THE CLASSES OF KAPPA IN PARAMECIUM AURELIA*

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SUMMARY

Kappas (bacterial symbionts containing R bodies) have been studied in 16 strains of Paramecium aurelia, syngens 2 and 4. All produce toxins capable of killing sensitive paramecia. The first major class, the 51 group (consisting of the kappas of strains 51, 116, and 298), has R bodies which, when the pH is lowered below 6.5, unwind reversibly from the inside to form a tight tube; the outside end of the R-body ribbon forms an acute angle; no sheath surrounds the R body. The phage-like structures of the 51 group are helical; no capsomere-like structures are present; isolated R bodies do not have any killing activity. The second major class, the 7 group (strains 7, 576, Bl 166-1, 249, 1041, 310, 1039), has R bodies which unwind irreversibly from the outside to form a loose twisted ribbon whose outside tip is blunt, irregular or finger-like; a single membraneous sheath covers the R body. Phage-like structures in the 7 group are spherical; capsomere-like structures are present; isolated R bodies show killing activity. The third major class, the 562 group (strains 562, 517, 511), is similar to the 7 group except that there is no sheath, no capsomeres, and free R bodies are almost or completely inactive. The phage-like structures are spherical, and unlike those of the 7 group, do not stick to the R bodies. In addition to these 3 major classes there are 3 strains which show important differences from the major classes and also differ among themselves. 1038 is like 7 in all respects except that the phage-like structures are helical and isolated R bodies are not very active. 5117, presumably a mutant of 51, has spherical phage-like structures and 7 type-R bodies which are inactive when isolated. 570 is the only known mate-killer with R bodies; it has spherical phage-like structures. It is noted that strain 1039 of the 7 group has very few phage-like structures, virtually all of which are empty. Since free 1039 R bodies are highly active, it is likely that intact phage-like structures are not essential for the toxic action on sensitives. The spherical and helical phage-like structures are probably very closely related because 51, which has helical structures, apparently gave rise to 5117, which has spherical structures. Likewise the kappa symbionts of 1038 with helical phage-like structures are virtually identical to the kappas of the 7 group with spherical phage-like structures. The presence of phage-like structures in all strains with R bodies suggests that R bodies may be a product of phage activity. Strain 570, a mate-killer whose symbionts contain R bodies, provides a link between kappas and mus, the mate-killer symbionts. The symbionts of 570 are the only ones containing R bodies which are non-toxic when encountered by sensitives in the medium in which the paramecia are cultured.

INTRODUCTION

The object of this paper is to survey the different kinds of kappas which are found in the cytoplasm of Paramecium aurelia. Kappas are bacterial endosymbionts and are perhaps the best studied of the numerous symbiotic bacteria found within the cyto-

* This paper is dedicated to Professor Tracy M. Sonneborn on the thirty-fifth anniversary of his discovery of mating types.
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plasm of eukaryotic cells. Kappas are unique among bacteria in that a certain fraction of every population contains R bodies, i.e. refractile inclusions consisting of a thin ribbon of protein wound into a tight roll. By an unknown mechanism kappas find their way out of their host paramecium and are released into the medium. If a paramecium of a sensitive strain ingests a kappa containing an R body it may be killed. Strains of paramecia bearing kappa are therefore called killers. All killers are resistant to the strains of kappa which they bear. After sensitives ingest kappa, kappas within food vacuoles begin to break down, the R body suddenly unrolls into a long straight tube or twisted ribbon, and the food vacuole membrane breaks down (Jurand, Rudman & Preer, 1971). The R body and its unrolling have been implicated in the killing process. The production of R bodies in kappa particles is closely associated with the production of microscopically visible phage-like structures (J. R. Preer & Preer, 1967; J. R. Preer & Jurand, 1968; Grimes & Preer, 1971; J. R. Preer, Preer, Rudman & Jurand, 1971). The symbiotic interactions and adaptations between phage, bacterium, and eukaryote cell have apparently resulted in a remarkable series of adaptations in the genetic apparatus of the phage, the kappa and the paramecium (see reviews by Sonneborn, 1959, and J. R. Preer, 1968, 1971). Variations in the morphology of the R body and phage-like structures in kappas from known strains of paramecia, together with variations in the nature and action of the killing substance, have suggested the need for the present survey of the properties of the different kappas in selected strains of paramecia of diverse origins.

