield of $h\ r^+$, which appeared to be homogenous, formed atypical plaques and proved on isolation to differ from any known mutant of *T2H*. It seems reasonable to suppose that these exceptional bursts contained progeny stemming from mutants contaminating the parental stocks of virus. On the other hand, the one exceptional burst following low multiplicity of infection, together with the failure to find similar bursts among the crosses involving *h* and *r13*, suggest that a different interpretation should be looked for. Genetic tests which might have clarified this point are lacking. For the present we conclude, as a first approximation, that recombinants arise only in those bacteria in which both parental types of virus succeed in multiplying.

It may be added here, because the question arises in connection with these exceptional bursts, that no correlation can be seen between the proportion in mixed bursts of the total virus containing the *h* allele, and the proportion of the recombinant virus containing the *h* allele. We have therefore omitted this datum from the tables.

DISCUSSION

In collecting and analyzing the data just described, we have had in mind the following questions. Does genetic exchange occur in the course of matings between viral particles, or is it the expression of a mechanism of growth such as that visualized by LURIA (1947), according to which the multiplying units in the cell are not phage particles, but simpler structures derived from them? Can the linkage relations represented in fig. 1 be interpreted in terms of linear chromosome-like structures? Are the genetic exchanges reciprocal, as one expects for simple cases of crossing over, or must one look for an alternative mechanism more intimately connected with the mode of reproduction of the virus?

It was soon apparent that the data for crosses between linked and unlinked factors tended to give different answers to these questions, and we were led to consider a model based on two distinct mechanisms of exchange. The necessity for this arises from the following facts.

First, the linkage data indicate a limitation at about seven percent to the proportion of wild type found in crosses between linked factors, which is dif-

ficult to reconcile, in terms of a single mechanism, with the existence of a second class of crosses yielding about 15 percent of wild type.

Second, the correlation between proportions of the two recombinants in the cross $h \times r7$, and the lack of a corresponding correlation in the cross $h \times r1$, is incompatible with a single mechanism for the two crosses. It must be recalled, however, that the correlations found are too weak to be wholly convincing.

Third, LURIA's (1947) evidence for a mechanism of independent multiplication and transfer of subunits of the virus, and ours for a system of linkage, require dissimilar types of interpretation.

The model to which these considerations seem to lead is described below, but we do not consider that we have decisive answers to any of the questions originally posed. The remainder of this discussion is of value only insofar as it clarifies the questions, and systematizes the experimental results so far obtained.

The two linkage structures bearing the markers $r1$ and h, respectively, are assumed to be examples of the class of independently multiplying subunits of the virus whose minimal number LURIA and DULBECCO (1949) estimate at about 25. The reconstitution of virus from these units must be regulated in such a way that each particle receives one representative of each kind of unit. In the cross $h \times r1$ the choice between h and h^+, and between r and r^+, is decided nearly at random to yield on the average 37 percent of recombinants and 63 percent of the parental types in bacteria yielding equal numbers of the two parents. The deficit of recombinants below 50 per cent is unexplained, but may be thought of as an effect of incomplete mixing between neighboring clones of multiplying virus in the cell.

According to this hypothesis one expects from the cross $h \times r1$ proportionate yields of the two recombinants in a single burst to be:

$$\mathrm{p}(h^+r^+) = \mathrm{m}\left(\frac{\mathrm{n}(h^+)}{\mathrm{n}(h^+) + \mathrm{n}(h)}\right)\left(\frac{\mathrm{n}(r^+)}{\mathrm{n}(r^+) + \mathrm{n}(r)}\right) \tag{4}$$

$$\mathrm{p}(hr) = \mathrm{m}\left(\frac{\mathrm{n}(h)}{\mathrm{n}(h^+) + \mathrm{n}(h)}\right)\left(\frac{\mathrm{n}(r)}{\mathrm{n}(r^+) + \mathrm{n}(r)}\right) \tag{5}$$

where the expressions on the left refer to proportions of recombinant virus, the corresponding expressions on the right refer to the intra cellular yields of the respective unit linkage structures, and the coefficient m expresses the fraction of the intracellular virus which may be regarded as a random mixture of the two parental types, the remainder being considered unmixed. If the structures carrying the markers h, h^+, r, and r^+ grow independently in the cell, their yields will fluctuate independently, and no correlation will be expected between the numbers of the two recombinants in sufficiently small yields of virus. This expectation is borne out by the data for viral yields from single bacteria. The average yields of the two recombinants are equal, however, showing that the several unit structures grow at equal rates. According to equations (4) and (5), the proportionate yield of recombinants should not be

influenced by burst size unless the latter has an effect on the fraction m. According to the data it does not, and m is a parameter having the average value 37/50. This interpretation requires that 26 percent of either kind of parental virus in the cell should multiply in effective isolation from the other parent.

