REFERENCES

1. KUBITSCHEK, H. E. 1970. *J. Theor. Biol.* **28**:15.
2. KUBITSCHEK, H. E. 1971. *Cell Tissue Kinet.* **4**:113.
3. KUBITSCHEK, H. E. 1968. *Biophys. J.* **8**:792.
4. KOCH, A. L. 1969. *J. Theor. Biol.* **23**:251.
5. SHOWACRE, J. L. 1968. *In* Methods in Cell Physiology. D. M. Prescott, editor. Academic Press, Inc., New York. 147.

H. E. KUBITSCHEK
Division of Biological
and Medical Research
Argonne National Laboratory
Argonne, Illinois 60439

Reactivity of Purine and Pyrimidine Bases toward Singlet Oxygen

Dear Sir:

Despite extensive studies of photodynamic reactions (1) involving nucleic acids and their component bases, there is presently only a limited understanding on the molecular level of the mechanism of this process. This reaction, which requires a dye, visible light, and molecular oxygen, produces appreciable destruction of guanine moieties and, to a smaller extent, thymine moieties in nucleic acids, most probably by some oxidative pathway (2). A large number of efficient sensitized oxidations of organic compounds are initiated by dye sensitization. The active species is presently believed to be an excited form of molecular oxygen, singlet oxygen $O_2(^1\Delta_g)$, formed by energy transfer from an excited molecule of sensitizer (S) to ground state molecular oxygen (3):

$$S + h\nu \rightarrow S^*,$$

$$S^* + O_2(^3\Sigma_g) \rightarrow S + O_2(^1\Sigma_g^+ \text{ and/or } ^1\Delta_g).$$

For this reason it has been suggested that singlet oxygen may be the active intermediate in aerobic photodynamic reactions (4).

F. R. Hallett et al. (5), tested this hypothesis by reacting a large number of purine and pyrimidine compounds with singlet oxygen generated by chemical reaction of NaClO and H_2O_2. A good correspondence between the reactivity of these compounds in photodynamic reactions and reactions with chemically produced singlet oxygen was found; however, interpretation of the results of this study was somewhat complicated because of side reactions between the substrates and chemical species other than singlet oxygen present in the solution, and rigorous pH control could not be accomplished by this method. For instance, the reactivity of different bases toward chemically produced singlet oxygen apparently decreases with the increase in pH, in direct contradiction to the experimental observations in the photodynamic effect.

The purpose of this study was to determine the sensitivity of nucleic acid components towards singlet molecular oxygen generated *externally* by microwave discharge of an oxygen stream (6). This procedure offes the advantage of a cleaner system, free of interferences due to side reactions.

Ultrapure oxygen (Matheson Co., Inc., East Rutherford, N. J.) was mixed with helium and passed along a 13 mm O.D. quartz tube where it was subjected to a stabilized electrodeless microwave discharge produced by a tunable wave guide. The latter was connected to a 2450 MHz Raytheon microwave generator (Raytheon Co., Microwave and Power Tube Div., Waltham, Mass.) equipped with a Bendix power meter (Bendix Corp., Dayton, Ohio). The discharge was operated at about 80 w input power. Such a discharge produces atomic oxygen and electronically excited molecular oxygen in the $^1\Delta_g$ and $^1\Sigma_g^+$ states. A small mercury reservoir was placed before the discharge and mercuric oxide was deposited immediately downstream from the discharge. This and the mercury vapor acted as efficient scavengers of atomic oxygen and ozone (7). Indeed, periodic checks showed the absence of these species.

Oxygen was passed through the discharge at 3–5 torr partial pressure, and the total pressure was built up to 40 torr with helium. The gas emerging from the discharge was bubbled through the reaction solution which was contained in a three-neck flask cooled in an ice bath and provided with a reflux condenser. The distance between the discharge zone and the reactor was 25 cm. Pumping was accomplished using a conventional vacuum pump.

