LETTERS TO THE EDITOR

[Brief letters to the Editor that make specific scientific reference to papers published previously in the BIOPHYSICAL JOURNAL *are invited. Receipt of such letters will not be acknowledged but those containing pertinent scientific comments and scientific criticisms will be published.]*

Dear Sir:

In this issue of the *Biophysical Journal* we have described a method of denaturing DNA in the presence of formaldehyde under conditions which, we believe, cause little damage to the denatured polynucleotide strands (1). Using this technique we have obtained two experimental results of sufficient interest to merit brief publication.

1. *Analysis of X-Ray Damage to DNA.* In an earlier paper we have shown that single breaks present in native DNA can be made evident by denaturation in the presence of formaldehyde (2), *i.e.*, interruptions in individual strands which are concealed by the contiguity of the apposed hydrogen-bonded strand are made evident by the sedimentation distribution of the denatured DNA. In this way it was shown that the process of degradation of the acridine orange–DNA complex by visible light resembled the action of DNAase on DNA rather than that of sonic irradiation. Since the former degradation proceeds through single-strand and the latter through double-strand scission it was concluded that visible light produces single-strand breaks in the acridine orange–DNA complex—although an additional double-strand mechanism could not be excluded. We have since found that the denaturation procedure used during those experiments introduced some hydrolytic breaks in the polynucleotide strands, although this finding does not in any way invalidate our conclusions.

Similar experiments have now been conducted on DNA degraded by biological doses of x-rays (several thousand roentgens) under aerobic and anaerobic irradiation. The standard denaturing technique (1) was used. From the change in median sedimentation coefficient on denaturation of the DNA it was concluded that under both aerobic and anaerobic conditions, degradation proceeds by single-strand scission. Furthermore, although under anaerobic conditions a greater dose was required to reduce $s_{20,w}$ to a given value, the decrease in $s_{20,w}$ upon denaturation was the same for samples irradiated with or without oxygen.

2. *UV Inactivation.* Marmur and Grossman (3) have shown that doses of ultraviolet irradiation within the range that inactivates transforming principle introduce interstrand cross-links which prevent strand separation upon the denaturation of hybrid DNA. It was assumed that at least some of these cross-links were responsible for lethal and mutagenic effects and that the cross-link could partially be identified with the thymine dimer which can be isolated from UV-irradiated DNA. It has been shown, however, that the thymine dimer probably is a result of a reaction between adjacent thymines within a single strand (4). Our denaturation procedure clearly can be used to look for cross-links in DNA isolated from irradiated T7 phage, since the coupled strands can be detected as material sedimenting at 65S ahead of the main DNA peak (1).

Ultraviolet irradiated T7 phage was titered for survivors, and then denatured DNA was released from the phage at pH 12 and heated at 70°C in 12 per cent HCHO (1). For a surviving phage fraction of 1 per cent, none of the DNA sedimented at 65S; the boundary was indistinguishable from that of DNA from unirradiated phage. Hence there was neither cross-linking nor strand breakage. At lower survival levels (0.1 per cent),

18 ± 5 per cent of the material sedimented at 65S. At higher doses, cross-linking increased, and strand breakage produced a somewhat spread boundary. At a survival of 10^{-4} per cent probably every DNA-molecule contained both a cross-link and a break.

From these results it is obvious that neither interstrand cross-linking nor strand breakage can account for the lethal effect of ultraviolet radiation on T7 phage.

REFERENCES

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