Killer strains of paramecia were first described by Sonneborn (1938). Kappa was recognized genetically as a cytoplasmic factor responsible for the killer phenotype by Sonneborn (1943). The cytological recognition of kappa by J. R. Preer (1948) was followed by various cytological, biochemical, and physiological studies by a number of workers. They firmly established that kappa is a bacterium (see recent papers by Kung, 1970, 1971). Many aspects of kappa and its relation to paramecia were studied, and it was found that there are numerous kinds of killers which owe their killing activity to a number of rather different bacterial symbionts, which include not only kappa, but also mu, lambda, and others. Their extensive studies are summarized in the reviews by Sonneborn and Preer cited above.

In a companion paper to the present one, Beale, Jurand & Preer (1969) surveyed the classes of endosymbionts in *P. aurelia* and defined kappas as those symbionts which can develop R bodies. R bodies were first observed with the light microscope by J. R. Preer & Stark (1953), then with the electron microscope by Dippell (1958) and Hamilton & Getner (1958). J. R. Preer, Siegel & Stark (1953), Smith (1961) and Mueller (1963) showed that only kappas containing R bodies are toxic, and L. B. Preer & Preer (1964) showed that free R bodies of stock 7 are able to kill. Mueller (1962) found that R bodies could suddenly achieve a filamentous form, and later Anderson, Preer, Preer & Bray (1964) showed that this process consisted of 'unwinding' of a roll of ribbon. Descriptions of the R bodies of strains 7, 51 and 562 have been given in previous papers (J. R. Preer, Hufnagel & Preer, 1966; J. R. Preer & Preer, 1967; L. B. Preer, 1969).

Structures which resemble phages were discovered first in strains 7 and 51 (J. R.
Classes of kappa in Paramecium

Table 1. Strains and their origins*

<table>
<thead>
<tr>
<th>Stock no.</th>
<th>Place collected</th>
<th>Stock no.</th>
<th>Place collected</th>
</tr>
</thead>
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<td>Pinehurst, N.C.</td>
<td>517</td>
<td>Gif, France</td>
</tr>
<tr>
<td>51</td>
<td>Spencer, Ind.</td>
<td>562</td>
<td>Milan, Italy</td>
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<tr>
<td>51ml</td>
<td>Mutant of 51 isolated by R. Dippell</td>
<td>570</td>
<td>Georgia, U.S.S.R.</td>
</tr>
<tr>
<td>116</td>
<td>Bloomington, Ind.</td>
<td>1038</td>
<td>Syktyvkar (Komi A.S.S.R),</td>
</tr>
<tr>
<td>249</td>
<td>Chipola River (U.S. 90), Fla.</td>
<td>1039</td>
<td>U.S.S.R.</td>
</tr>
<tr>
<td>298</td>
<td>Empire Range, Panama</td>
<td>1041</td>
<td>Lomonosov, U.S.S.R.</td>
</tr>
<tr>
<td>310</td>
<td>New Zealand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>511</td>
<td>Edinburgh, Scotland</td>
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</tbody>
</table>

* All of the strains belong to syngen (variety) 2 except 51, 51ml, 116 and 298 which belong to syngen 4.

Preer & Preer, 1967). Their association with R body-bearing kappas and usual absence in kappas free of R bodies was demonstrated by J. R. Preer & Jurand (1968) and Grimes & Preer (1971). The phage-like structures of strain 562 have been isolated and estimated to contain about 80% as much DNA and about the same amount of protein as phage T2 (J. R. Preer et al. 1971). Although infection has not been demonstrated, their fine structure and chemical composition leave little doubt that they are phages, perhaps defective.