It will be noticed that the expressions (4) and (5) reduce to mk if one makes the approximations mentioned in connection with equation (3). This provides a theoretical basis for the correction we have applied to yields of recombinants in crosses between unlinked factors. An analogous justification for its use in crosses between linked factors will appear in the discussion to follow.

The predictions for the cross $h \times r7$ are different from the preceding case, because here the markers are situated on homologous linkage structures, so that recombination requires something like crossing over, which in turn requires something like synapsis. The expected fractional yields of recombinants are

$$\mathrm{p}(h^+r^+) = \mathrm{p}(hr) = \tfrac{1}{2}\ \mathrm{msc}, \qquad (6)$$

in which m has been defined previously, s is a fraction independent of m expressing frequency of pairing, and c is a crossover frequency. In order to make c independent of m and s it is evidently sufficient to define the product ms as the fraction of the viral yield made up of particles in which the marked unit has descended from an unlike synapsed pair. The application of (6) leads to ambiguity if exchanges can occur between descendants of unlike synapsed pairs (HERSHEY and ROTMAN 1948). In what follows this difficulty does not appear to be very serious, but no rigorous analysis has yet been attempted.

For the cross $h \times r7$, if exchanges are reciprocal, one expects the correlation between proportions of the two recombinants to be disturbed only by fluctuations in relative growth of the two exchange products, in contrast to the cross $h \times r1$, where the correlation is subject to fluctuations in the growth of four independent units. That these fluctuations are individually considerable is shown by the variations in relative and total viral yields in mixedly infected bacteria. A weak but probably significant correlation between proportions of the two recombinants is nevertheless visible in the cross $h \times r7$. No such correlation can be seen in the cross $h \times r1$. If the mechanism of exchange for these two crosses were the same, the greater correlation would be expected in the cross $h \times r1$, which gives the larger yield of recombinants.

The predictions for the cross $h \times r13$ are the same as for $h \times r7$ except as modified by the much smaller yield of recombinants, presumably owing to a smaller frequency of crossing over. We have shown that in this cross the recombinants come from two or three individual exchanges per bacterium, and that there is little growth of recombinants subsequent to exchange. These circumstances ought to be favorable for testing the hypothesis of reciprocal exchange. The data are nevertheless inconclusive of this point.

The experiments provide information about the sequence of events in the cell. A mechanism of exchange limited to an initial phase of multiplication is ruled out by the following consideration. If exchange occurred at a time when there were few replicas in the cell, any cross yielding a small average number of

recombinants would show some individual bursts containing no recombinants and others containing a very large proportion, especially at low multiplicity of infection. Instead one finds a comparatively uniform yield of recombinants, and the distribution of their proportions is not affected by the multiplicity of infection.

Also if exchanges occurred freely throughout the period of multiplication of the virus, one would expect considerably greater variations in yields of recombinants than we have found. For instance, in the cross *h*×*r13*, in which there are only two or three exchanges per bacterium, the variations in yields of recombinants are not much greater than those expected to result from a random variation in the number of exchanges alone. Moreover, most of the bacteria yield only a few recombinants, so that little growth can have occurred subsequent to exchange. The conclusion is unavoidable that the exchanges are limited to the terminal phase of multiplication, or at any rate that recombinants are prevented from multiplying appreciably in most of the bacteria.

It is remarkable that the variations in proportions of recombinants are so little dependent on the degree of linkage (as between *h*×*r7* and *h*×*r13*), or on the postulated mechanism of exchange (as between the above and *h*×*r1*). The coefficients of variation in proportions of individual recombinants among single bursts are, for *h*×*r1*, about 40 percent; for *h*×*r7*, about 60 percent; and for *h*×*r13*, about 100 percent. This circumstance also supports the inference that the exchanges are limited to a late phase of multiplication.

