

## Chemical and Physiological Studies on Paramecin and Kappa<sup>1</sup>

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Paramecin is the end product of the system gene K-Kappa-paramecin which is present in some stocks of *Paramecium aurelia* of varieties 2 and 4. The killer and sensitive characters have been most fully studied in variety 4 of stock 51 by Sonneborn and his associates(2).

When sensitive stocks of variety 4 are exposed to fluid in which the killer stock 51 has grown they develop characteristic morphological changes. A slight hump appears after several hours on the aboral surface near the hind end of the body. This hump enlarges while the anterior end of the body gradually wastes away and the posterior part is pushed into the humped region. The animals then become smaller and finally die. Sensitives can be mated to killers without any evidence of injury, if mating begins soon after the two kinds of paramecia are brought together, if the conjugant pairs are removed to fresh culture fluid soon after they unite, and if the two members of each pair are put into separate culture dishes soon after conjugation has been completed.

The killer phenotype is manifested only when there is present in the cytoplasm a factor designed as "kappa". In the absence of kappa the phenotype is invariably sensitive. The presence of kappa is related to the genetic constitution. Clones of killers always have in the nucleus a dominant gene K, either in homozygous or in heterozygous condition. If the allele k is substituted for gene K, the kappa which is initially present in the cytoplasm soon disappears. Once kappa has disappeared from the cytoplasm it cannot be brought back by restoring gene K. Sensitive stocks, therefore, might have either allele, K, or k, for kappa is not initially producible by any known gene.

The relations between K and kappa are illustrated by the following. When a cross is made between killers and sensitives both of which are homozygous for K, each member of a conjugant pair usually gives rise to a clone of the same character as the parent from which it derives the bulk of its cytoplasm. Normally there is no cytoplasmic exchange between the mates, hence usually the killer conjugant produces a killer clone and the sensitive conjugant produces a sensitive clone. Under certain conditions, however, exchange of cytoplasm does take place between the mates. When this happens, both mates produce killer clones. When cytoplasm is introduced into a sensitive animal containing the

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<sup>1</sup>Contribution No. 459 from the Department of Zoology, Indiana University. Supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research, The National Institute of Health, the Rockefeller Foundation (research on Protozoan genetics) and Indiana University.

This paper was presented as part of a Symposium on Selective Toxicity.

gene K, kappa is maintained and multiplied thereafter in all subsequent vegetative and sexual reproduction. The function of gene K seems to be to control the maintenance and increase of kappa when some is already present, although it cannot start the production of kappa when none is initially present.

Biochemical investigations on the nature of paramecin have shown the following: 1. Paramecin is unstable at each pH ranging from pH 5 to pH 9.5. Paramecin is most stable at a pH 8.5. Even at this pH approximately 15% of its original activity is lost after one hour incubation at 30° (2); 2. Paramecin is inactivated by treatment with pepsin, chymotrypsin and desoxyribonuclease. Its activity is not affected by treatment with ribonuclease. It can therefore be concluded that biological activity of paramecin is associated with a desoxyribonucleo-protein (3). Preer (1) has shown that desoxyribonucleic acid is a component of the cytoplasmic factor kappa. The three components of the gene-kappa-paramecin-system have therefore in common that all contain desoxyribonucleic acid.

Respiratory studies, using the Cartesian diver technique, have revealed that there exist striking and significant differences in the respiration of animals possessing kappa and those which lack this cytoplasmic factor. Comparisons were made between sensitives and killers grown under identical conditions. The first group, stock 29.7, is a sensitive stock, having the recessive genotype. It has no kappa in its cytoplasm and it cannot support the growth of kappa. The 51.7 stock used for comparison has kappa in its cytoplasm and can maintain it due to the presence of the dominant killer gene. The  $Q_{O_2}$  of animals of stock 29.7 was found to be  $0.457 \pm 0.037$   $m\mu l$ /animal-hour, as compared to  $0.871 \pm 0.090$   $m\mu l$ /animal-hour for animals of stock 51.7. However, the possibility that this difference might be due to a stock difference is not excluded, since the two stocks have different genetic backgrounds. A further comparison shows that the sensitive animals which differ from the killer animals only in the absence of kappa in the cytoplasm also have a respiratory rate approximately one half as great as that of the killer animals, the  $Q_{O_2}$  being  $0.510 \pm 0.032$   $m\mu l$ /animal-hour and  $0.899 \pm 0.085$   $m\mu l$ /animal-hour respectively. The respiration of a culture isogenic with 51.7 killers was also compared with the respiration of 51.7 killers. Stock 186.7 was obtained by a series of successive backcrosses of 29.7 sensitives by 51.7 killers, and differs from the killer stocks in the presence of the recessive gene at the killer locus. Again the 51.7 killer stock respire at approximately double the rate of the 186.7 stock, the  $Q_{O_2}$  in this instance being  $0.962 \pm 0.054$   $m\mu l$ /animal-hour, and  $0.546 \pm 0.042$   $m\mu l$ /animal-hour for the two stocks.

To summarize, here we are dealing with a genetic system in *Paramecium aurelia*, that on the one hand produces an antibiotic, and that on the other hand induces an immunity to this antibiotic. Both the production of the antibiotic and the expression of immunity are

under control of a cytoplasmic factor. Whether an animal is immune to the antibiotic paramecin is dependent upon the concentration of the cytoplasmic factor. Sonneborn (1) has shown that the concentration of kappa can be reduced to such an extent that the resulting paramecia are resistant non-killers. If the concentration of kappa is still further reduced the animals become sensitive non-killers.

Selective antibiosis and respiratory rate are both correlated with the cytoplasmic factor. Our observations to date are mostly confined to the overall processes of respiration and do not take into account possible finer differentiations. That these exist is demonstrated by the following: the overall respiration of KK sensitives, 186.7 sensitives and KK killers is differently influenced by azide. The respiration of KK sensitives is inhibited 50% by  $10^{-3.5}$ M azide, that of 186.7 sensitives is not inhibited by the same azide concentration, while the respiration of the KK killers is enhanced by this concentration of azide. Definite different enzymatic sequences must take place in these stocks to account for the difference in behavior towards the inhibitor. It is possible that these different enzymatic reactions direct selective antibiosis. The postulates of comparative biochemistry, stating that all living things utilize essentially the same reactions in their life processes would still be valid. Sensitives and killers would differ because some additional reactions take place in the killers caused by the presence of the cytoplasmic factor. It might perhaps be possible that selective antibiosis is due to the existence of hitherto unsuspected genic and cytoplasmic differences between different stocks.

#### Literature Cited

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