MATERIALS AND METHODS

Most of the techniques used in this study have already been described: (1) collecting and cultivating paramecia (Sonneborn, 1970); (2) measurement of killing activity (J. R. Preer et al. 1953); (3) methods for the purification of kappa, summarized by Preer in Sonneborn, 1970; (4) isolation of R bodies using ultrasonication and centrifugation (J. R. Preer & Preer, 1967); (5) electron microcopy (J. R. Preer & Jurand, 1968); and (6) isolation of the phages by centrifuging in a cesium chloride gradient (J. R. Preer et al. 1971). In addition, the method of staining whole paramecia for observing symbionts in the light microscope (Beale & Jurand, 1966) was used with a modification to remove lipid droplets from the cytoplasm of the animal. This was done by pipetting several drops of acetone on to osmium-fixed animals and allowing the preparation to dry before adding the stain. For observation of whole kappa in the electron microscope, the negative staining procedure was modified to render kappa less opaque by treating it with 2% sodium deoxycholate for 5 min, then centrifuging the kappa to eliminate the deoxycholate before negative staining.

Although the standard strain 16 (syngen 1, Baltimore, Maryland) was often used as a sensitive for killing tests, strain 152 (syngen 3, New Haven, Connecticut) was more often used; it is sensitive to all known killer strains of P. aurelia (M. V. Schneller, personal communication). The strains of killers used in this study were obtained, for the most part, from the collections of Sonneborn and Beale. Special thanks are due to D. V. Osipov for making available strains 1038, 1039 and 1041 from the U.S.S.R. A list of the strains and their origins appears in Table 1.

THE CLASSES OF KAPPA

All of the 14 syngens of P. aurelia were surveyed for symbionts containing R bodies (kappas), and only in syngens 2 and 4 were kappas found; in these syngens they proved to be very common. In some localities, such as Edinburgh, Scotland, syngen 2 is quite common and almost every individual of P. aurelia isolated contains kappa.
Three major groups of kappa emerge from this study along with 3 unique types, each represented by only a single strain. The results are summarized in Table 2 and will be described below.

The 5/ group

All of the kappas of this group induce pre-lethal aboral humps in the sensitives they kill. They are found exclusively in syngen 4, and they occur in strains 51, 116 and 298. In addition, the syngen 4 hump killers from Australia described by Stevenson (1970) appear to belong to the same group. Their R bodies are distinctive and markedly different from all other groups in unwinding from the inside to form a tightly coiled tube (Fig. 1), in having the outer (as well as the inner) end of the ribbon in form of a regular acute angle, and in unwinding in response to lowering the pH below 6.5 and rewinding when the pH is raised above 6.5. Their helical phage-like structures (Figs. 2, 3) readily detach from the unrolling R bodies. Breaking open the isolated kappa symbionts results in immediate loss of all killing activity. It is a unique characteristic of the kappas of this group that the outer cell wall layers of the kappas often appear wavy and sometimes separated from the cell membrane in sectioned material (Fig. 4), indicating perhaps a specific fixation artifact. The cell wall appears much more fragile than in other groups, and lysis may be achieved with low concentrations of sodium deoxycholate and brief ultrasonication. The surface of the kappas is such that they do not adsorb to the ion exchange resin ECTEOLA. Since other constituents of homogenates of paramecium are adsorbed, the kappas may be purified using a column of ECTEOLA (Smith, 1961). Widmayer (1968) showed that strain 5111142, a weak hump killer derived from stock 51, is very similar to 51. She found, however, that the R bodies of 5111142 are smaller, and do not unroll as readily as those of 51.