The hypothesis stated permits one to examine further the structure of the linkage units. Since crosses between *r13* and any of the mutants belonging to the group closely linked to *r7* yield about the same proportion, seven percent, of wild type (HERSHEY and ROTMAN 1948), it might be supposed that one crossover between the distant markers is always accompanied by several others, so that 50 percent of the progeny of synapsed pairs of the units *r7* and *r13*, for example, would be recombinant types. If this supposition is correct the terms of equation (6) can be evaluated by setting $c = 0.5$ and $p(h^+r^+) = 0.07$. This gives 0.28 for the average fraction ms of virus descending from unlike synapsed pairs. If this fraction is assumed to be the same in other crosses involving the same linkage structure (the cross *h r7*×*r13* reported in this paper suggests that it is), one can write for them

$$p \text{ (wild type)} = 0.14\ c, \tag{7}$$

where c is the appropriate crossover frequency and the proportion of wild type is experimentally measured. The data for the three point crosses involving *r2*, *r3*, and *r6* (HERSHEY and ROTMAN 1948) are examined from this point of view in table 9. The proportions of wild type have been calculated for random crossing over between unit linear structures, using the crossover frequencies given by (7). It will be seen that the data are entirely compatible with the hypothesis tested. Additional tests of this kind are needed, however.

It will have been noticed that the average yield of recombinants in crosses between distant linked factors is very nearly half that found for unlinked factors. DR. M. DELBRÜCK has pointed out to us that this relationship can be

understood in terms of random pairing between homologous structures. In the simplest case one visualizes unrepeated pairing, that is, pairing limited to a phase in which there is no multiplication, and during which no structure finds more than one partner. For this case, the frequency of synapsis in equation (6) is simply the ratio of the number of unlike homologous pairs to the total number of homologous pairs. This ratio can be written

$$s = \frac{2ab}{(a + b)(a + b - 1)} \tag{8}$$

where a and b are the respective numbers of the two unlike homologous structures. Inspection of (8) shows that this ratio is essentially 2k as given by equation (3) when a+b is large compared to unity. Instead of equation (6) we have, therefore,

$$p \text{ (wild type)} = mkc, \tag{9}$$

from which the proportion of either recombinant expected in crosses between distant linked factors can be computed as follows.

The parameter m, taken to be the average fraction of virus randomly mixed in the cells at the time of reconstitution of virus from subunits, was found to

TABLE 9

Three Point Linkage Tests of Linear Structure

The symbol *r2,3* refers to the double mutant containing *r* alleles at the loci *r2* and *r3*, etc.
c_1 is the crossover frequency for the region between *r2* and *r3*.
c_2 is the crossover frequency for the region between *r3* and *r6*.
The locus *r3* is assumed to lie between *r2* and *r6*.
The factor 0.14 is explained in the discussion.

CROSS	FRACTIONAL YIELD OF WILD TYPE		COMPUTATION
	EXPECTED	FOUND	
r2×r3	—	0.020	$c_1=0.020/0.14=0.14$
r3×r6	—	0.014	$c_2=0.014/0.14=0.10$
r2×r6	0.030	0.024	$0.14[c_1(1-c_2)+c_2(1-c_1)]$
r2,3×r6	0.012	0.008	$0.14\ c_2(1-c_1)$
r3,6×r2	0.018	0.014	$0.14\ c_1(1-c_2)$
r2,6×r3	0.002	0.003	$0.14\ c_1c_2$

be 37/50 in crosses between unlinked factors. In (9) we require the corresponding fraction at the time of pairing, and assume this to be the same. The average of k for bacteria giving mixed viral yields (table 4) is 0.21, or 0.19 if one includes the ten percent of bacteria yielding only one type of virus. If k and m vary independently, their mean product is the same as the product of means, or 0.14 averaged over all bacteria. The average yield of either recombinant from equation (9), for factors sufficiently far apart so that c=0.5, is accordingly seven percent, computed solely from the data for crosses between unlinked factors. This is the maximum actually found in crosses

between linked factors (HERSHEY and ROTMAN 1948). Equation (9) also predicts, in agreement with the data for single bursts, proportionality between yields of recombinants and k.

The agreement supports the inferences previously drawn that the markers *r7* and *r13* are attached to the same linkage unit, and that the frequency of crossing over between them is 0.5. It suggests that the pairing itself is complete without appreciable repetition, and occurs at random except that about 26 percent of the units of each kind are effectively segregated from their opposite numbers. The measure of this segregation, m, is on this view the same for crosses between linked and unlinked factors. On the other hand, this interpretation cannot be rigorously correct, because one can show by multiple factor crosses (HERSHEY and ROTMAN 1948) that repeated exchanges, or exchanges among three viral particles, occur. An estimate of the amount of repeated pairing has not yet been attempted, except that the considerations just offered suggest either that it is small, or that random pairing is limited to a small proportion of the population.

It follows from equation (9) that the interpretation in terms of orderly pairing accords with the fact, otherwise very puzzling, that the proportion of recombinants is not affected by size of burst even in crosses between linked factors.

