THE *E. COLI* B-RECEPTOR FOR THE PHAGE T5

II. ELECTRON MICROSCOPIC STUDIES

by

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INTRODUCTION

In a previous communication[1] methods of extraction and purification were described of a lipoglycoproteid complex which represents a minor component of the cell wall of *E. coli* B and shows a specific inactivating effect upon the *E. coli*-phage T5. The active material was obtained in pure form and assumed to function *in situ* as receptor site for the phage named above.

From a study of the kinetics of interaction between this receptor substance and T5 it was deduced that 1 mole of receptor must be equivalent to one mole of phage—or, in other words, that one particle of the receptor substance could combine with *only one* T5 particle (and *vice versa!*), rendering the phage particle incapable of infecting a B-cell. Taking into account that probably all phages are adsorbed to their host cell by the tip of their "tail" as has been shown for phage T2[2], it could be expected that electron micrographs of a T5 suspension completely inactivated by adding the specific receptor substance, ought to show T5 particles all with one receptor molecule attached to their tails. Expectations appeared to be supported by some preliminary electron micrographs taken at the Max-Planck-Institut für Virusforschung in Tübingen. A more thorough investigation with improved techniques was carried through at the Département de Biophysique de l'Institut de Physique in Geneva. The results, confirming rather strikingly all previous deductions, are given here.

MATERIALS AND METHODS

Bacteria. Escherichia coli, strain B and a mutant of it, resistant to T5 (B/1,5).
Bacteriophage. Strain T5 of the coli-phage T-series.
Preparation of receptor substance etc. l.c.[1].
Electron microscopic techniques.
(a). *Filtration method* (KELLENBERGER[3,4]). A 0.2% solution of parlodion in amyl-acetate is poured on a pre-dried agar plate and removed immediately. When the amylacetate evaporates a thin parlodion film is formed on the agar. A small volume of water spread on the film will be sucked into the agar quite rapidly. Anything suspended in the water and too large to pass through the pores of the film will stay on top of it. Care should be taken to adjust pH, salt concentration *etc.* of the agar gel to the respective conditions prevailing in the suspension to be filtered. After filtration and fixation in formalin vapour, the film is floated on a solution of lanthanum nitrate which prevents any water from diffusing backwards to the surface of the film. Further treatment as usual.

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This method does not eliminate effects of surface tension on the objects carried on the film, but makes it unnecessary to dilute or wash them in distilled water in order to remove salts or other diffusible material. Up to the last moment the objects stay in their proper medium.

(b). *Freeze drying method* (WILLIAMS[5]). Objects must be suspended in a buffer which will evaporate completely *in vacuo*. Our material was thoroughly dialyzed against ammonium benzoate. The suspension was then sprayed on a collodion membrane supported by a cooled (−80° C) metal plate. For spraying, an "Aerograph" spray pencil was found to be convenient. Sublimation of ice and benzoate was then effected as described by WILLIAMS.

(c). *Shadowing*. All preparations shown here were shadowed — those of Fig. 1 and 2 with a Pd-Pt-Au-alloy, the others with UO_2.

(d). *Electron microscope*. RCA, type EMU/2D.

EXPERIMENTAL RESULTS

1. *T5-receptor substance*: Micrographs were taken of samples of the pure material, suspended in buffer, according to two different methods. Fig. 1 shows the shadowed receptor particles as they are left on the supporting collodion membrane after the suspending liquid has been filtered through it. They appear as rather flattened spheres of more or less uniform diameter, showing a tendency to fuse where they lie in chance groups.

Fig. 2 shows the same receptor particles dried upon the collodion membrane from the frozen state. They keep a perfectly spherical shape, and there is no doubt about the uniformity of their size. Diameters were measured accurately and showed a very narrow distribution around a mean of 31.5 mμ. From sedimentation measurements in the ultracentrifuge a diameter of 30.7 mμ had been calculated earlier[1].