In all experiments, substrate concentrations of about 10^{-4} in different buffers were used. Since collisional deactivation of $O_2(^1\Sigma_g^+)$ is very effective (8), it can be assumed that the gas entering the reaction vessel consisted only of $O_2(^1\Delta_g)$ and ground state $O_2(^3\Sigma_g^+)$. The presence of $O_2(^1\Delta_g)$ in the gas stream was determined by monitoring the emission at 12,700 A with a germanium detector.

The per cent of destruction for a variety of purines and pyrimidines by singlet oxygen was calculated by the loss of absorbance at the λ_{max} (95% confidence limits). Control solutions, exposed to an oxygen stream with the discharge off, showed no spectral changes.

TABLE I

EFFECT OF SINGLET OXYGEN ON PURINE AND PYRIMIDINE BASES*

Base	% destruction		
	Phosphate buffer pH = 6.8	Tris buffer pH = 8.5	Carbonate buffer pH = 10.5
Uric acid	65‡	77‡	83‡
Guanine	21	56§	69‡
Guanosine	23	53§	69‡
Thymine	5	35	60§
Thymidine	3	16	50§
Uracil	4	10	28
Uridine	3	3	5
Theophylline	18	75§	57‡
Theobromine	7	23	58§
Xanthosine	37	48‡	58‡
Hypoxanthine	5	12	17
Inosine	3	3	3
Caffeine	5	16	35
Cytosine	3	3	5
Cytidine	3	3	3
Adenine	3	3	3
Adenosine	3	3	4

* Time of exposure 3 hr unless otherwise specified.
‡ Time of exposure 45 min.
§ Time of exposure 90 min.

The results of the exposure of several purines and pyrimidines and corresponding nucleosides to singlet oxygen at different pH's are summarized in Table I.

As additional evidence that the reaction observed was due to singlet molecular oxygen, the oxidation of guanosine at pH = 6.8 was carried out in the presence of 1,4-diazabicyclo[2.2.2] octane (DABCO), a known singlet oxygen inhibitor (9). In this case, under identical experimental conditions, less than 10% guanosine was destroyed as compared with 23% in the absence of DABCO.

While guanine, thymine, uracil, guanosine, thymidine, uric acid, theobromine, theophylline, xanthosine, and to a smaller extent caffeine and hypoxanthine have been reported to be affected in dye-sensitized oxidation, adenine, cytosine, adenosine, cytidine, uridine, inosine were reported unchanged (2, 10–15). Our results in Table I are in good agreement with these. It also is of interest to compare these results with those reported for the same reaction using singlet oxygen produced chemically by the reaction of H_2O_2 and NaClO (5). With one exception, thymidine, the present results with externally generated $O_2(^1\Delta_g)$ correspond very well with the results obtained in chemical studies.

Guanine breakdown seems to be the basic mechanism involved in biological deactivation by the photodynamic effect, and our experimental study shows that guanine is the most susceptible moiety of nucleic acids when the different components are treated separately with singlet oxygen.

Mechanistic studies of singlet oxygen chemistry have shown that singlet oxygen attack upon an unsaturated compound is electrophilic (3). This suggests that the greater the electron donor ability of the molecule, the better the chances are that singlet oxygen will react with a specific molecule. The energy of the highest occupied molecular orbital (HOMO) is a good measure of the electron donor ability of the molecule. Among the constituents of the nucleic acids, the calculated energy of the HOMO of guanine is lowest (16). This means that guanine should be the best electron donor among these bases and consequently the best substrate for singlet oxygen reaction. Furthermore, the electron donor ability of the guanine-cytosine pair has been calculated to be much higher than that of the adenine-thymine pair (16). These facts may explain the selective destruction of guanine in nucleic acids exposed to the photodynamic effect. A final consideration regards the observed increase in reactivity in photodynamic as well as in singlet oxygen oxidations with the increase in pH. Since at higher pH purine and pyrimidine bases exist in the anion form, which is a more electron-rich species, the electrophilic attack of singlet oxygen is facilitated.

