ysin, since the respective lytic endpoints of 2.1 μg and 1.56 μg for 2-percent and 1-percent red blood cell suspensions were identical in both buffered and nonbuffered saline systems.

The results of these experiments demonstrated that the lipoid is sufficiently different from the lipopolysaccharide as not to be characterized, in the traditional sense, as endotoxin; and that its toxic effects cannot be ascribed to the presence of lipopolysaccharide. However, inasmuch as these two materials appear to be unrelated, the comparable lethal potencies in both Namru and Balb/C mice of the lipoid and lipopolysaccharide, together with certain similarities in their toxic action, are worthy of note. Although these data offer insufficient grounds for the conclusion that the lipid moiety of endotoxin is implicated in its lethal effect on mice, they do demonstrate that bacterial lipids can have significant toxic activity.

M. S. REDFEARN

University of California, Naval Biological Laboratory, Naval Supply Center, Oakland, California 94614

References and Notes

1. O. Westphal and O. Luderitz, *Angew. Chem.* **66**, 407 (1954).
2. M. E. Webster, J. G. Sagin, M. Landy, A. G. Johnson, *J. Immunol.* **74**, 455 (1955); M. Landy, A. G. Johnson, M. E. Webster, J. G. Sagin, *ibid.*, p. 466; E. Ribi, B. H. Hoyer, K. C. Milner, T. D. Perrine, C. L. Larson, G. Goode, *ibid.* **84**, 32 (1960).
3. A. Boivin, I. Mesrobeanu, L. Mesrobeanu, *Compt. Rend. Soc. Biol.* **114**, 307 (1933); W. R. J. Morgan and S. M. Partridge, *Biochem. J.* **34**, 169 (1940); C. G. Freeman, *ibid.* **37**, 601 (1943); W. F. Goebel, F. Binkley, E. Perlman, *J. Exptl. Med.* **81**, 315 (1945); A. Bendich and E. Chargaff, *J. Biol. Chem.* **166**, 283 (1946).
4. A. A. Miles and N. W. Pirie, *Brit. J. Exptl. Pathol.* **20**, 278 (1939).
5. E. Neter, H. Anzai, E. A. Gorzynski, A. Nowotny, O. Westphal, *Proc. Soc. Exptl. Biol. Med.* **103**, 73 (1960); E. Mihich, O. Westphal, O. Luderitz, E. Neter, *ibid.* **107**, 816 (1961); O. Westphal, *Ann. Inst. Pasteur* **98**, 789 (1960).
6. E. Ribi, W. T. Haskins, M. Landy, K. C. Milner. *J. Exptl. Med.* **144**, 647 (1961); E. Ribi, W. T. Haskins, M. Landy, K. C. Milner, *Bacteriol. Rev.* **25**, 427 (1961); W. T. Haskins, M. Landy, K. C. Milner, E. Ribi, *ibid.* **114**, 665 (1961); K. Fukushi, R. L. Anacker, W. T. Haskins, M. Landy, K. C. Milner, E. Ribi, *J. Bacteriol.* **87**, 391 (1964).
7. O. G. Lien, E. Isaeff, C. F. Tedford, *Univ. of California, Naval Biological Laboratory, Res. Stat. Rept. No. 18, App. B, Sect. VI* (1955).
8. G. J. Hildebrand, M. S. Redfearn, Y. Seys, *Proc. Soc. Exptl. Biol. Med.* **113**, 902 (1963).
9. I thank K. B. DeOme, Cancer and Genetics Laboratory, University of California, for the Balb/C mice, and also Mrs. Margaret Johnston for excellent technical assistance. This work was sponsored by the Office of Naval Research and the Bureau of Medicine and Surgery, United States Navy, under a contract between the Office of Naval Research and the Regents of the University of California.

30 April 1964

Proton Tunneling in Radiation-Induced Mutation

Abstract. *The equilibrium proton distribution and tunneling rate in the N–H . . . N hydrogen bond of the guanine-cytosine base pair have been calculated quantum mechanically for the ground state, a charge-transfer excited state, and positive and negative ionic states. These results are consistent with the idea that tautomeric rearrangement can be a cause of radiation induced mutation or carcinogenesis.*

It has long been recognized that the hydrogen bonds connecting the nucleotide bases of the two strands of the DNA molecule are crucially involved in the transfer and retention of genetic information. Under normal conditions, the bonds cause the bases of the two strands to be paired, guanine with cytosine (G–C) and adenine with thymine (A–T), so that the DNA replication process produces duplicates of the genetic coding of the replicating molecules. Improper pairing of the bases during replication would result in alteration of the genetic coding, thereby possibly producing mutations.