The 7 group

The 7 group (strains 7, 576, Bl 166-1, 249, 1041, 310, 1039) is the largest and most diverse class. Pre-lethal effects may differ from one killer strain to another, and they include spinning, vacuolization (or simply swelling and formation of corpses), and paralysis. R bodies of all groups except the 5/1 group are similar and are of the 7 type: they unwind from the outside to form a loose twisted ribbon (Fig. 5); the outer end of the ribbon does not end in an acute angle; unrolling is irreversible, often occurring spontaneously. The outer end of the R body ribbon in a number of strains such as 7 and 576 is blunt or irregular (Fig. 5). In others, such as strains 249 and 1039, it may show long, finger-like projections (Fig. 6). As far as is known, a membranous ‘sheath’ is present in all members of this group. It appears in sections as an extension of the outermost coil of the ribbon and extends in one direction only (Fig. 7). It can sometimes be seen adhering to the outer end of the ribbon in negatively stained preparations of the unrolled R body. Phage-like structures are spherical and tend to stick to the inner end of the R body ribbon (Fig. 8). Subunits, presumably protein, of the order of 10 nm in diameter, are seen in the electron microscope and referred to as capsomeres or capsomere-like structures (Figs. 9, 11) because of their resemblance to the capsomeres of phages and their association with the phage-like structures in kappa. They
Table 2. *The classes of kappa*

<table>
<thead>
<tr>
<th>Class</th>
<th>Strain</th>
<th>Syngen</th>
<th>Type</th>
<th>Sheath</th>
<th>Phage-like structures</th>
<th>Activity</th>
<th>Isolation technique</th>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>R body</td>
<td></td>
<td></td>
<td>Kind</td>
<td>Sticky</td>
<td>Capso-</td>
<td>Killing</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td>Isolation</td>
<td>technique</td>
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<td>technique</td>
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<tr>
<td>51</td>
<td>51</td>
<td>4</td>
<td>51</td>
<td></td>
<td>Hel.</td>
<td>Hump</td>
<td>ECTEOLA wash</td>
</tr>
<tr>
<td>116</td>
<td>4</td>
<td>51</td>
<td>4</td>
<td>4</td>
<td>Hel.</td>
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<tr>
<td>298</td>
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<td>4</td>
<td>4</td>
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<tr>
<td>7</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td></td>
<td>Sph.</td>
<td>Spin.</td>
<td>Filter-paper eluate</td>
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<tr>
<td>576</td>
<td>2</td>
<td>7</td>
<td>+</td>
<td></td>
<td>Sph.</td>
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<td>Filter-paper eluate</td>
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<tr>
<td>B1166-1</td>
<td>2</td>
<td>7</td>
<td>+</td>
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<td>Spin.</td>
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<tr>
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<td>2</td>
<td>7</td>
<td>+</td>
<td></td>
<td>Sph.</td>
<td>Spin.</td>
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<tr>
<td>1041</td>
<td>2</td>
<td>7</td>
<td>+</td>
<td></td>
<td>Sph.</td>
<td>Vac. Par.</td>
<td>Filter-paper eluate</td>
</tr>
<tr>
<td>310</td>
<td>2</td>
<td>7</td>
<td>+</td>
<td></td>
<td>Sph.</td>
<td>Vac. Par.</td>
<td>Filter-paper eluate</td>
</tr>
<tr>
<td>1039†</td>
<td>2</td>
<td>7</td>
<td>+</td>
<td></td>
<td>Sph.</td>
<td>Vac. Par.</td>
<td>Filter-paper eluate</td>
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<tr>
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<td>562</td>
<td>2</td>
<td>7</td>
<td></td>
<td>Sph.</td>
<td>Vac.</td>
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</tr>
<tr>
<td>511</td>
<td>2</td>
<td>7</td>
<td>+</td>
<td></td>
<td>Sph.</td>
<td>Vac.</td>
<td>ECTEOLA wash</td>
</tr>
<tr>
<td>1038</td>
<td>1038</td>
<td>2</td>
<td>7</td>
<td></td>
<td>Hel.</td>
<td>Spin.</td>
<td>Filter-paper eluate</td>
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<tr>
<td>51m1</td>
<td>51m1</td>
<td>4</td>
<td>7</td>
<td></td>
<td>Sph.</td>
<td>Spin.</td>
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<tr>
<td>570</td>
<td>570</td>
<td>2</td>
<td>7</td>
<td></td>
<td>Sph.</td>
<td>M.K.</td>
<td>ECTEOLA eluate</td>
</tr>
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</table>

Hel., helical; hump, aboral humps; m.k., mate-killer; par., paralysis; sph., spherical; spin., spinning; vac., vacuolization.

