Summary—The treatment of mice with two alkylating agents, i.e. Tris-(2-chlorethyl) amine hydrochloride and 1,6 *bis*(2-chlorethyl-amino) 1,6 deoxy-D-mannit dihydrochloride, leads to profound damage to desoxyribonucleoprotein complex in thymus and spleen, manifested in a distinctly increased sensitivity of DNP to the action of heparin.

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REFERENCES

1. Z. M. BACQ and P. A. ALEXANDER, *Fundamentals of Radiobiology*. Pergamon Press, New York (1961).
2. W. C. J. ROSS, *Biological Alkylating Agents*. Butterworths, London (1962).
3. G. P. WHEELER, *Cancer Res.* **22**, 651 (1962).
4. P. A. ALEXANDER, *Nature, Lond.* **169**, 226 (1952).
5. P. BROOKES and P. D. LAWLEY, *Expl. Cell Res. suppl.* **9**, 521 (1963).
6. G. P. WHEELER, *Fedn Proc.* **26**, 885 (1967).
7. L. A. ELSON, *Radiation and Radiomimetic Chemicals*. Butterworths, London (1963).
8. L. J. COLE and M. E. ELLIS, *Radiat. Res.* **7**, 508 (1957).
9. M. SKALKA, J. MATYÁŠOVÁ and V. CHLUMECKÁ, *Folia biol., Praha* **11**, 113 (1965).
10. M. SKALKA and J. MATYÁŠOVÁ, *Folia biol., Praha* **11**, 378 (1965).
11. J. MATYÁŠOVÁ and M. SKALKA, *Neoplasma* **13**, 433 (1966).
12. M. SKALKA and J. MATYÁŠOVÁ, *Nature, Lond.* **212**, 1253 (1966).
13. M. SKALKA and J. MATYÁŠOVÁ, *Int. J. Radiat. Biol.* **11**, 193 (1966).
14. U. HAGEN, *Nature, Lond.* **187**, 1123 (1960).
15. J. F. SCAIFE, in *The Cell Nucleus—Metabolism and Radiosensitivity*, p. 309. Taylor & Francis, London (1966).

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The non-reactivity of 1,2-fluorenoquinone-2-acetimide with deoxyribonucleic acid and soluble ribonucleic acid*

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IN A STUDY of the relationship of the combination of carcinogens with tissue components to the induction of tumors by these agents, the possibility that 1,2-fluorenoquinone-2-acetimide (FQI) might react with nucleic acids has been examined. FQI, a possible metabolite of the carcinogen 2-acetylaminofluorene, and other *o*-quinone imides combine irreversibly with free amino groups of protein to give stable adducts exhibiting increased absorption in the u.v. region and new absorption maxima in the visible region.[1] In order to see whether similar reactions occur with nucleic acids, soluble RNA and native and heat-denatured DNA were characterized after exposure to FQI under conditions which were shown to give rise to combination of this compound with protein.

DNA from calf thymus (Calbiochem, Los Angeles, Calif.) and soluble RNA from yeast (Calbiochem, Los Angeles, Calif.) were exposed to FQI by adding solutions of the quinone imide in dioxane to buffered solutions of the nucleic acids at room temperature and allowing them to stand overnight at 4°. The nucleic acids were then reisolated from the incubation mixture by successive extractions

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with equal volumes of phenol[2] and ether[3] followed by precipitation with ethanol.[2] The purified material was characterized by ultraviolet and visible spectroscopy and phosphorus analysis.[4] Determination of the base composition of formic acid hydrolysates was accomplished by a paper chromatographic method.[5] Thermal transition profiles of the nucleic acid solutions were obtained by recording the increase in absorbancy as the temperature was increased linearly 1·1°/min. The sedimentation behavior of DNA in sucrose gradients (5–20% sucrose in 0·14 M sodium chloride–0·015 M citrate, pH 7) was determined by centrifuging samples for 16 hr at 22,000 rpm in an SW 25·2 rotor (Spinco Division, Beckman Instruments, Palo Alto, Calif.) at 4°. The distribution of DNA was determined by continuous u.v. spectroscopy of the gradient as it flowed through a recording spectrophotometer.

