THE INFLUENCE OF HOST RESISTANCE ON VIRUS INFECTIVITY AS EXEMPLIFIED WITH BACTERIOPHAGE

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Parker (1) has shown that the results of infectivity measurements with vaccinia virus may be interpreted as a Poisson distribution of single infective particles among aliquots of the virus obtained by dilution. Thus, if it may be assumed that there exists a quantity of virus invariably necessary and invariably sufficient to produce a lesion in the skin of the rabbit, the behavior on dilution requires this quantity to be a single indivisible particle.

However, if the possibility exists that some independently varying factor influences the appearance of lesions in the inoculated sites, the Poisson distribution is inapplicable, and a different conclusion is reached. In this case the results can only be interpreted as an indication of a particular kind of dose response among the animals tested. Bryan and Beard (2) have called attention to the fact that the single particle response curve has considerable resemblance to the hyperbolic curves characteristic of certain drugs (per cent of positive responses plotted against dosage). Their discussion gives the impression that the reverse is also necessarily true. Actually, two separate requirements must be met if the response to a drug is to resemble the response to single infective particles. First, the distribution of resistance among the animals must be of the extreme skew type, resulting in an approximately symmetrical distribution of logarithms of individual effective doses. In addition, the dispersion of these doses must have a particular value, *e.g.* the ratio between the dose affecting 64 per cent of the population and the dose affecting 16 per cent must be 10.6. Gaddum (3) has collected data for a number of drugs fulfilling the first of these two requirements. In his Table I, twenty-five examples are cited, together with the dispersion measures obtained. The latter are expressed as the standard deviation of the logarithms of individual effective doses, which, for the single particle response curve, is approximately $\frac{1}{2}$ log 10.6 or 0.51. The values tabulated for the twenty-five measurements fall between 0.04 and

0.91, among which only those for acetonitrile, dysentery toxin, and pneumococcus antibody (protective effect), all tested on mice, lie in the neighborhood of 0.51. Apparently it is quite possible for a drug to approximate the single particle response, but it can only do so by a coincidence, dependent on the properties of the drug and also largely influenced by the choice of animal, and the experimental procedure.

We have found a record of three substances tested on the human skin (4), which is of interest in this connection. Of these three, mercuric iodide in

TABLE I

Effect of Volume of Agar on Infectivity of Phage

Aliquot of phage	4 cc. agar			8 cc. agar		
	Actual count	Expected $n = 1$	Expected $n = 2$	Actual count	Expected $n = 1$	Expected $n = 2$
$\times 10^{-1}$ cc.						
8.0	±800	847	7600	463	414	3700
2.7	296	282	847	138	138	414
0.9	94	(94)	(94)	46	(46)	(46)
0.3	32	31	10	13	15	5
0.1	7	10	1	6	5	<1

Twenty times the aliquot of phage shown, in 1.0 cc. of broth, was mixed with 4.0 cc., 18–24-hr broth culture of a susceptible coliform species. After allowing the mixture to stand 5 minutes. at room temperature, 0.5 cc. amounts were mixed with 4.0 and 8.0 cc., respectively, of 0.7 per cent nutrient agar, and poured into Petri dishes containing 15 cc. of solidified 1.0 per cent agar. Plaques were counted after 18–24 hrs. at 37°C. The counts shown are the mean of three plates.

The expected counts for $n = 1$ are computed on the assumption that plaques result from single particles; *i.e.*, their number is proportional to the concentration of phage. The expected counts for $n = 2$ are computed on the assumption that plaques result from the coordinate action of two particles, so that their number is proportional to the square of the concentration of phage. In either case, the figures in parentheses are the basis for calculation of the remaining values.

The method of plating used here has not previously been described. It was adopted by the authors several years ago to obtain satisfactory counts with small plaque phages and will be discussed in detail in a forthcoming publication.

aqueous solution shows no resemblance to the behavior of the virus, the per cent response curve being sigmoid with respect to dosage. Of the remaining two, applied in vaseline, chrysarobin approximates fairly well, and mercuric chloride extremely well, to the single particle curve. The data for the latter are reproduced on a suitable scale in Fig. 1. It may be seen that there is very little difference between this series of tests with mercuric chloride, where it is inconceivable that the irritation is produced by a single particle, and the results of a typical titration of vaccinia virus.

The correspondence between the result with mercuric chloride and the probable distribution of single particles is not very remarkable, however,

since the data shown were deliberately selected to achieve this end. Moreover, nothing is known about the reproducibility of this particular set of results and, judging from comparisons made with other materials (3), one would not in general expect to obtain a similar coincidence with mercuric chloride if the tests were carried out under any other conditions.

