This work was for the greater part carried out at the Biochemical Department of the Technological University, Delft, under the supervision of Prof. W. Berends.

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* NAD, NADP, NADH and NADPH represent, respectively, the oxidized nicotinamide adenine dinucleotide, its phosphate and both the reduced stages. ATP is adenosine-5′-triphosphate; AMP is adenosine-5′-monophosphate.

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Deoxyribonucleic Acid Base Composition of Kappa and *Paramecium aurelia*, Stock 51

CERTAIN stocks of *Paramecium* contain cytoplasmic particles called kappa[1]. Kappa-bearing *Paramecia* (killers) are capable of killing other *Paramecia* (sensitives) and are resistant to the toxic agent which they produce[1]. The killer trait requires both the cytoplasmic particles and the dominant *K* gene for the maintenance of kappa, and is one of the most extensively studied examples of nucleo-cytoplasmic relations on a biological level[2–5].

In stock 51, kappa is of two morphological types, the so-called bright particle (which contains a refractile *R* body) and a non-bright particle which lacks this body[3]. The bright particle is associated with its ability to kill sensitive *Paramecia*[4]; the non-bright particle with the ability to convert sensitive paramecia of the proper genotype to killer animals[5]. In different stocks of killer animals, different kinds of killing are exhibited, characteristic of a particular stock; in stock 51, the most extensively investigated killer, the affected animals show an aboral hump before death[1].

The origin and phylogenetic relations of kappa to other organisms, to paramecia, to other cytoplasmic particles of paramecia, and to other DNA-containing particles of other organisms needs to be investigated using as many traits as possible. Since the equivalence of base composition of DNA would seem to be a minimum requirement for extensive base sequence homology and therefore genetic compatibility[6], a knowledge of the average base composition of the DNA of both host and particle might serve as a valuable trait. The present study reports the determination of the base composition of the DNA of kappa, of killer and sensitive *Paramecia*, and of *Aerobacter aerogenes*, used in the cultivation of the *Paramecia*.

The DNA base composition were determined using the cæsium chloride density gradient centrifugation technique. The buoyant density of DNA is linearly related to its mole per cent guanine plus cytosine (G + C) content and requires only 1–2 μg DNA in the purified state or as the cell lysate[6]. The samples were each centrifuged together with *Pseudomonas aeruginosa* ^{15}N DNA (used as a reference of known density) in approximately 7 M cæsium chloride at 44,770 r.p.m. until equilibrium was obtained in the Spinco Model *E* analytical ultracentrifuge (22 h). The banded DNA was photographed using ultra-violet absorption optics and the negatives traced with a Joyce-Loebl microdensitometer. The results are given in Table 1.

Lysates of killer *Paramecia* showed at least three well-defined bands. Each preparation of kappa (purified by the method of Smith[7]), of the strain of *A. aerogenes* used, and of the sensitive *Paramecia* used (isogenic with the killer, but lacking kappa) gave only a single band. Three bands were found in the lysate of the killer organisms at buoyant densities of 1·715, a minor or 'satellite' band at 1·696, and a major band at 1·689 g/c.c. corresponding respectively to the buoyant density of the single bands of DNA of *A. aerogenes*, kappa, and *Paramecia* found when each was run separately. Using a stock 51 *Paramecia* bearing a mutant kappa particle 51_{m43} (ref. 8) the DNA band of the host (1·689 g/c.c.) appeared to be the 'satellite' band with respect to kappa DNA (1·696 g/c.c.). This is not surprising since these particles are known to reach an average concentration of 3,000 per animal.

Table 1. COMPARISON OF MEAN GUANINE–CYTOSINE CONTENT OF DNA OF KAPPA, *Paramecium aurelia* AND *Aerobacter aerogenes* *

	Buoyant density of DNA (g/c.c.)	Percentage G + C	DNA preparation ‡
Killer paramecia †	1·716, 1·696, 1·689	56, 36 29	lysate
A. aerogenes	1·715	56	purified
Kappa	1·696	36	lysate
Sensitive paramecia †	1·689	29	part purified

* The mole per cent guanine plus cytosine content was estimated by density gradient centrifugation of DNA in cæsium chloride, using ^{15}N-labelled *Pseudomonas aeruginosa* DNA as the reference (density, 1·742 g/c.c.).
† Stock 51, killer *Paramecia* were grown on 0·15 per cent infusion of baked lettuce plus 0·1 per cent calcium carbonate inoculated 1 day before use with *A. aerogenes* (ref. 12). The *p*H of the medium was adjusted to a value of 6·5–7·5 prior to use; the sensitive animals were cultured axenically (ref. 13).
‡ Lysates were prepared by suspending 1×10^5 *Paramecia*, or 2×10^8 kappa, in 0·1 M ethylenediamine tetraacetate (EDTA) plus 0·15 M sodium chloride, *p*H 8·0, and adding sodium lauryl sulphate to a final concentration of 3 per cent; DNA was purified by Marmur's method (ref. 14).

On the basis of the mole per cent G + C content predicted from the buoyant density of the DNA, the results indicate that the DNA of the *Paramecia* is sufficiently different from kappa that extensive base sequence homology seems unlikely. It should be pointed out, however, that the presence of unusual bases in the DNA could affect the relation of the buoyant density of the DNA and the base composition[6,9]. The possibility of an odd base has not been excluded.

The base composition of the DNA of the *Paramecia* studied was in agreement with the range of mole per cent G + C contents for related ciliated protozoa (22–35 per cent)[10]. The DNA base composition of kappa, which strains gram negative, is close to certain *PPLO* organisms[6] and the cytoplasmic particle (polar bodies) of the flagellate *Strigomonas oncopelti*[11]. Whether these similar base compositions are fortuitous or indicative of a close taxonomic relation is not known at the present time.

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Effect of Cortisone on Collagen Formation in the Chick Embryo

CORTISONE given to adult animals decreases the amount of alkali-soluble[1] and neutral salt-soluble[2,3] collagen in the skin. In the urinary excretion of hydroxyproline, however, no effect has been found[4,5]. In the chick embryo, a marked increase in the content of free hydroxyproline occurs in the tissues 6–10 days after a single injection of