THE INHERITANCE OF REQUIREMENTS FOR ADSORPTION COFACTORS IN THE BACTERIAL VIRUS T4[1]

THOMAS F. ANDERSON

Eldridge Reeves Johnson Research Foundation, University of Pennsylvania, Philadelphia 4, Pennsylvania

Received for publication January 26, 1948

Previous work has shown (Anderson, 1945, 1946, and 1948*a*) that many strains of the bacteriophage T4 are not adsorbed on their host, *Escherichia coli* strain B, unless the virus has been activated by a cofactor such as L-tryptophan. However, all preparations of T4 so far studied contain fractions that form plaques on bacterial smears growing on synthetic medium agar to which no cofactor has been added. The appearance of such plaques suggests that inhomogeneities with respect to cofactor requirements exist in the virus preparations.

The results reported here show that our stocks are, indeed, inhomogeneous with respect to cofactor requirements. The virus particles which form plaques efficiently in the absence of added cofactor can be removed from the stocks by adsorption on the host. Particles that are inefficient in producing plaques without added cofactor are not removed by such treatment. Analyses of individual virus clones arising from particles of different types show that cofactor characters (requirement and nonrequirement) are inherited by members of virus clones.

MATERIALS AND METHODS

The host for T4, *E. coli* strain B, has been described previously (Anderson, 1945), as has the synthetic ammonium lactate medium (F) and the nutrient medium (N) on which the host was grown in this work. All plates used for assay were incubated at 37 C.

Individual plaques were picked up with medicine droppers equipped with 2-mm tips and rubber bulbs. With the bulb slightly compressed, the tip was pushed through the agar containing the desired plaque and moved a bit to free the agar from the underlying glass of the petri dish. Then, when the bulb was released, atmospheric pressure forced the cylinder of agar containing the plaque into the dropper. The agar cylinder was then ejected into 1 ml of F medium in a small sterile tube. After 20 minutes the fluid was found to contain between 10^7 and 10^9 virus particles, belonging to a single clone of T4 particles. After incubation at 52 C for 20 minutes, to kill the infected bacteria but not the free virus, the samples were assayed on F agar and N agar, all dilutions being made in liquid F medium.

The original parent strain of T4 had been isolated by Demerec and Fano (1945) from a single plaque or clone appearing on B grown on N agar. These authors

[1] This work was supported by a grant from the Medical Research Division of Sharp and Dohme, Inc., and by Contract N6 ori 168 TO II between the Navy Department and the Trustees of the University of Pennsylvania.

very kindly gave a sample of their stock preparation to the author. It contained 2×10^9 plaque-forming particles per ml on B grown on N agar, and only 4×10^5 plaque-forming particles per ml on B grown on F agar. One stock preparation used, T4 (5/10/45), owed its origin to a single plaque isolated from F agar on which B infected with T4 (activated by the cofactors in N medium) had been plated. In subsequent serial passages on F medium on estimated 10^{29} phage particles would have been formed if all the original particles had multiplied equally. Fifteen hundred ml of F medium containing 10^{11} B in a shaker flask were then seeded with 7×10^9 of these phage particles. After aeration overnight, the culture had lysed. The virus was concentrated in the Sharpless supercentrifuge at 45,000 rpm and filtered. The 350 ml of filtered, concentrated virus, T4 (5/10/45), contained 3.9×10^{11} plaque-forming particles per ml on N agar and 2.6×10^8 plaque-forming particles per ml on F agar at 37 C. The characters of the virus particles in plaques formed by this stock under various conditions were determined in the initial experiments.