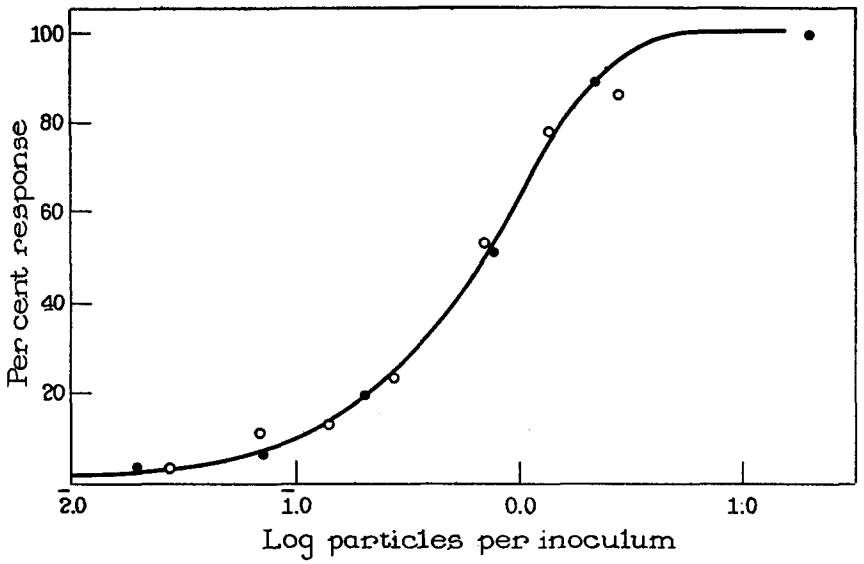

FIG. 1. Titration data obtained with vaccinia virus, mercuric chloride, and ideal infectious particles. The curve represents the response to single infectious particles predicted by Poisson's distribution:

$$\text{Average No. per inoculum} = -2.3 \log \frac{\text{negative inoculations}}{\text{total inoculations}}$$

The open circles are the data for the control animals in experiment 6 of Sprunt and McDearman (5) with vaccinia.

The closed circles are the data of Percival (4) for mercuric chloride in vaseline applied to the skin of thirty-five humans.

In either case the average number of particles per inoculum has been arbitrarily fixed at 0.69 for the 50 per cent response, reckoning the other doses proportional to concentration.

By contrast, recent observations with vaccinia reported by Sprunt and McDearman (5) have revealed a remarkable consistency in the behavior of this virus. They found that the resistance of rabbits could be increased by non-specific means so that the minimal reacting dose of virus was 30 to 50 per cent larger than for untreated rabbits. Nevertheless, the form of the titration curve obtained in treated and untreated rabbits was identical. Data from one of their experiments are included in Fig. 1. The deviation

from the one particle curve shown is systematic and might be attributed to a small degree of variation among the animals tested. On the other hand, if their data are to be interpreted as the coincidence of a geometric distribution of resistance having a standard deviation of approximately (log dose) 0.51, it seems remarkable that the operative procedures employed have affected the dispersion measure so little.[1]

In any case, it is apparent that the interpretation of the results of the titration of virus must rest for the present on the rather subjective criterion of inherent plausibility. On the basis of their own observations, Sprunt and McDearman (5) suggested, with some misgivings, that the effect of heightened resistance might be to decrease the fraction of virus particles capable of producing lesions, but that for either group of animals this fraction was constant and revealed itself in a one particle curve. Inasmuch as this conclusion seems at first sight contradictory, we are presenting some analogous results of our own with bacteriophage, of which the interpretation is clear.

It can be shown that the number of plaques produced on an agar plate, seeded with a given mixture of bacteriophage and bacteria, can be varied by changing certain conditions of bacterial growth, but that in all cases the individual plaques originate from the independent action of single particles of phage. In the experiment recorded in Table 1, two levels of "host resistance" were obtained by plating the mixtures of phage and bacteria in two different volumes of 0.7 per cent agar. Similar variations can be obtained by changing the concentration of agar (6), the nutrient composition of the medium, or the strain of bacterium employed. In the experiment shown here, the observed counts obtained with different aliquots of phage are in excellent agreement with the expected counts if each plaque results from one particle, whereas if even two particles were necessary, very different results would be obtained. The agreement is equally good for the two media, in spite of the twofold differences in the actual count. It is unlikely that any ambiguity is concealed by this agreement.

The finding with bacteriophage can be stated as follows: the probability that a given phage particle will produce a plaque varies with the conditions imposed, but under given conditions this probability is constant and inde-

[1] Actually, there may have been some effect not revealed by the chi^2 test employed by the authors. The average maximal per cent deviation from the theoretical curve for the six groups of untreated animals is 10.1 ± 1.6; for a similar number of treated animals it is 19.0 ± 3.4. All occur in the same region of the curve as illustrated in Fig. 1. The ratio of this difference to its standard error is 2.5, corresponding to 80:1 odds against obtaining this difference by accident.

pendent of any association between particles. It follows that no *a priori* objection can be made to the seemingly paradoxical interpretation suggested by Sprunt and McDearman of their results with vaccinia. The analogy with the bacteriophage is not, of course, to be construed as a confirmation of their findings.

Our own conclusion has been stated above in such a way as deliberately to avoid the fundamental question which arises concerning results of this kind. Do those particles which succeed in producing a plaque under given conditions do so because they possess some property distinguishing them from the remainder of the particles, or only because the success or failure of all the particles is determined by numerous local influences which fluctuate in a random manner? This question is irrelevant to the purely statistical interpretation of the data, but it suggests the possibility of attacking the more interesting problem of heterogeneity within the virus population itself.

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