In spite of the good correlation between singlet oxygen and dye-sensitized oxidation of purine and pyrimidine bases, it is premature to conclude that photodynamic oxidation of biological molecules is a simple reaction between "free" singlet oxygen and the substrate. This explanation would be contradicted by such experimental observations as the increased efficiency of acridine orange in sensitized oxidation of tobacco mosaic virus (TMV) RNA in comparison with the oxidation of free bases (17). Most probably the mode of approach of the sensitizer to the oxygen-sensitive residue in the ordered stereochemical structure of nucleic acids plays a critical role.

Received for publication 10 May 1971.

REFERENCES

1. SPIKES, J. D., and R. LIVINGSTON. 1969. *Advan. Radiat. Biol.* **3**:29.
2. SIMON, M. I., and H. VAN VUNAKIS. 1962. *J. Mol. Biol.* **1**:488.
3. GOLLNICK, K. 1968. *Advan. Photochem.* **6**:1.
4. FOOTE, C. S. 1968. *Science (Washington).* **162**:963.

5. HALLETT, F. R., B. P. HALLETT, and W. SNIPES. 1970. *Biophys. J.* **10**:305.
6. WAYNE, R. P. 1969. *Advan. Photochem.* **7**:311 and references therein.
7. BADER, L. W., and E. A. OGRYZLO. 1964. *Discuss. Faraday Soc.* **37**:46.
8. OGRYZLO, E. A. 1968. *Advan. Chem. Ser.* **77**:133.
9. OUANNES, C., and T. WILSON. 1968. *J. Amer. Chem. Soc.* **90**:6527.
10. SIMON, M. I., and H. VAN VUNAKIS. 1964. *Arch. Biochem. Biophys.* **105**:197.
11. WASKELL, L. A., K. S. SASTRY, and M. P. GORDON. 1966. *Biochim. Biophys. Acta.* **129**:49.
12. SASTRY, K. S., and M. P. GORDON. 1966. *Biochim. Biophys. Acta.* **129**:42.
13. FRIEDMAN, P. A. 1968. *Biochim. Biophys. Acta.* **166**:1.
14. SUSSENBACH, J. S., and W. BERENDS. 1965. *Biochim. Biophys. Acta.* **95**:184.
15. ZENDA, K., M. SANEYOSHI, and G. CHIHARA. 1965. *Chem. Pharm. Bull.* **13**:1108.
16. PULLMAN, B., and A. Pullman. 1963. Quantum Biochemistry. Interscience Publishers Ltd., London.
17. SASTRY, K. S., and M. P. GORDON. 1966. *Biochim. Biophys. Acta.* **166**:32.

I. ROSENTHAL
J. N. PITTS, JR.
Department of Chemistry
University of California
Riverside, California 92502

Application of Neural Network Models

Dear Sir:

One can hardly help but be impressed by the elegance and completeness of model neural network studies such as the one described by Harth and Edgar (1967); however, it seems that perhaps the technique of neural modeling could be used in a somewhat more mundane manner to study economically the consequences of some of the hypotheses presented in the interpretation of recent experimental results. In particular, some of the different hypotheses concerning the biochemistry of learning and memory could perhaps be modeled and studied. An example of how this might be done is given here.

First, a basic network would be needed in which to incorporate the model memory system. Roberts (1968) described a rather simple computer neural network model which seems reasonably adequate. Very briefly, the model is a 10×10 array of abstract elements, whose values are 0, 1, or 2, depending on whether the element is inactive, refractory, or active. The program cyclically calculates the state of each element as a function of the element states of the previous cycle and external stimulation. The stimulus felt by any element is proportional to first, the proximity of its active neighbors; second, a factor determined by the postulated memory mechanism; and third, a random stimulus which produces, in effect, a fluctuating threshold. If the calculated stimulus exceeds a preset threshold, the element becomes active in that cycle, refractory in the following cycle, and then can be again active or inactive two cycles later. Network instability (continuous alteration by all elements between the active and refractory state) is avoided by allowing activity to pass only unidirectionally from a row of 10 "input" elements to a row of 10 "output" elements and by use of a certain percentage of inhibitory elements, i.e., elements which contribute a negative stimulus. For more details of the model, see the original description by Roberts (1968).

The next step would be to design a memory storage system which would model some of the