

Fine Structure of Kappa in *Paramecium aurelia*.* † By L. D. HAMILTON AND M. E. GETTNER.‡
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Inhibition of the cytoplasmic factor kappa by 2,6-diaminopurine in *Paramecium aurelia* (1, 2) led us to study the fine structure of this protozoan and the effects of 2,6-diaminopurine on kappa.

P. aurelia, variety 4, stock 51 killer, mating type VII (51-7K), and the identical organism without kappa, stock 51 sensitive (51.7 S), were grown at 27°C. in a volume of timothy hay infusion inoculated with *Aerobacter aerogenes*; this medium supported two fissions per day. Standard procedures were used to fix, embed, and section the paramecia. In brief, concentrated organisms were fixed by the addition of an equal volume of 2 per cent OsO₄ in tap water. After dehydration in graded alcohol, they were embedded in a mixture of 75 per cent *n*-butyl methacrylate and 25 per cent methyl methacrylate polymerized with 2,4-dichlorobenzoyl peroxide for 48 hours, sectioned on a modified Spencer rotary microtome and examined in the electron microscope (RCA model EMU-2B) with intermediate lens and 25 μ objective aperture.

Kappa bodies were identified in the electron microscope by their presence in killers and absence in sensitives, and by a reasonable correspondence of their size and shape to previous observations on kappa in the light microscope.

In transverse section (Fig. 1), the most striking feature of kappa bodies is a variable number of dense concentric toroids about a central circular or ovoid core of less homogeneously dense material. These toroids are usually eccentrically, occasionally centrally situated in an ill-defined matrix of wispy, less dense stringy material. The matrix is enclosed by two dense limiting membranes similar to those enclosing mitochondria in *P. aurelia*. There are, also, occasional irregular aggregations of dense material in this matrix and other elements reminiscent of the microvilli (3) or tubules (4) of

mitochondria. Some of these elements are continuous with the inner of the two limiting membranes (*cf.* reference 3).

In longitudinal section (Fig. 3) the dense lamellae lie parallel on either side of a band of less dense material that is the central circular or ovoid core in cross section. In this plane the lamellae are not continuous at their ends, but occasionally in more oblique sections they appear to be so. In other sections lamellae appear in slightly curved parallel array (Fig. 2).

These observations on the dense lamellae suggest that they consist of toroids concentrically arranged around a solid central cylinder, or of a double spiral consisting of side-by-side dense and less dense lamellae (Swiss roll). Such a structure could well be built up of sub-units joined together side by side as are the protein sub-units of tobacco mosaic virus, forming a series of concentric helices, with the pitch of the helix so small that no periodicity is visible in lamellae in longitudinal section; *i.e.*, in the electron micrographs obtained. The suggestion that cylindrical lamellae are composed of large numbers of sub-units of equal size comes from the observation of the effects on killers of 500 μg./ml. 2,6-diaminopurine; some killers were made sensitives as indicated by susceptibility to test killers; kappa bodies almost doubled in size; and the dense concentric lamellae and central core appeared to be fragmenting into many small, and in the main, regular and equal particles (Fig. 4).

It is probable that this lamellated structure corresponds to the refractile body seen in kappa by phase microscopy (5). In our electron micrographs, as in phase microscopy, this structure seems occasionally to lie free of surrounding matrix and sometimes to be passing through the limiting membranes. The kappa body has been reported to be Feulgen-positive and also positive for arginine, but cytochemical tests on the nature of the refractile body have been inconclusive (5); observations in the ultraviolet do not exclude the presence of a basic nucleoprotein.

Such a concentric lamellated structure is not unique in cytoplasm. The arrangement of the lamellae resembles that seen in the dictyosomes and acroblasts in the male germ cells of the cricket (6), and in the mitochondria of the male germ cells of *Helix* (7). Like arrays of concentric lamellae

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are seen in the first stages of cortical granules of *Arbacia* oocytes (8) and in large round cytoplasmic bodies about the size of mitochondria in the renal proximal and cortical collecting tubules of newborn mice (9). In such mice these lamellated structures may be an alternative form of mitochondria.

Kappa bodies are as a rule randomly distributed throughout the cytoplasm but occasionally are concentrated near the macronucleus. In the light of similarities between the kappa body and mitochondria, it is tempting to speculate that kappa may represent a mitochondrion infected with an alien particle. The observation that killers have a lower cytochrome oxidase activity (10) may mean that there are fewer mitochondria in killers than in sensitives which is consistent with this hypothesis.

On the other hand, the observations that the fine structure of vaccinia virus consists of a central ellipsoidal body surrounded by a number of concentric lamellae (11), and the similarity of the lamellated structures seen in the nuclei of HeLa cells infected with RI-APC virus (12) suggest that it is more probable that kappa is a pure virus. The selective inactivation of kappa by streptomycin, chloromycetin, nitrogen mustard, 2,6-diaminopurine, and other agents encourages this

belief, although this latter argument is weakened by streptomycin inactivation of chloroplasts (13).