To serve as a control, nucleic acids which had been incubated with dioxane alone were isolated and characterized as described above. In addition, a second series of control experiments were carried out to demonstrate that the experimental systems afforded favorable conditions for the combination of FQI with macromolecules. FQI in dioxane, or dioxane alone, was added to solutions which contained bovine serum albumin (Pentex, Kankakee, Ill.) in amounts equivalent by weight to the nucleic acid concentrations used in these experiments. The proteins were purified by prolonged dialysis against dioxane:phosphate buffer[1] and ion-exchange chromatography on DEAE-cellulose.[6] Combination of FQI with protein was revealed by comparison of the spectra of FQI- and dioxane-treated proteins. In each system treatment with FQI resulted in the appearance of an absorption maximum near 460–470 mμ and in increased absorption in the ultraviolet region.[1]

Incubation mixtures of FQI with DNA became turbid, as did mixtures of FQI in dioxane and buffer, whereas FQI–albumin mixtures remained clear and showed a characteristic color change.[1] Isolation of FQI-treated DNA by extraction with phenol and ether followed by precipitation with ethanol yielded a white fibrous material. Some properties of a typical preparation are shown in Table 1. No

TABLE 1. PROPERTIES OF DNA AND sRNA EXPOSED TO FQI*

	DNA†		Heat-denatured DNA‡		sRNA§	
	Control	FQI-treated	Control	FQI-treated	Control	FQI-treated
λ_{max} (mμ)	258	258	260	260	258	258
λ_{min} (mμ)	230	230	234	234	230	230
A_{max}/A_{min}	2·19	2·12	1·66	1·64	2·17	2·14
A_{max}/A_{280} mμ	1·82	1·82	1·88	1·86	2·12	2·09
$E_{(P)max}$‖	9300¶	9700¶			5800	5700
Hyperchromicity**	38	38			28	28
Tm††	86	86				
Base composition (mole %)						
G	23·7	23·4	19·4	19·5	26·3	25·1
A	29·2	29·5	29·4	29·6	22·6	23·4
C	20·2	21·5	21·8	22·0	28·9	28·9
U	26·9	25·6	29·3	29·0	22·2	22·7

* FQI in dioxane was added to solutions of nucleic acids in citrate buffer at room temperature. Dioxane alone was added in the control experiments. The nucleic acids were purified and analyzed as described in the text.

† FQI (5 mg) in dioxane (5 ml) was added to DNA (5 mg) in 0·14 M NaCl–0·015 M citrate (pH 7, 20 ml). Spectral properties were determined in the NaCl-citrate buffer.

‡ FQI (5 mg) in dioxane (5 ml) was added to DNA (5) mg in 0·01 M sodium phosphate—0·001 M citrate (pH 8, 20ml) which had been denatured by heating in a boiling water bath for 10 min and rapidly cooled in ice.[2] Spectral properties were determined in 0·1 N NaOH.

§ FQI (8 mg) in dioxane (2 ml) was added to sRNA (8 mg) in 0·14 M NaCl–0·015 M citrate (pH 8, 8 ml). Spectral properties were determined in the NaCl-citrate buffer.

‖ Molar extinction coefficient based on nucleic acid phosphorus.

¶ In this case spectral properties were determined in 0·1 N NaOH in which the A_{max} was 260 mμ.

** Expressed as the percentage increase in extinction as the temperature was increased from 23° to 99°.

†† Temperature at which the hyperchromicity is one-half maximum.

real differences were noted in the spectral properties, thermal transition profiles, base composition, or sedimentation behavior of DNA exposed to FQI and DNA isolated from control experiments. These data indicate that, within the limits of the techniques employed in this work, FQI had neither combined with nor altered DNA. It is improbable that a modified nucleic acid was lost in the process of purification, since recoveries in these experiments of both FQI-treated and control nucleic acids as judged from ultraviolet absorption and phosphorus analysis, were uniformly high (88–94 per cent).