2. *T5-preparation*. In order to obtain clear pictures and unambiguous results with mixtures of T5 and its receptor substance it was essential to use T5-preparations of maximum purity. A combination of differential centrifugation with an application of

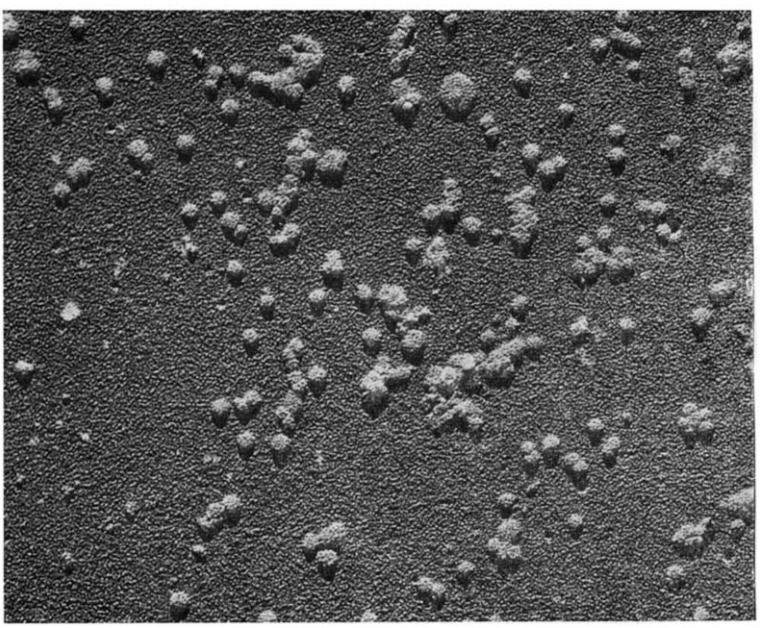

Fig. 1. Suspension of pure T5-receptor. Filtration method. Particles flattened by surface tension. × 60,000.

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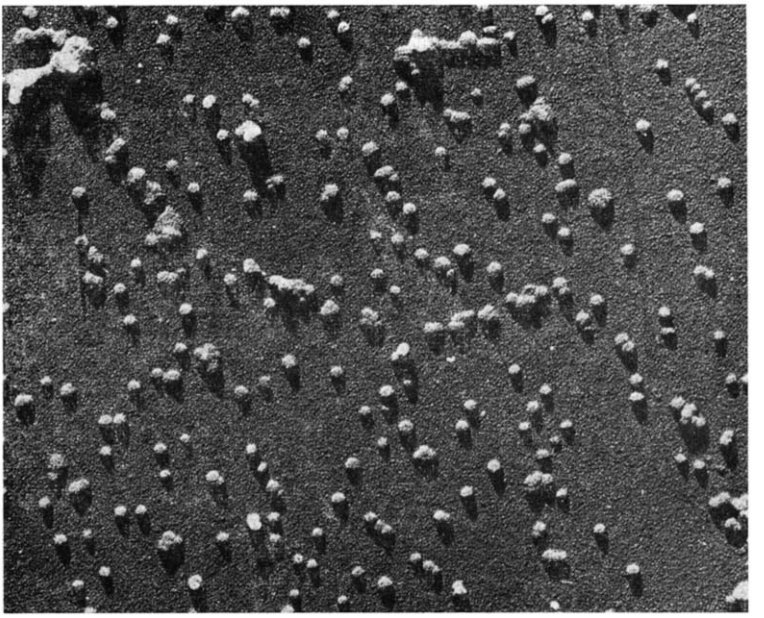

Fig. 2. Suspension of pure T5-receptor. Freeze drying method. × 60,000.