RESULTS

Analysis of individual plaques of T4. Four plaques were isolated from each of two F and two N plates used in the above assay of T4 (5/10/45). When analyzed, six of the clones obtained from F agar formed almost as many plaques on F agar as on N agar. Two of them gave much lower assays on F agar than they did on N agar. All of the plaques isolated from N agar gave much lower assays on F agar than on N agar (table 1A). It thus appeared that the stock T4 contained at least two types of phage. One required no added cofactor for efficient production of plaques on B on synthetic agar at 37 C. The other type, present at 1,000 times the concentration of the former, was quite inefficient in producing plaques in the absence of added cofactor. Furthermore, it may be noted that plaques like nos. 2 and 3 of table 1, requiring added cofactor for the efficient production of plaques, occasionally appear on synthetic medium at 37 C. Similar plaques were obtained in the assays of cofactor-requiring clones like 5 to 8 and 13 to 16 inclusive.

The question arose: Do these plaques arising on F agar from cofactor-requiring stocks contain predominantly cofactor-requiring particles or not? This was tested by analyzing 20 individual plaques isolated from F and N plates used in the analysis of plaque no. 15. The results given in table 1B show that whether they arise on F or on N agar, clones originating from cofactor-requiring stocks require cofactor.

Do clones arising on N agar from populations such as clone 10, which do not require cofactor, retain this characteristic? Analyses of 10 plaques from F plates and 10 plaques from N plates used in the analysis of clone 10 are given in table 1C. It is seen that the nonrequirement is retained by clones arising on N agar. The cofactor requirements are inherited by T4.

Is L-tryptophan an adequate cofactor for strains arising from T4 (5/10/45)? Fifty-six plaques isolated from T4 (5/10/45) plated on N agar were assayed on F agar, on F agar containing 20 mg L-trytophan per liter, and on N agar. The

ratios of assays on F agar to assays on N agar were all less than 0.005. The ratios of assays on F agar plus trytophan to assays on N agar were all greater than 0.9.

TABLE 1

Analyses of single plaques formed by particles in T4 (5/10/45) stock

PLAQUES FROM F PLATES		PLAQUES FROM N PLATES	
Clone no.	Ratio Assay on F agar / Assay on N agar	Clone no.	Ratio Assay on F agar / Assay on N agar
A. Single plaques from T4 (5/10/45) stock			
1	0.62	5	0.000019
2	0.0004	6	0.000011
3	0.0002	7	0.00006
4	0.83	8	0.000002
9	0.6	13	0.000004
10	1.01	14	0.000002
11	0.9	15	0.0037
12	0.9	16	0.0037
B. Analyses of plaques from clone 15			
15-1	0.00006	15-11	0.0001
15-2	0.00004	15-12	0.0004
15-3	0.0002	15-13	0.004
15-4	0.000006	15-14	0.002
15-5	0.000017	15-15	0.001
15-6	0.00007	15-16	0.003
15-7	0.00008	15-17	0.0002
15-8	0.007	15-18	0.001
15-9	0.0004	15-19	0.005
15-10	0.00012	15-20	0.0003
C. Analyses of plaques from clone 10			
10-1	0.77	10-11	0.93
10-2	0.58	10-12	0.74
10-3	0.64	10-13	0.41
10-4	0.53	10-14	0.68
10-5	1.3	10-15	0.93
10-6	1.25	10-16	0.71
10-7	0.35	10-17	0.9
10-8	1.1	10-18	2.5
10-9	1.6	10-19	0.95
10-10	0.9	10-20	1.14

It is concluded that the majority of the clones arising on N agar from T4 (5/10/45) contain particles which can utilize L-tryptophan as a cofactor.

Previous work has shown that once a T4 particle requiring activation by a cofactor is adsorbed on B, the resulting virus-host complex is efficient in forming plaques on F agar at 37 C (Anderson, 1945). In order to determine whether

such plaques contain virus particles that require cofactor or not, a series of such plaques were analyzed. The virus particles in them were found to have low efficiencies of plaque formation on F agar.