Watson and Crick (*1*) have suggested that the genetic coding may be perturbed by the presence of nucleotide bases appearing in the unusual, or "rare," tautomeric form. For example, the rare tautomer of guanine pairs with thymine instead of cytosine. Löwdin (*2*) pointed out that rare tautomers may be produced in pairs by proton rearrangements within the hydrogen bonds connecting a base pair, and that replications involving such rearranged base pairs would perpetuate genetic coding alterations.

We next consider the factors affecting proton rearrangements. The proton in a hydrogen bond is subject to a potential which in ordinary cases has two minima of unequal depth at the normal and tautomeric equilibrium positions, separated by a maximum which acts as a barrier to rearrangement. As indicated by Löwdin, the energy of the protonic motion is ordinarily less than the barrier height, and the proton may get from one minimum to the other by quantum mechanical tunneling. The tunneling process is very sensitive to the shape of the barrier, and particularly to its height and breadth. This suggests that mutagens, and possible carcinogens, may act by affecting the protonic potential, and particularly the barrier.

One way in which the protonic potential can be affected is by forming electronically excited, or ionized, states. Several calculations have been made of the π-electronic structure of the ground and excited states of nucleotide base pairs (*3*, *4*). Rein and Ladik (*4*) found, from an approximate self-consistent field calculation, that a charge-transfer excited state of the G–C base pair seemed relatively favorable for a proton rearrangement. The ground state, and all states considered for the A–T pair, appeared less favorable. Thus, radiation capable of producing this excited state would be expected to have a significant influence on the production of tautomeric base pairs and, thence, mutations. In the absence of radiation, rearrangements would have to take place in the ground electronic state. Löwdin has remarked that in that case the energetically most favorable process may be the simultaneous tunneling of two hydrogen bonds in the G–C base pair.

We have extended the study of the proton rearrangements by making calculations of the protonic potential function by methods which consider both the π-electrons of the bases and the σ-electrons directly involved in the hydrogen bonding. We have obtained approximate self-consistent field solutions for the potential of the N–H . . . N bond of the G–C base pair in its ground state, and several excited states and ionic forms. The excited states are subject to rather severe approximations, but we believe they are nevertheless qualitatively useful. Full descriptions of the quantum mechanical methods and calculations will appear elsewhere (*5*). By certain methods, conveniently summarized by Löwdin (*6*), we have derived from the protonic potential for each electronic state the equilibrium proton distribution among the tautomeric

Table 1. Tunneling time and tautomeric equilibrium constant of the ground, excited, and ionic states of the guanine-cytosine base pair; K is equal to the ratio of C_{rare} to C_{normal} where C is the population number.

State	τ (sec)	K
Ground	2.7×10^{-10}	2.4×10^{-23}
Excited	6.2×10^{-6}	2.3×10^{-5}
Positive ion	3.3×10^{-4}	3.1×10^{-2}
Negative ion	1.2×10^{-6}	8.0×10^{4}

forms, and the tunneling rate. These data are listed in Table 1 for the ground state, the lowest charge-transfer excited state, and the ground states of the anion and cation of the G–C base pair. The tunneling time, τ, is that needed for the departure from tunneling equilibrium to be reduced to $1/e$ of its original value; the equilibrium constant K is the ratio of the populations of the two tautomeric forms.

The data of Table 1 confirm the notion that the probability of an isolated proton rearrangement in the ground electronic state is negligibly small. However, appreciable equilibrium populations of the rare tautomeric form are estimated for both the ionic and excited states, thereby permitting such states to provide opportunities for mutation or carcinogenesis. The effect is most marked for the anion, where the rare tautomeric form is the favored arrangement. However, the cation and excited state both have a sufficiently high rare tautomer population to yield significant mutation rates.

The table also indicates that the proton tunneling is possible from a kinetic point of view. The times required to establish equilibrium are small relative to lifetimes which may be expected for the ions and excited state (which consists of both singlet and triplet spin states whose energy difference was ignored in the present study, and of which at least the triplet would have an appreciable lifetime). Thus, G–C pairs which are not in the ground state of the neutral species at the replication time would have sufficient time to reach the tautomeric equilibrium.

Finally, let us note that the states facilitating proton rearrangements may be reached by the action of radiation in several ways. For example, negative ions could be produced by the capture of free electrons which may be present in irradiated systems. Nonradiative processes, such as donor-acceptor interactions between DNA and chemical mutagens, may also be involved. Such processes might in particular facilitate the formation of the ionic states we have discussed.