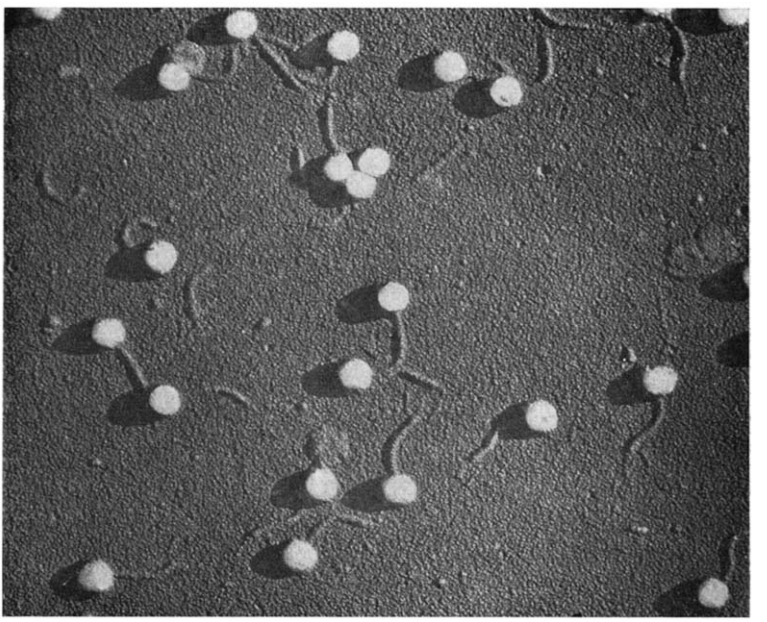

Fig. 3. Sample of the highly purified T5-suspension which has been used in all experiments. Filtration method. × 60,000.