It has been seen that the linkage data support fairly well the idea of linear structure, but independent evidence for crossing over is meagre. According to any simple model of reciprocal exchange, a correlation between proportions of sister recombinants in individual bursts would be expected. This expectation has been only partially realized, and the question arises whether the linkage data themselves require the crossover hypothesis. The following model, suggested by DR. A. H. STURTEVANT, shows that they do not, and also shows that the question of reciprocity is closely connected with the question whether the exchanges are material transfers.

Suppose that the replication of linear structures occurs zipperwise along the pattern from one end to the other, but that the partners separate prematurely to yield fragmentary replicas. Additions to the fragments are subsequently possible only after pairing with the same or another homologous structure, which in mixedly infected bacteria could belong either to the same or a different parental line. Genetic recombination in a two factor cross will depend, then, on the contingency that the two marked regions of a given replica be laid down one after the other on homologous structures from the two unlike parents. With simple assumptions, all the consequences of the crossover hypothesis (equation (6)) follow from this model, except that the independent origin of the two recombinants provides an additional source of independent variation in their numbers.

The complications peculiar to this model have to do principally with the evidence that exchanges occur only during the terminal phase of growth. These complications are not very serious if one assumes that during early stages of growth the probability is great that a fragment will be started and completed on patterns belonging to the same parental line; that is, that the

mixing of the cell contents is relatively incomplete, and the distance between unlike clones relatively great, for small total populations. It has to be stipulated further that the terminal mixing is independent of the final concentration of virus in the cell, to account for the lack of dependence of proportion of recombinants on burst size. Some hypothesis of this sort may prove useful if further experiments fail to strengthen the present evidence for reciprocal exchange.

It is notable that two very different lines of evidence, ours and that of LURIA (1947), have led to the idea of independently multiplying subunits of the virus. Our results differ from LURIA's only in calling for a system of linkage superimposed on the set of independent units. It remains to be seen whether a combination of genetic and radiological techniques bears out the present conclusions, and perhaps leads to an identification of the radiation-sensitive units with the linkage structures.

SUMMARY

Genetic recombination between two viruses differing by two mutational steps has been studied by infecting bacteria with the pair, and counting the numbers of the four types of virus found in yields from single bacteria. The crosses so examined include $h \times r1$ (unlinked), $h \times r7$ (linked), and $h \times r13$ (closely linked), where h refers to a mutant of altered host range, and *r1*, *r7*, and *r13* are different mutations producing the same alteration in type of plaque. The reverse crosses, $h\,r \times$ wild type, were also studied. The results may be summarized as follows.

Nearly all mixedly infected bacteria yield both parental types of virus and two recombinants, according to the scheme $h+r = h\,r+$ wild type. The ten percent or so of bacteria yielding only one of the parental types seldom or never yield any recombinants. The rest of the bacteria always yield two recombinants, except for the occasional absence of one or both in the crosses between closely linked factors.

The average yields of the two recombinants in any one cross are the same, and are independent of the direction of exchange, so that reverse crosses involving the same pair of mutant factors yield the same number of recombinants. The proportionate yields of recombinants from individual bacteria are independent of burst size, and of the total multiplicity of infection, but depend on the relative yields of the two parental types. The effect of the latter is not marked, however, and the variations from bacterium to bacterium must be chiefly the result of variations in the number of genetic exchanges and in the growth of recombinants subsequent to exchange. These variations may be described by saying that one finds a moderately skewed distribution, with mode less than the mean, and with mean and standard deviation dependent on the linkage relations as follows: for $h \times r1$, 15 ± 6, for $h \times r7$, 7 ± 4, for $h \times r13$, 1 ± 1, expressed in round numbers as percent of either recombinant in the total yield of virus.

A weak but moderately convincing correlation between the proportionate

yields of the two recombinants in individual bacteria is discernible in the cross *h*×*r7* and its reverse, but not in the other crosses.

In the cross *h*×*r13* only two or three genetic exchanges occur during the multiplication of the virus in a single bacterial cell. These exchanges take place near the end of the period of multiplication of the virus.

A hypothesis is outlined which is compatible with the genetic data and with the results of LURIA concerning reactivation of irradiated virus in bacteria receiving two or more individually noninfective particles. The hypothesis is an extension of that of LURIA, according to which one visualizes genetic interaction not between two viral particles, but between two sets of independently multiplying chromosome-like structures. Genetic exchange occurs either by reassortment of these structures, or by something like crossing over between homologous pairs, depending on the structural relation between the genetic factors concerned. The interpretation made brings the linkage relations into superficial agreement with the requirements of linear structure, but there is little evidence that the genetic exchanges are reciprocal, and accordingly little evidence that they are material exchanges.

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