The majority of the virus particles in the T4 (5/10/45) formed plaques of the fuzzy r+ type (Hershey, 1946). In order to obtain clear, more easily counted plaques for other work, a single clear plaque of the r type was isolated from an N agar plate on which T4 (5/10/45) had been assayed. It was added to a shaker flask containing actively growing B ($\sim 10^8$ per ml) in 1,500 ml of F medium. The lysate assayed 3.6×10^{10} particles per ml and $\sim 10^5$ particles per ml on N and F agar, respectively. Concentrated by differential centrifugation at 4,000 rpm and 10,000 rpm, the partially purified virus was filtered. Designated T4r (3/10/47), the resulting concentrate assayed 3×10^{11} particles per ml and 4×10^5 particles per ml on N and F agar, respectively.

Individual plaques from this more homogenous stock were also analyzed. A series of 8 F plates were poured with various total numbers (between 2.5×10^8 and 5×10^9) of virus particles together with a parallel series of 5 N plates, containing 40 to 400 particles from T4 (3/10/47). After incubation at 37 C for 18 hours, contact prints were made of each of the petri dishes to record the sizes of the plaques. A series of 40 plaques of varying sizes and morphologies were then picked from the F plates, and 10 plaques from the N plates. The suspended plaques were assayed on F agar and on N agar. As may be seen from figure 1, a fair correlation between the assays on N agar and the diameters of the plaques on F agar was obtained. On the other hand, no correlation between the numbers of virus particles in the plaques and the cofactor requirement of the predominating virus was noted. Nor did we detect consistent correlations between other aspects of the morphologies of these plaques (such as fuzziness or halos) with the cofactor requirements of the virus contained in the respective clones.

Nine of the 40 plaques taken from F agar gave assays on F agar at 37 C that were comparable to their assays on N agar. The remaining 31 plaques gave very low counts on F as compared to N agar. As may be seen from figure 1, no correlation between plaque size and the character of the virus was obtained.

Since a total of 10^8 virus particles had to be plated to produce about 80 plaques on F agar, the efficiency with which the cofactor-requiring fraction forms plaques on F agar may be estimated to be about

$$\frac{31}{40} \times \frac{80}{10^8} = 6 \times 10^{-7}$$

As was to have been expected, none of the plaques isolated from N agar plates gave comparable assays on F and N agar. Further analyses of this material are given in a following paper (Anderson, 1948*b*), in which the effect of temperature and nutrient on plaque formation by these clones was studied.

Absorption of T4 stocks on B. Clearly the plaques isolated from F agar are of two types: some contain predominantly cofactor-requiring virus, others contain virus that appears not to require added cofactor. The question arose: Do all

the plaques that arise on F medium originate from cofactor-requiring types and owe their character to early mutations of the virus to nonrequirement followed, perhaps, by selection of these types? Or do the nonrequiring clones arise from