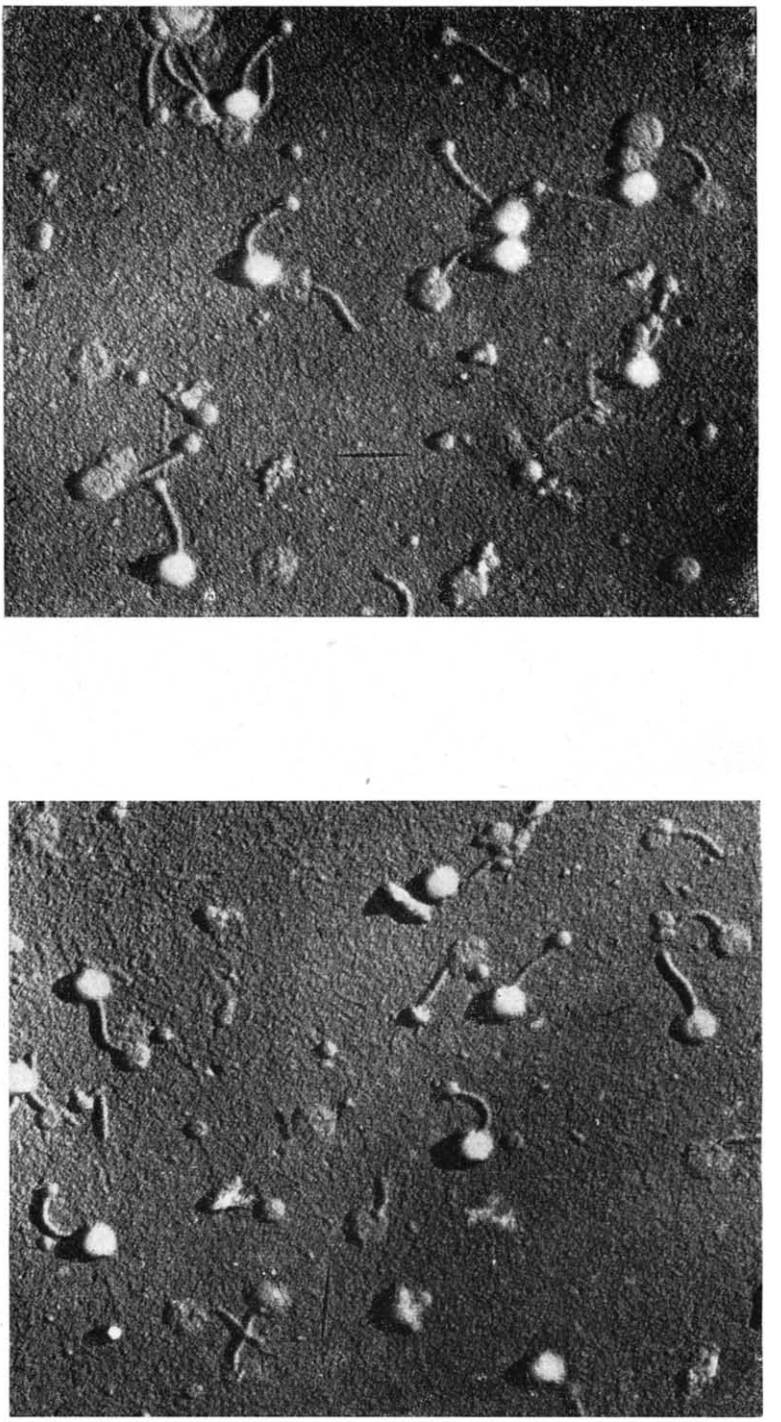

Fig. 4 and 5. Suspension of T5, more than 99 % inactivated by adding T5-receptor. Phage particles and ghosts, with receptor spheres attached to tails. × 65,000.