* 51-type R bodies unwind from inside, are induced to unroll and reroll by lowering and raising pH, form a tight tube when unrolled, and the outside end of the roll forms an acute angle. 7-type R bodies unwind from outside, are unaffected by pH, form a loose twisted ribbon when unrolled, and outer end of roll is blunt or irregular in shape. Some R of 116 and 298 appear to be of the 7 type and have spherical phages and no capsomeres.

† *Very few* phage-like structures and almost all empty.
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are present in varying numbers in all members of the 7 group. Isolated R bodies of this group show varying degrees of toxicity in different stocks. All kappas of group 7 belong to syngen 2 and unlike the 51 group all are irreversibly bound to ECTEOLA. They can be isolated in very pure form by adsorbing them to filter paper columns at low ionic strength and pH and eluting at high ionic strength and pH (J. R. Preer et al. 1966). Strain 1039 is unusual in that its phage-like structures are rare and almost always empty (Fig. 10). This strain, like 1041, has an unusually large number of capsomeres (Fig. 11).

The central core enclosed by the R body in the kappas from this as well as other groups is often seen in sections to consist of material different from the surrounding cytoplasm of kappa (Fig. 12). This material may in some cases be capsomeres, in others helical phage-like structures or perhaps some other material. It cannot be adequately characterized in sectioned material and is observed much better after negative staining.

Phages which appear to have tails have been observed occasionally in strains 249, 576 (Fig. 13), 1039 and 1041. In 1041 a thin strand of material, probably DNA, has been observed issuing from the tail (Fig. 14).

The R bodies of most of the kappas of the 7 group tend to be somewhat smaller than the dimensions cited for 7, a mean length of 14.3 and width of 0.5 μm (Anderson et al. 1964). Lengths and widths of about two-thirds these dimensions are more common in most strains.

The 562 group

All the members of this group (562, 517, 511) cause vacuolization. They have been found only in syngen 2. Their R bodies are of the 7 type but they possess no sheath. Phage-like structures (see Fig. 15 for location of phages in intact kappa) do not stick to the R body, but are found in the medium, often in very large numbers, making them the best subjects for isolation (Fig. 16) (J. R. Preer et al. 1971). Capsomeres are absent and isolated R bodies are inactive or only very weakly active. The kappas of this group do not adsorb to filter paper or ECTEOLA, hence they may be isolated using ECTEOLA, as in the case of 51. Phage-like structures with tails occur rarely in 562 (Fig. 17).

1038

The kappas of 1038 (Fig. 18) resemble those of the 7 group in most respects except that 1038 kappas contain helical (Fig. 19), rather than spherical phage-like structures and isolated R bodies are not very active. Strain 1038 is a member of syngen 2.

51m1

This mutant of 51 kappa (Dippell, 1950) has an unusual combination of characteristics. It is unlike 51 in that it causes spinning (rather than humps) in sensitives. It has 7-type (rather than 51) R bodies with numerous capsomere-like structures (Figs. 20, 21), and spherical phages (rather than helical) (Fig. 22) which sometimes stick to the R bodies. The outer layer of the cell wall does not tend to separate from the cell membrane as it does in 51, but appears more like the walls of the kappas of the
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other groups. On the other hand, \textit{51m1} kappas are isolated by taking advantage of the fact that, like \textit{51}, they do not adsorb to ECTEOLA. Also, like \textit{51}, isolated R bodies are completely inactive.

It is interesting to note that a small portion of the kappa particles isolated from cultures of \textit{116} and \textit{298} (\textit{51} group) contain 7-type R bodies. In prolonged ultrasonication they represent increasingly larger proportions of a mixed suspension of kappa types, due to the greater fragility of the \textit{51} type kappas. It is presumed that these kappa particles in \textit{116} and \textit{298} are mutant kappas, just as \textit{51m1} is thought to be a mutant of \textit{51}.