The increase in reactivity toward some reagents, which accompanies the denaturation of DNA by heat,[3, 7] prompted experiments with DNA which had been denatured by heating in solutions of low salt concentration.[2] The pH was raised from 7 to 8 in these experiments, a procedure which was found to increase the combination of quinone imides with protein.[1] DNA isolated from such experiments exhibited no changes in either spectral properties or base composition due to exposure to FQI (Table 1).

Experiments with soluble RNA permitted higher concentrations of nucleic acids to be used and offered additional possibilities for reactive sites. Soluble RNA isolated after exposure to FQI at concentrations of nucleic acid four times greater than those used in the DNA experiments was also unchanged with respect to spectral properties, base composition or thermal transition behavior when compared with control preparations (Table 1).

The experiments reported here have provided no evidence that the *o*-quinone imide FQI combines with or alters nucleic acids. The fact that the methods used in this study have detected the combination of other fluorene compounds with nucleic acids[3, 8] strengthens the conclusion that, in contrast to its ability to react with protein,[1] FQI does not react with or modify DNA or RNA. The reactivity of FQI is, therefore, similar to that of the related *o*-quinone imine,[2] but differs from the reactivities of *N*-2-fluorenylhydroxylamine[8, 9] and *N*-acetoxy-2-acetylaminofluorene,[3, 10] which react with both protein and nucleic acid. The N atom of N-acetoxy-2-acetylaminofluorene has been shown to react with the 8-carbon of guanine[11] and a similar mechanism has been proposed for reaction of N-2-fluorenylhydroxylamine with nucleic acids.[8] Combination of *o*-quinone imides[1] and *o*-quinone imines[6] with nucleophiles, however, is believed to result from activation of the fluorene nucleus. These considerations prompt the suggestion that combination of derivatives of the carcinogen 2-acetylaminofluorene with nucleic acid may be favored by activation of the N atom rather than of the aromatic ring.

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REFERENCES

1. C. C. IRVING and H. R. GUTMANN, *J. biol. Chem.* **234**, 2878 (1959).
2. C. M. KING and E. KRIEK, *Biochim. biophys. Acta* **111**, 147 (1965).
3. E. C. MILLER, U. JUHL and J. A. MILLER, *Science* **153**, 1125 (1966).
4. W. R. MORRISON, *Analyt. Biochem.* **7**, 218 (1964).
5. G. R. WYATT and S. S. COHEN, *Biochem. J.* **55**, 774 (1953).
6. C. M. KING, H. R. GUTMANN and S. F. CHANG, *J. biol. Chem.* **238**, 2199 (1963).
7. M. BEER, S. STERN, D. CARMALT and K. H. MOHLHENRICH, *Biochemistry, N.Y.* **5**, 2283 (1966).
8. E. KRIEK, *Biochem. biophys. Res. Commun.* **20**, 793 (1965).
9. P. H. GRANTHAM, E. K. WEISBURGER and J. H. WEISBURGER, *Biochim. biophys. Acta* **107**, 414 (1965).
10. P. D. LOTLIKAR, J. D. SCRIBNER, J. A. MILLER and E. C. MILLER, *Life Sci.* **5**, 1263 (1966).
11. E. KRIEK, J. A. MILLER, U. JUHL and E. C. MILLER, *Biochemistry, N.Y.* **6**, 177 (1967).

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Effects of triamcinolone on isolated rat-liver mitochondria

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ON REVIEWING effects and actions of steroid hormones, triamcinolone (a synthetic glucocorticoid) seems peculiar in two respects. First, when used for a long term treatment of rheumatoid arthritis or