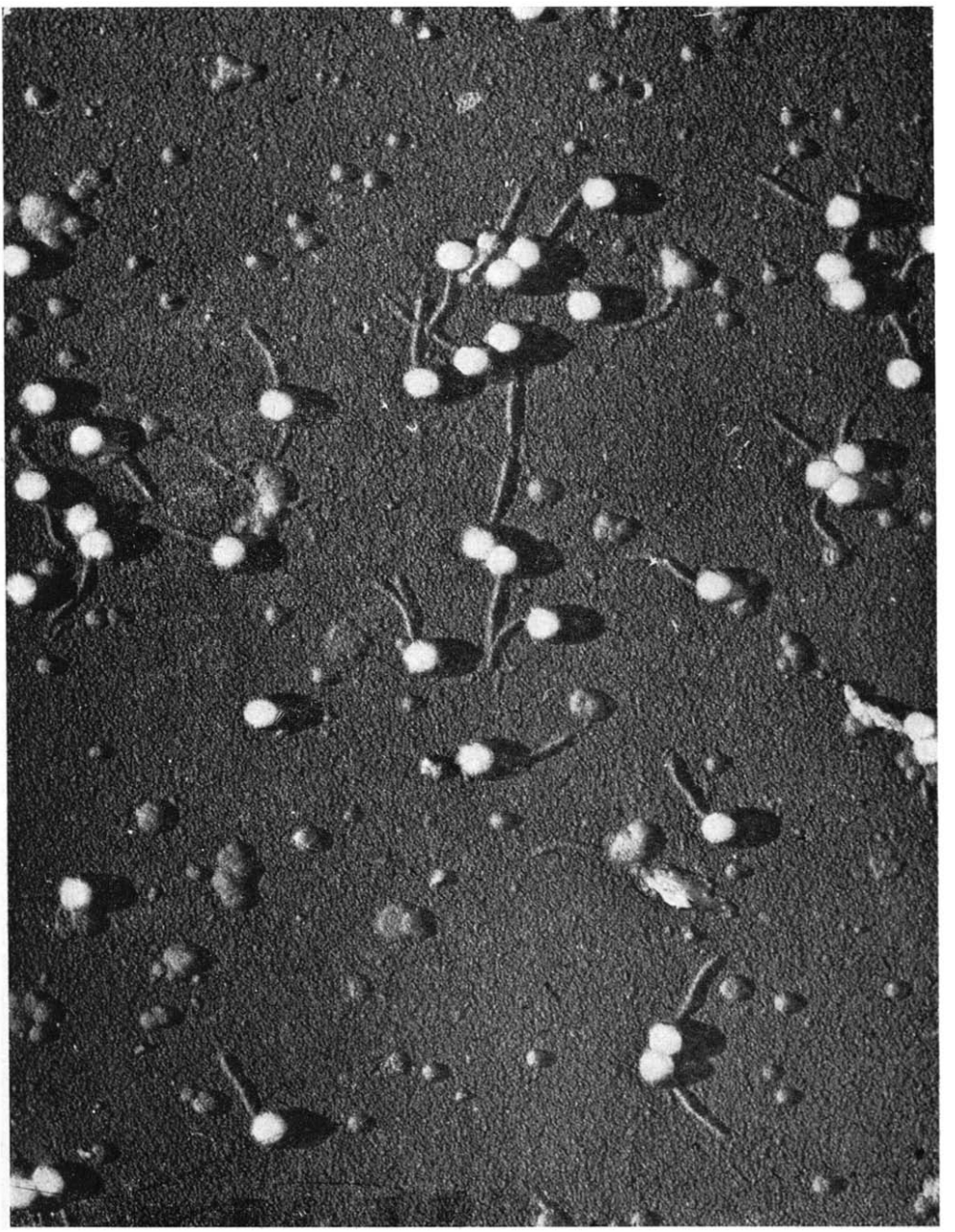

Fig. 6. Mixed suspension of T5 and receptor-analogous, inactive B/1,5-substance. Phages not inactivated. Spheres not attached to phage tails. × 60,000.

anti-B-serum to B-T5-lysates was found to yield very satisfactory T5-preparations of high titer, free of T5-ghosts and bacterial debris. The anti-serum precipitates and thereby removes amorphous material and small globular particles which resemble morphologically those of the T5-receptor substance and may in fact have something to do with it (see DISCUSSION).

Fig. 3 shows a sample of the T5 preparation used throughout the following experiments.

3. *Mixtures of T5 with T5-receptor substance.* $2.47 \cdot 10^{11}$ T5-particles in 0.9 ml of Bacto Tryptone were mixed with 2 "units" (definition see *l.c.*[1]) of receptor (in 0.1 ml Bacto Tryptone), which is enough for the titer of active phage particles to drop to $3 \cdot 10^{9}$ within 45 minutes. Samples of the mixture were then prepared for electron microscopy according to the filtration method. Figs. 4 and 5 represent two out of several dozen different micrographs. They all show T5-ghosts and T5-particles, all of which have one spherical receptor molecule attached to the tips of their tails—and nowhere else. Except in very scarce and dubious cases, two tails attached to the same receptor molecule were never found. With mixtures only partially inactivated by adding less receptor, the fraction of free T5-particles on the micrographs was always proportional to the fraction of still active T5 as determined by titration. A more direct confirmation of the validity of previous[1] assumptions on the mechanism of T5-inactivation by its receptor substance, based upon entirely indirect methods, cannot be wished for.

Furthermore the micrographs give a clear answer to a question which could not be answered easily by using indirect methods. It had been found[1] that in T5-suspensions inactivated with T5-receptor substance, some but not all of the phage-DNA was released into the medium. There were two possibilities: either the combination with receptor caused all of the T5-particles to eject part of their DNA, the rest being kept inside the protein ghosts—or it caused only part of the T5-particles to lose all of their DNA, whereas the rest lost none, in spite of having combined with receptor. From our pictures it can be seen that the latter explanation is the valid one. On the micrographs we see empty ghosts (often with the receptor sphere still sticking to their tails!) and unemptied T5-particles, seemingly unaltered, though equally adorned with a receptor molecule. Whether this difference in behaviour is due to an inhomogeneity of the T5-population or of the "population" of receptor particles remains to be explained. It may be, for instance, that the triggering capacity of a receptor particle is destroyed more easily than the attaching capacity.