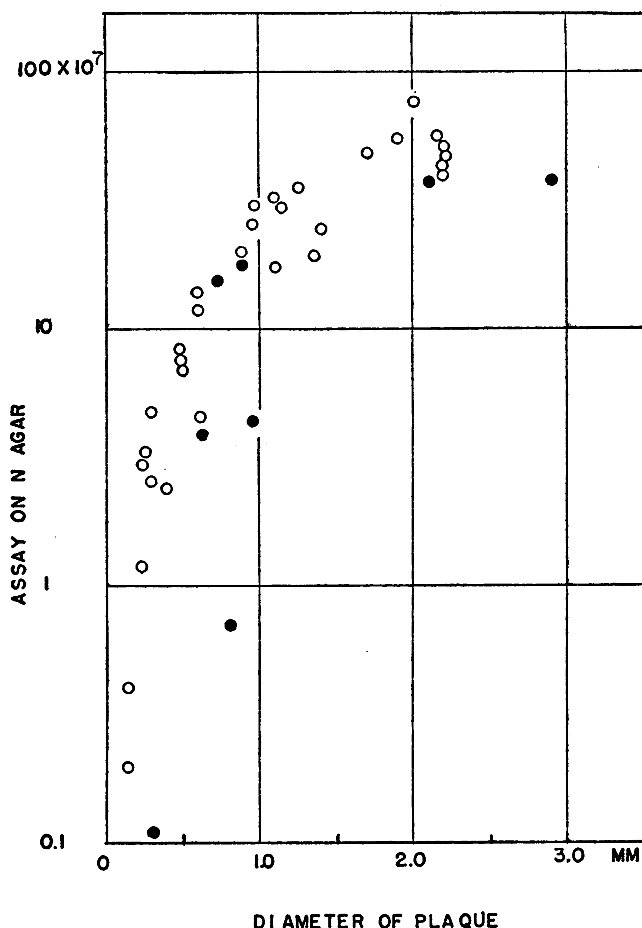

FIG. 1. CORRELATION BETWEEN THE NUMBERS OF VIRUS PARTICLES IN F PLAQUES (ORDINATES) WITH THE MEASURED DIAMETERS OF THE PLAQUES (ABSCISSAE)

Two types of plaques are distinguished in the graph: ○, plaques containing a predominance of particles that require cofactor; ●, plaques containing a predominance of particles that do not require added cofactor for the efficient production of plaques on F agar at 37 C. No correlation between cofactor requirement and plaque size was noted.

particles in the initial population that do not require added cofactor for efficient plaque production and inherit this character from them? If the latter situation exists it should be possible to remove the nonrequiring type from a population by adsorbing it on the host in the absence of added cofactor, and so reduce the assay of the stock virus on F agar. This was done as follows:

To 10 ml of a 5-hour, aerated, 37 C B culture in F medium (5×10^9 bacteria

per ml) was added 0.03 ml of T4 (5/10/45). After 10 minutes had been allowed for adsorption, the mixture was assayed on F and on N agar. The B + adsorbed T4 was then removed by centrifugation and filtration. This treatment reduced the assay of the filtrate on F agar by 95 per cent, whereas that on N agar was unchanged. An analogous experiment in which the T4 suspension was absorbed on B three times in succession resulted in the removal of 98.7 per cent of the T4 that formed plaques on unfortified F agar. We conclude that virus of the non-requiring type pre-existed in T4 (5/10/45).

In three analogous experiments, T4 (5/10/45) was mixed with 24-hour B grown to saturation in aerated F culture at 37 C. With such B cultures adsorption is slow (Delbrück, 1940), and negligible fractions of the particles forming plaques on F agar were removed. However, the addition of 0.8 μg L-tryptophan per ml to these absorption mixtures enhanced the adsorption of these particles. In the three experiments, centrifugation of adsorption mixtures containing tryptophan removed 98, 93, and 93 per cent, respectively, of the T4 that was able to form plaques on F agar. The assays on N agar were again unchanged by absorption in the presence of these minimal amounts of tryptophan.

The results of these experiments indicate that the fraction of T4 that forms plaques on F agar at 37 C can be removed from a population of T4 virus particles. It has been further demonstrated that this fraction has an enhanced rate of adsorption on the host in the presence of L-tryptophan.

DISCUSSION

We have found that the virus in our stock cultures of T4 can be divided into at least two types—one producing plaques efficiently on minimal media at 37 C and the other doing so only in the presence of an added adsorption cofactor such as L-tryptophan. The response of the stock cultures to tryptophan would at least in part be determined by the proportions of the various types of virus which they contain.

Delbrück (1948) has added at least two types to the deficient forms of T4. T4,11 is like our tryptophan-deficient strains, but unlike ours its adsorption is strongly inhibited by traces of indole. The adsorption of another, T4,12, is also strongly inhibited by indole, and requires Ca^{++} in addition to a cofactor like tryptophan for adsorption. His T4,1 requires no added cofactor at 37 C, but the effect of temperature on plaque formation has not been studied (Anderson, 1948*b*).