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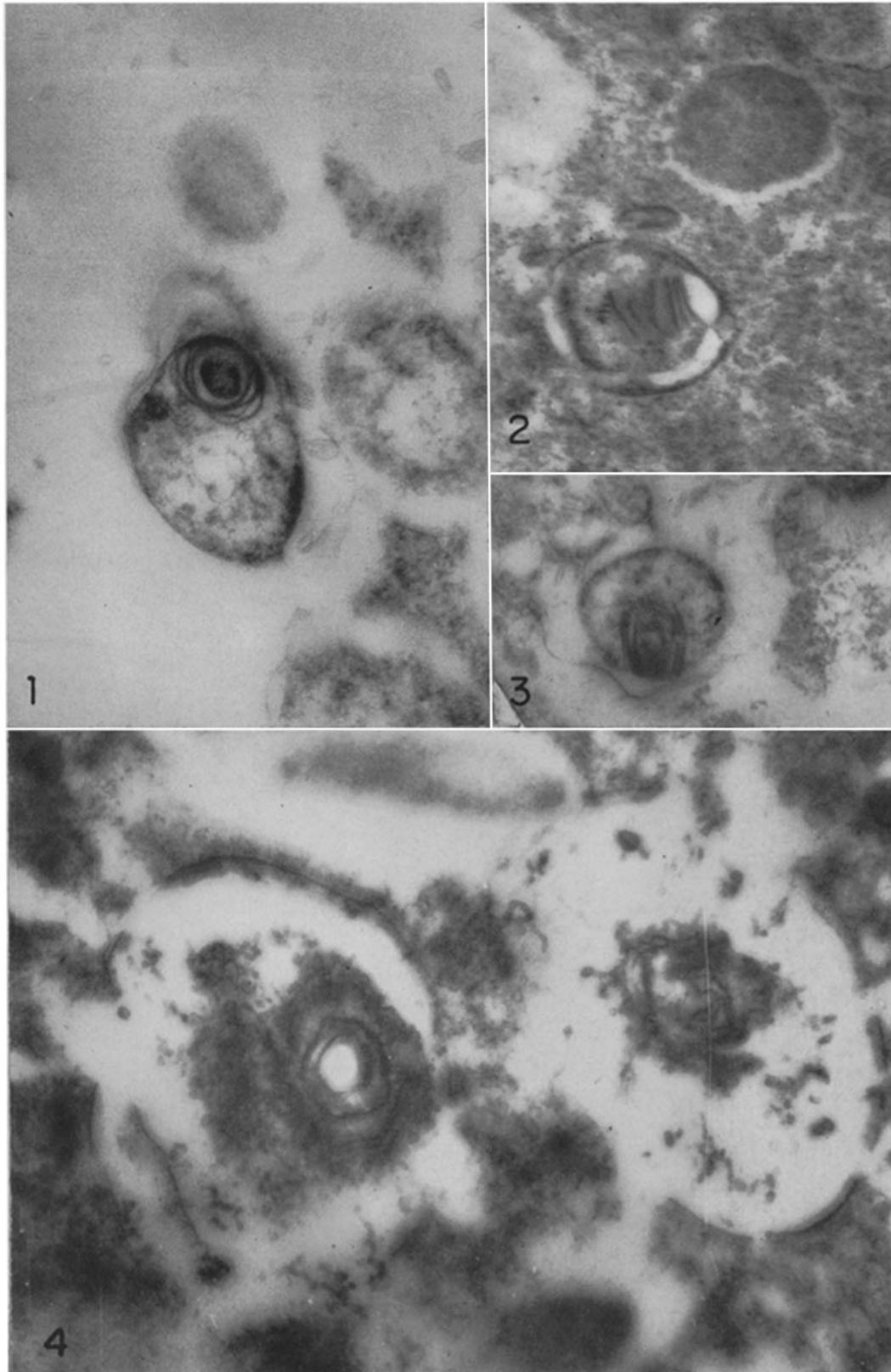
EXPLANATION OF PLATE 58

FIG. 1. Kappa body. Note dense concentric toroids about a central core, wispy less dense stringy material, and evidence of two dense limiting membranes enclosing body. $\times 44,000$.

FIG. 2. Oblique section through a kappa body. The concentric toroids are seen in parallel curved array. Note evidence of double limiting membrane. $\times 33,000$.

FIG. 3. Longitudinal section of kappa in which lamellae lie parallel on either side of band of central core material. In this slightly oblique section the lamellae at one end appear continuous. $\times 33,000$.

FIG. 4. Kappa bodies after treatment with $500 \mu\text{g./ml.}$ 2,6-diaminopurine. Note the doubling in size (some mitochondria in the treated *P. aurelia* are similarly affected, others remain unchanged). The dense concentric toroids are seen disintegrating into many small particles. $\times 33,000$.



(Hamilton and Gettner: Structure of kappa in *Paramecium aurelia*)