The network of entangled DNA-threads ejected from the ghosted T5 can be seen on many of our micrographs, where it forms a more or less confluent layer as a background. Fortunately—for our main purpose of clearly elucidating the 1:1-relationship between receptor and T5-particles—the DNA-layer is only occasionally thick enough for the phage tails (with attached receptor spheres) to be buried in it completely.

Originally it was intended to employ the polystyrene latex procedure[6] for a direct count of receptor molecules per "unit" under the electron microscope. Such a count had been made earlier by means of a titration method, but had yielded a value which appeared too low[1]. An independent way of obtaining this value was desirable, especially since it could give an answer to the question of what fraction of the particles of a physicochemically pure receptor preparation is really receptor-active. However, the direct count was unfeasible for us because of an unexpected damaging effect on the activity of the receptor, apparently exerted by the Difco Tryptone media generally in use at the Geneva laboratory. This effect is absent in Difco Nutrient Broth (standard medium in Tübingen) which was not available during the time of our collaboration. The tryptone effect requires further investigation. It did not really affect the rest of our experiments—except that on the micrographs

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of T5 + receptor-mixtures there was always a considerable surplus of free receptor particles which had become inactive in the suspension before they had a chance to combine with T5-particles.

4. *Receptor-analogous, inactive lipoglycoproteid from B/1,5.* The extraction and purification of this material which is chemically analogous to the T5-receptor substance from B but inactive against T5, has also been described[1]. Under the electron microscope it looks at first absolutely like the T5-receptor substance. However, exact measurements of the diameters of the spheres revealed a somewhat wider distribution of values around the mean than was the case with the T5-receptor particles. Also the mean was found to be slightly higher, namely 36.5 mμ. The spherical molecules of the B/1,5-substance, together with admixed T5, can be seen in Fig. 6.

5. *Mixtures of T5 with B/1,5-substance.* Mixtures were prepared as described under 3, using an amount of B/1,5-substance equal to the amount of receptor substance employed in the above case, as judged by the nitrogen content. No inactivation of T5 was observed in 45 minutes. The mixture was then put under the electron microscope after preparation according to the filtration method. Tailtips of T5-particles were seen everywhere to be free of attached spheres although the spheres of the B/1,5-substance were scattered freely and in large amounts all over the preparation (Fig. 6). It may be concluded that not even reversible combination occurs between T5 and the receptor-analogous lipoglycoproteid from B/1,5.

DISCUSSION

The observations as such, reported above, may seem too simple and straightforward to need further discussion. However, there are a few additional points which ought to be made. First of all one may ask whether the fact that one receptor sphere reacts with only one T5-particle means that such a sphere, though symmetric in shape, is nevertheless functionally asymmetric, *i.e.* carries the actual receptor site as one tiny spot on its otherwise inactive surface. This may be so if one considers that *in situ* the sphere is probably imbedded rather deeply into the coli cell wall, showing but a small part of its surface to the outer world. Only here then would there be a real need for the specific receptor configuration. From a chemical point of view on the other hand, it seems somewhat easier to visualize a spherical macromolecule which is also symmetric in its micro-structure. But if this were true in our case, then more than one phage particle ought to find space for attachment on one sphere—*except* if the phage particle attaching first would somehow change the rest of the sphere's surface in a fraction of a second, rendering it inactive against any newcomer! Only if two T5-particles were to hit the same sphere at almost the same moment would they have a chance to both be fixed. Perhaps the rare cases on the micrographs where one has the impression of seeing two (and never more than 2) T5-particles or ghosts sticking to the same sphere with their tails, can be interpreted in this way. Since in virus-receptor-interaction there are certainly complex and rapidly-occurring events involved on both sides[7], it may well be worthwile to look for direct experimental proof of the "changed surface hypothesis".