The strains of T4 that are efficient in forming plaques on F agar probably arose by mutation of the cofactor-requiring type during its multiplication on B. To prove this we should have to show that the proportion of this fraction is highly variable in a series of cultures initiated by particles that are identical in their cofactor deficiency (Luria and Delbrück, 1943; Luria, 1945). The irregularities in the efficiencies of plating isolated deficient clones on F agar, as observed in table 1, may well be a reflection of this mutation effect. However, it did not seem worth while in the absence of more detailed knowledge of the mechanism of virus proliferation, plaque formation, and the factors influencing selection of

mutant strains to investigate the rate of mutation of T4 at this time (Hershey, 1946).

It may seem odd that the cofactor-requiring fractions should be able to form *any* plaques on B grown on unfortified F agar. However, it should be pointed out that the bacterial synthesis of tryptophan or other cofactors could well result in the presence of enough cofactor in the agar to activate small fractions of the deficient virus for adsorption and the initiation of plaque formation.

The fractions that are efficient in the production of plaques on F agar at 37 C probably require cofactor for activation too, but can utilize more effectively the low concentrations provided by the bacterial metabolism. The fact that L-tryptophan enhances their rate of adsorption on old bacteria lends support to this idea and at the same time provides a possible explanation for the slowness of virus adsorption on nonproliferating cells in exhausted media. Further indications of the true requirements of these fractions came from studies of the decreased efficiency with which they form plaques on F agar at low temperatures as described in the following paper (Anderson, 1948*b*). A survey of the effects of nutrients and temperature on the rates of adsorption of other viruses on bacteria that have passed the stationary stage of growth might well uncover unsuspected cofactor phenomena in their activities.

SUMMARY

A stock preparation of the bacteriophage T4 contained 3.9×10^{11} plaque-forming particles per ml on its host *Escherichia coli* strain B, grown on Difco nutrient agar (N), but only 2.6×10^{8} particles per ml forming plaques on B grown on ammonium lactate agar (F).

Individual plaques or clones from this stock appearing on N agar were suspended in F medium and assayed. They gave much lower counts on F agar than on N agar, and this characteristic persisted in subclones from these plaques, whether formed on F or on N agar.

The majority of individual clones appearing in assays of the stock on F agar, as well as their subclones, whether they arose on B on F or on N agar, produced as many plaques on F agar as they did on N agar.

The particles in the stock that are efficient in forming plaques on F agar can be removed by absorption on the host in F medium, but more effectively in the presence of the adsorption cofactor for T4, L-tryptophan.

We conclude that the degree of cofactor requirement is inherited in clones of T4.

REFERENCES

ANDERSON, T. F. 1945 The role of tryptophane in the adsorption of two bacterial viruses on their host, *E. coli*. J. Cell. Comp. Physiol., **25**, 17–26.

ANDERSON, T. F. 1946 Morphological and chemical relations in viruses and bacteriophages. Cold Spring Harbor Symposia Quant. Biol., **11**, 1–13.

ANDERSON, T. F. 1948*a* The activation of the bacterial virus T4 by L-tryptophan. J. Bact., **55**, 637–649.

ANDERSON, T. F. 1948b The influence of temperature and nutrients on plaque formation by bacteriophages active on *Escherichia coli*, strain B. J. Bact., **55**, 659–665.

DELBRÜCK, M. 1940 Adsorption of bacteriophage under various physiological conditions of the host. J. Gen. Physiol., **23**, 631–642.

DELBRÜCK, M. 1948 Biochemical mutants of bacterial viruses. *In press.*

DEMEREC, M., AND FANO, U. 1945 Bacteriophage-resistant mutants in *Escherichia coli.* Genetics, **30**, 119–136.

HERSHEY, A. G. 1946 Spontaneous mutations in bacterial viruses. Cold Spring Harbor Symposia Quant. Biol., **11**, 67–76.

LURIA, S. E. 1945 Mutations of bacterial viruses affecting their host range. Genetics, **39**, 84–99.

LURIA, S. E., AND DELBRÜCK, M. 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics, **28**, 491–511.