It was shown by BEER AND ZOBEL[5] that DNA fibrils were not visible in the electron microscope unless either stained with uranyl acetate or shadowed. The unstained fibrils were not visible within the shadow of a polystyrene latex particle although easily seen on either side of it. In general this was found to be the case in the present study. In some unstained preparations DNA molecules could be seen with difficulty, contrast being extremely low (Fig. 2).

Direct evidence for the uptake of cesium ions by DNA was obtained by the use of radioactive CsCl. Collodion films were prepared on pieces of glass 1 × 1 cm, washed with Photoflo and distilled water, then exposed either to *M. lysodeikticus* DNA (1 μg in 0.05 ml ammonium acetate) or to the solvent alone.

The excess liquid was pipetted off after 10 min and replaced with a 1% CsCl solution containing ^{137}Cs (specific activity 0.1 μC/μmole). After a further 30 min the CsCl was removed and the samples washed thoroughly with distilled water and allowed to dry.

The samples exposed to DNA took up radioactivity equivalent to 0.2 μg of Cs. Control samples took up less than 1/20 of this amount. The amount of radioactivity taken up by the DNA, 0.2 μg Cs/μg DNA is a minimum estimate since much of the DNA may not have adhered to the film.

Exposure to CsCl does not appear to destroy the biological activity of DNA, at least in the case of pneumococcal transforming principle[6]. The molecules seen after exposure to CsCl are therefore probably undamaged DNA molecules.

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On the mechanism of ultraviolet-induced mutations

It is generally acknowledged that ultraviolet and ionizing radiations may be mutagenic either by direct action on DNA or through different indirect effects, for instance by producing reactive substances able to interact with DNA or base analogs susceptible to be incorporated into DNA. However, while the ionizing radiations are on the whole non-selective, the irradiation with ultraviolet light is more specific. Thus, ultraviolet radiation is not ionizing, induces relatively few chromosome breaks, and its absorption is limited essentially to conjugated molecules. In fact, the similarity between the

absorption spectrum of DNA and the action spectrum of mutations[1] suggests that it is the absorption of the ultraviolet light by the purine and pyrimidine bases of DNA which may constitute an essential cause of ultraviolet-induced mutations.

As an essential consequence of the absorption of ultraviolet radiations, the purine and pyrimidine bases of DNA are raised into excited states. In these circumstances, a reasonable hypothesis capable to account for the enhancement of mutagenesis by ultraviolet irradiation consists in postulating that the irradiation increases the chances of the events which are responsible for the general occurrence of mutations, in particular for spontaneous mutations. (This point of view has, in fact, been advocated by ALEXANDER AND STACEY[2] as valid for all kinds of mutagenic agents.) Now, following an original suggestion by WATSON AND CRICK[3], developed in recent years in particular by FREESE[4], spontaneous mutations may originate from base-mispairings due to the involvement of rare tautomeric forms of the compounds. Explicit quantum-mechanical calculations have led to the prediction, that the base which from that view-point should be the most readily involved in mutagenesis is cytosine[5,6].

The calculations, based on the Hückel molecular-orbital method[7], have now been extended to the first excited state of the purine and pyrimidine bases (the involvement of the higher excited states being less probable in chemical phenomena because of the rapidity of internal conversion) with the view of investigating the influence of the excitation on the relative tendencies of the compounds to exist in rare tautomeric forms. The essential varying factor responsible for this tendency being (as indicated already in refs. 5 and 7) the relative value of the resonance energies of these bases, these quantities have been evaluated for the first excited state of the molecules and compared with the same quantities determined previously for the ground state. The results are summed up in Table I for the essential tautomeric transformations susceptible to be involved in the mispairing of the bases and thus in mutations.

It is seen from Table I that (1) the gain of resonance energy accompanying the lactam–lactim tautomerization of uracil, thymine and guanine is greater in the first excited state of these molecules than in their ground state, (2) the loss of resonance energy accompanying the amino–imine tautomerization of cytosine and adenine is smaller in the first excited state of these molecules than in their ground state. Consequently, the ease of the tautomeric transformation into the rare form should be greater for all these compounds in their excited states and this situation may account, at least in part, for the enhancing effect of ultraviolet radiations on the rate of mutagenesis.

TABLE I

VARIATION OF RESONANCE ENERGIES (ΔR) UPON TAUTOMERIZATION

The values are given in β-units.

Tautomeric transformation	*Compound*	*ΔR in ground state*	*ΔR in first excited state*
Lactam–Lactim	Uracil	0.22	0.43
	Thymine	0.22	0.40
	Guanine	0.32	0.36
Amino–Imine	Cytosine	—0.13	—0.01
	Adenine	—0.27	—0.14

The same mode of approach may also be used for the evaluation of the influences of some indirect effects of irradiation upon the rate of mutations. Thus, as is well known (see for example ref. 8), one of the most important products of the ultraviolet irradiation of the bases of the nucleic acids are the hydroxy-hydro derivatives of the pyrimidines, in which the elements of water have been fixed at the 5–6 C–C bond of these bases (and the dimer of thymine, in which the 5–6 C–C bond is also saturated). Although the hydration itself is not a mutagenic transformation it represents an example of an ultraviolet-induced abnormal component. Of course, the tautomeric equilibrium may be displaced in these components and if the displacement is in the direction of the rare form, the chances of the miscoupling of bases and thus of mutation will be increased. That such may actually be sometimes the case is indicated by the calculations carried out for the hydro-hydroxy derivative of cytosine, which show that the loss of resonance energy on passing from the amino to the imino form is reduced by about 0.06 β in the hydrated derivative of the compound. On the contrary, the tautomerization of uracil should be more difficult in the hydrated form.

Another structural change produced by irradiation which may also be responsible for the increased mutagenesis is the modification of the electronic charge of the nitrogen atom of the bases which is engaged in their glycosidic linkage. *E.g.* an increase of its net positive charge signifies an increase in the rate of the enzymic or acidic hydrolysis of the linkage[9], a situation which may facilitate the incorporation of a wrong base or the breaking of the chain. Calculations carried out for the distribution of electronic charges in the first excited state of the molecules indicate that such an increase should actually accompany the excitation of the pyrimidines (but not that of the purines). *E.g.* the net positive charge of N-1 of thymine passes from 0.307 *e* in the ground state to 0.487 *e* in the first excited state, and that of cytosine from 0.361 *e* in the ground state to 0.415 *e* in the first excited state.

With the usual restrictions concerning the validity of Hückel-type calculations for the study of specific excited molecular states, the results are illustrative of the numerous ways in which ultraviolet radiations may induce or increase physico-chemical changes leading to mutagenesis.

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