ROBERT REIN
FRANK E. HARRIS*
Quantum Chemistry Group, Uppsala University, Uppsala, Sweden

References and Notes

1. J. D. Watson and F. H. C. Crick, *Nature* **171**, 964 (1953).
2. P. O. Löwdin, *Biopolymers Symposia No. 1*, (1964), p. 161; *Rev. Mod. Phys.* **35**, 724 (1963); *Electronic Aspects of Biochemistry* (Academic Press, New York, 1964), p. 167.
3. J. Ladik, *J. Theoret. Biol.* **6**, 201 (1964).
4. R. Rein and J. Ladik, *J. Chem. Phys.* **40**, 2466 (1964).
5. R. Rein and F. Harris, *ibid.*, in press.
6. P. O. Löwdin, Technical Report QB7 from Uppsala Quantum Chemistry Group (1964).
7. We thank Prof. P. O. Löwdin for the opportunity of studying his work prior to its publication, and for his interest in our work. Supported by the USPHS under grant CA-06850 to Uppsala University.
* Permanent address: Department of Chemistry, Stanford University, Stanford, Calif.

23 July 1964

Effects of Polycyclic Aromatic Carcinogens on Viral Replication: Similarity to Actinomycin D

Abstract. *When incorporated into a nutrient overlay, the carcinogenic hydrocarbons benzo*[a]*pyrene and 7,12-dimethylbenz*[a]*anthracene inhibit plaque formation by herpes virus and vaccinia virus, DNA viruses, but not by Sindbis virus, an RNA virus. These carcinogens also decrease herpes and vaccinia virus yields in liquid medium, without affecting Sindbis virus yields. Four structurally related, but noncarcinogenic polycyclic hydrocarbons, namely benzo*[e]*pyrene, pyrene, benz*[a]*anthracene and anthracene, have no inhibitory effect on DNA virus replication. Taken together with the known inhibition of interferon production, these effects on virus growth resemble the action of actinomycin D and hence provide evidence for a selective interaction of these carcinogens with DNA.*

The polycyclic aromatic carcinogens 3-methylcholanthrene, 7,12-dimethylbenz[*a*]anthracene (DMBA), and benzo[*a*]pyrene (BaP) (*1*) inhibit interferon synthesis in tissue cultures of rat cells infected with Sindbis virus (*2*, *3*, and an unpublished observation). Similarly, the antibiotic actinomycin D prevents formation of interferon in cells infected with an RNA virus (*4*). The latter finding suggests that the synthesis of interferon is DNA-dependent and consequently coded by the genome of the cell—not by the virus. Actinomycin D exerts its activity by forming complexes with DNA, thereby arresting the synthesis of DNA-dependent RNA (*5*). This explains why growth of DNA viruses is actinomycin-sensitive, while that of most RNA viruses is not (*5*, *6*). The similarity of effect of actinomycin D and polycyclic carcinogens on interferon synthesis suggested that carcinogens might combine selectively with the DNA of the cellular genome, thus preventing synthesis of interferon, a cellular protein. If this were true, carcinogens, like actinomycin D, should inhibit replication of DNA viruses but not of RNA viruses. This hypothesis was tested by examining the influence of BaP and DMBA on the replication of vaccinia and herpes simplex viruses, DNA viruses, and Sindbis virus, an RNA virus. Structurally related but noncarcinogenic aromatic hydrocarbons were included in the experiment as controls.

The effects of DMBA, benzo[*a*]anthracene (BA), and anthracene, and of BaP, benzo[*e*]pyrene (BeP), and pyrene on plaque formation by vaccinia, herpes simplex, and Sindbis viruses were examined. Both DMBA and BaP are potent carcinogens; BeP, pyrene, and anthracene are not carcinogenic. According to many reports BA is not carcinogenic; according to others, it is a weak carcinogen (*7*, *8*). The compounds were diluted in the culture medium from a stock solution in acetone at 5000 μg/ml; in this way, a very fine suspension in the culture medium was obtained. The plaque assays were carried out as follows. Secondary cultures of rat-embryo cells were grown in plastic petri dishes, 55 or 85 mm in diameter. Before virus inoculation, the growth medium was removed, and 0.9 ml of an appropriate virus dilution was added to each culture. The inoculum fluid was removed after 1 hour, thus removing all unadsorbed virus. A nutrient overlay solidified with starch (*9*) was then added to each culture. This overlay contained the various hydrocarbons at various concentrations (Table 1). The cultures were then kept in an atmosphere of 5 percent CO_2 in air at 36.5°C for 3 to 5 days. Neutral red was added on the morning of the day the plaques were to be counted, 6 to 8 hours before the counts were made. The results of representative experiments are summarized in Table 1.

Plaque formation by the two DNA viruses was significantly inhibited by the carcinogenic compounds, but not by their noncarcinogenic counterparts. Plaque formation by Sindbis virus was unaffected by the carcinogens or by control compounds. Plaque numbers of