This leads to the second point: which way was the DNA actually released from the receptor-ghosted T5-particles? Did it really squeeze through the tail and past the receptor sphere which in many cases appears to stick still firmly to the ghost's tail tip and plug it up rather than open it? It does not seem too convincing an assumption. Perhaps the sphere does to the phage's protein coat what the phage's tail tip does to

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the sphere: it alters its structure (and thereby its DNA-permeability) at some distance from the point of attachment. Still it must be said that about half of the ghosts seem to have lost their receptor sphere in the course of events. This is perhaps a secondary effect, due to surface tension or other influences during preparation. Unfortunately, so far we have not been able to observe a T5-particle just halfway through the receptor-triggered process of emptying out its DNA. The particles had either not yet begun or finished completely, and one never could clearly see the DNA-threads on their way out.

Finally, attention may be drawn to a curious circumstance; one very rarely finds in crude T5-preparations, T5-particles with an attached receptor sphere. In addition one finds many more globular particles resembling T5-receptors, but free. Are these inactive receptor particles, released from the cell during lysis but almost 100% inactivated before an appreciable amount of the newly produced T5 could attach to them? For several reasons it does seem to us that some mechanism specifically inactivating the receptor of the reproducing phage before or at the very moment of lysis, is a rather general feature in phage production. The setting in of this receptor destroying process may even actually trigger lysis. There are other observations which point in the same direction and which are being pursued further[1,8].

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SUMMARY

Electron micrographs of pure T5-receptor substance extracted from *E. coli* B show spherical particles of uniform size. Their diameters correspond exactly to the value obtained from sedimentation measurements. Micrographs of T5-suspensions inactivated by receptor show that the inactivation is brought about by the attachment of a single receptor particle to the tailtip of each of the inactivated T5-particles. Frequently this attachment triggers ejection of the T5-particle's DNA. Obviously the infection mechanism for T5 is similar to the mechanism found with other tailed phages. Particles extracted from the T5-resistant Coli-Mutant B/1,5 have about the same diameter as the spheres of active receptor, but do not attach to T5-tails and do not inactivate this phage.

RÉSUMÉ

Nous avons mesuré, par microscopie électronique, les dimensions des récepteurs pour le bactériophage T5, extraits chimiquement des cellules de *E. coli* B et purifiés. Les valeurs obtenues correspondent exactement à celles déterminées par ultracentrifugations. Nous avons observé que le récepteur s'adsorbe sur le phage en se fixant sur l'extrêmité de la queue, chaque phage ne prenant qu'une particule de récepteur et *vice versa*. Environ 50% des phages ainsi inactivés éjectent leur ADRN et en se vidant donnent l'apparence de ghosts. Le mode d'infection de B par T5 semble donc être identique à celui étudié pour T2, à savoir adsorption par la queue et injection du ADRN. Les particules "réceptrices" extraites de bactéries résistantes au T5 (B/1,5), présentent des dimensions similaires à celles du récepteur actif, mais elles ne se fixent pas sur le phage et ne l'inactivent pas.

ZUSAMMENFASSUNG

Die Teilchengrösse der aus *E. coli* B extrahierten und gereinigten Rezeptorsubstanz für den Phagen T5 wurde elektronenmikroskopisch gemessen und gefunden, dass diese mit dem aus der Sedimentationskonstante sich ergebenden Wert übereinstimmt. Elektronenmikroskopische Aufnahmen mit Rezeptor inaktivierter T5-Suspensionen zeigen, dass das Phagenteilchen durch die

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Adsorption eines einzigen Rezeptorteilchens an seine Schwanzspitze inaktiviert wird. Dabei läuft häufig die Nucleinsäure des Phagenteilchens aus ihrer Proteinhülle aus. Daraus ist zu schliessen, dass auch T5-Teilchen (wie andere geschwänzte Phagentypen) infizieren, indem sie sich mit ihrer Schwanzspitze an die rezeptortragende Zellwand anheften. Eine dem Rezeptor chemisch analoge, aber inaktive Substanz aus der T5-resistenten Coli-Mutante B/1,5 zeigt praktisch die gleiche Teilchengrösse wie der aktive Rezeptor, wird jedoch nicht an die Schwanzspitzen von T5-Teilchen adsorbiert.

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