570

Strain 570 represents the only mate-killer in syngen 2. R bodies are very small (approximately 0.2 \textmu m in width and 5 \textmu m in length) and low in frequency (about 1–2 %). They have 7-type R bodies with no sheath or capsomeres, but with spherical phage-like structures (Figs. 23, 24). They differ from all kappas and in fact, from all endosymbionts of \textit{P. aurelia} except the macronuclear symbiont, \textit{alpha} in adsorbing to ECTEOLA, from which they may be eluted by solutions of high ionic strength and pH. Unlike all other symbiotic particles with R bodies, the R body-containing symbionts of this stock are not toxic when taken up from the medium by sensitive paramecia; they exert their toxic effect, like other mate-killers, only at conjugation.

At first it was thought that strain 570 might have a mixture of both mus (symbionts without R bodies which produce mate killing) and kappas. P. Wong (unpublished) in our laboratory undertook an experiment to test this possibility. He diluted the endosymbionts of strain 570 by rapid multiplication of the paramecia until the majority had no symbionts and the remainder presumably only one (J. R. Preer, 1948). Then by slowing down cell division he allowed the full complement of symbionts to regenerate. All new cultures containing symbionts derived in this way still showed the same low frequency of symbionts with R bodies and all were mate-killers. Therefore, in this strain no evidence for a mixed population of symbionts exists, and this conclusion is supported by observations with the electron microscope.

DISCUSSION

It is interesting that kappas, the symbionts with R bodies, are found only in syngens 2 and 4 where they are very common. Other classes of endosymbionts in \textit{P. aurelia} are also restricted to certain syngens.

Previous studies have shown that within a single strain there is a very high degree of correlation between the presence of phage-like structures and the presence of R bodies in kappa. The present study reveals further that when different strains are compared, there is a perfect correlation between the presence of R bodies and the presence of phage-like structures in the kappa symbionts. The hypothesis that R bodies are a product of the phage genome is thus strongly supported. If R bodies result from the invasion of bacterial symbionts by a special class of bacteriophages, then there must be great specificity in the kind of bacteria which can successfully be invaded and harbour R bodies.
If the spherical phage-like characteristic of the mutant kappa 51ml had arisen by mutation from the helical structures of strain 51 the implication could be that the 2 phage forms must be fundamentally very similar, although infection by an unrelated phage has not been ruled out. The hypothesis of a true interconversion between spherical and helical forms is supported by 1038 kappa, which has helical phage-like structures, yet is virtually identical in all other respects to the 7-type kappas which have spherical structures. It may be that the helical structures represent protein sub-units which are incompletely or improperly assembled, rather than complete phages. Indeed, a similar case has been reported for Venezuelan equine encephalitis virus by Klimenko et al. (1965).

The nature of the toxins produced by kappa remains mystifying. The involvement of the R body seems certain, for only kappas with R bodies are toxic, and isolated R bodies from many strains are toxic. No element smaller than a rolled R body has been shown to be toxic. Free phage-like structures from kappa, kappa extracts, unrolled R bodies, and R body fragments are all without effect. The results are consistent with the view that unrolling of the R body is necessary for killing; but beyond unrolling there must be a toxin to account for the specificity of the different prelethal effects. Such a toxin could be an easily inactivated part of the molecules which make up the R body ribbon or perhaps another unseen molecule absorbed to the ribbon. Since capsomere-like structures are absent in some strains and phage-like structures are virtually absent in 1039, no consistent correlation between toxic activity and these structures seems possible. It may be that the toxic substance is the phage, complete in some cases but incomplete in others, and in some instances – for example, 1039 – the capsomeres may be the effective toxins.

Whether the symbionts of the 570 mate-killer should be called mu (which lack R bodies in other syngens) or kappa because of the presence of R bodies is arbitrary. The symbiont in 570 is a true intermediate between mu and kappa. It would be of great interest to determine whether the R bodies of the 570 symbionts are involved in their mate-killing activity.

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ABBREVIATIONS ON PLATES

<table>
<thead>
<tr>
<th>c</th>
<th>capsomere-like structure</th>
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<tr>
<td>ca</td>
<td>aggregates of capsomere-like structures</td>
</tr>
<tr>
<td>co</td>
<td>central core material of R body</td>
</tr>
<tr>
<td>p</td>
<td>phage-like structure</td>
</tr>
<tr>
<td>R</td>
<td>refractile body in bright kappa particle</td>
</tr>
<tr>
<td>s</td>
<td>sheath to R body</td>
</tr>
<tr>
<td>g</td>
<td>grid-like pattern on unrolled R body</td>
</tr>
<tr>
<td>w</td>
<td>cell wall of kappa</td>
</tr>
</tbody>
</table>

All figures are electron micrographs. Figs. 1–3, 5, 6, 8, 9, 11, 13–17, 19–21, 23 are negatively stained preparations; Figs. 4, 7, 10, 12, 18, 22, 24 are sectioned material.

Fig. 1. 51 (syngen 4). R body (R) unrolling from inside to form tight tube. ×48,000.

Fig. 2. 116. Helical phage-like structures (p) associated with unrolled R body (R). ×150,000.
Classes of kappa in Paramecium
Fig. 3. 298. Helical phage-like structures associated with unrolled R body. × 150,000.

Fig. 4. 116 (syngen 4). Longitudinal section through kappa showing irregularities in cell wall (w) of kappa. × 60,000.

Fig. 5. 576 (syngen 2). R body unrolling from outside to form loose twisted ribbon. Note blunt outer end. × 20,000.

Fig. 6. 249 (syngen 2). Unrolled R body (R) with finger-like projections at outer end. × 100,000.

Fig. 7. 1039 (syngen 2). Longitudinal section through kappa with sheath (s) extending from one side of R body (R). × 60,000.

Fig. 8. 249. Unrolled R body with spherical phages (p) adhering to the inside, acute-angled end of R body ribbon. × 60,000.
Classes of kappa in Paramecium
Fig. 9. 1041 (syngen 2). Unrolled R body with numerous capsomere-like structures (c) adhering to it. Note that empty phages (p) stain negatively. The grid-like pattern (g) on R body is of unknown significance, but the dimensions of the grid are similar to those of capsomere-like structures. × 150,000.

Fig. 10. 1039. Longitudinal section through kappa. Note that only empty phages (p), which do not stain in sectioned material, are present. × 60,000.

Fig. 11. 1039. Inner end of an unrolled R body (R) with numerous capsomere-like structures (c) present, many aggregated in rows (co). × 150,000.

Fig. 12. 1041. Longitudinal section through kappa showing central core material (co) of R body (R). Note the sheath (s). × 60,000.
Classes of kappa in Paramecium
Fig. 13. 576. R body with adhering phages (p), some of which appear to have tails (arrows). \( \times 100,000 \).

Fig. 14. 1041. Phage in medium. Note fine strand of material, probably DNA, issuing from tail region. \( \times 200,000 \).

Fig. 15. 562 (syngen 2). Whole kappa symbiont pretreated with 2% sodium deoxycholate before negative staining, showing numerous spherical phages (p) outside R body (R). \( \times 45,000 \).
Classes of kappa in Paramecium

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15
Figs. 16, 17, 562. Purified spherical phages prepared by centrifugation in caesium chloride density gradient. Fig. 17 shows tail-like structure. \( \times 200000 \).

Fig. 18. 1038 (syngen 2). Longitudinal section through kappa with R body (\( R \)) devoid of spherical viruses. \( \times 60000 \).

Fig. 19. 1038. Unrolled R body with helical phage-like structures (\( p \)) adhering to it. \( \times 150000 \).
Classes of kappa in Paramecium
Fig. 20. 51m1. (syngen 4). Unrolled R body (R) with numerous capsomere-like structures (c) adhering to it. Note grid-like pattern (g) on R body similar to that in Fig. 9. x 100,000.

Fig. 21. 51m1. R body unrolling from outside (7- rather than 51-type R body). x 25,000.

Fig. 22. 51m1. Oblique section of kappa with spherical phages (7 type), rather than helical (51 type). x 60,000.

Fig. 23. 570 (syngen 2). Unrolled R body from mate-killer stock. x 30,000.

Fig. 24. 570. Longitudinal section through kappa from mate-killer stock showing R body (R) and spherical phages (p). x 60,000.