

TABLE 3

		Equiactive molar ratios
Acetylcholine bromide	$[(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{OCCH}_3]\text{Br}^-$	1
β -Carbomethoxyethyltrimethylammonium bromide	$[(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{COCCH}_3]\text{Br}^-$	15
Betaine ethyl ester chloride	$[(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{COCCH}_2\text{CH}_3]\text{Cl}^-$	660
4-Methylamyltrimethylammonium bromide	$[(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)]\text{Br}^-$	1,370
4-Acetoxyamyltrimethylammonium iodide	$[(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)\text{OCCH}_3]\text{I}^-$	7,500

likely that the shifts in position of the carbonyl group and adjacent oxygen are, in part, responsible for the decreased activity of the β -carbomethoxyethyltrimethylammonium ion and the betaine ethyl ester.

This brief summary of results that we have obtained, using the isolated heart of *V. mercenaria* as a test object, supports the view of Pfeiffer that an ether oxygen and carbonyl group, spatially disposed as in acetylcholine, are of special significance in determining the activity of acetylcholine analogues. However, in agreement with Ing, we would emphasize the importance of exactness of "fit," or "dovetailing," between all parts of the drug molecule and structures in the receptor molecule. It has been suggested earlier (?) that acetylcholine may act as a coenzyme for an enzyme that plays a role in the regulation of membrane polarity and permeability. This

further evidence of the significance of molecular configuration in the interaction of acetylcholine analogues and receptor substance is not inconsistent with the hypothesis.

References

1. ALLES, G. A., and KNOEFEL, P. K. *Univ. Calif. Pub. Pharmacol.*, **1**, 187 (1939).
2. BARNES, T. C., and BEUTNER, R. *Science*, **110**, 511 (1949).
3. HOLTON, P., and ING, H. R. *Brit. J. Pharmacol.*, **4**, 190 (1949).
4. ING, H. R. *Science*, **109**, 264 (1949).
5. PFEIFFER, C. C. *Science*, **107**, 94 (1948).
6. RAVENTÓS, J. *Quart. J. Exp. Physiol.*, **26**, 361 (1937).
7. WELSH, J. H. *Bull. Johns Hopkins Hosp.*, **83**, 568 (1948).
8. WELSH, J. H., and TAUB, R. *Biol. Bull.*, **95**, 346 (1948).
9. ———. *J. Pharmacol. Exp. Therap.*, **99**, 334 (1950).

The Photochemical Action of Ultraviolet Light on the Absorption Spectra of Nucleic Acid and Related Substances¹

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With the greater availability of materials capable of producing ionizing radiations, there has been an increase of interest in factors involved in the production of lethal effects in microorganisms and also of mutational changes in various types of living organisms. In view of the known cytochemical importance of nucleic acids, as well as their postulated significance, not only in the chromosomes but also in the cytoplasm, we have carried out experiments on the photochemical effect of ultraviolet radiation on nucleic acids and related substances, in the region of their major absorption, namely, about 260 m μ .

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Previous reports of investigations on spectral changes in nucleic acids produced by ultraviolet radiations have been conflicting. Heyroth and Loofbourow (2), using a photographic colorimetric method, showed a reduction in the selective absorption of deoxyribonucleic acid (DNA), dichloromethyl pyrimidine, uracil, and adenine. Caspersson (1) noted similar effects upon DNA, adenine, guanine, and sodium urate and stated that the effect was least in the nucleic acid, whose "constitution seemed to stabilize the bonds." Hollaender (3) observed no change in the absorption spectrum of a .5% solution of sodium thymonucleate after irradiation for 83 hr. On the other hand, Uber and Verbrugge (8), irradiating a $12.5 \times 10^{-5}M$ solution of the pyrimidine component of thiamine for 2 hr, noted complete loss of selective absorption. More recently, Sinsheimer and Hastings (7) found under conditions of their experiment a 63% loss in the maximum absorption of uracil and uridine solution (6.2 $\mu\text{g}/\text{ml}$) following 16 hr of irradiation at pH 7.0, which was largely reversible by changing the pH to 1.0. Preliminary studies by them indicated similar reversibility for cytidylic acid. They stated, however, that thymine, cytosine, adenine, guanine, adenylic acid, and guanylic acid are not decomposed under the same conditions. Studying tobacco mosaic virus, Oster and McLaren (6) irradiated for 21 hr a .3% solution of

nucleic acid derived from the virus and observed a 10% increase in absorption, which they reconciled with the fact that enzymatic degradation of nucleic acid is accompanied by an increase in absorption at 260 m μ (4, 5). Oster and McLaren also irradiated a dilute solution of virus nucleic acid (8×10^{-5} g/ml) for 3 min and observed a similar increase in absorption.

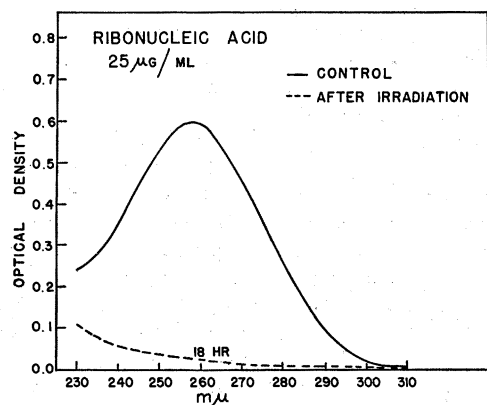


FIG. 1.

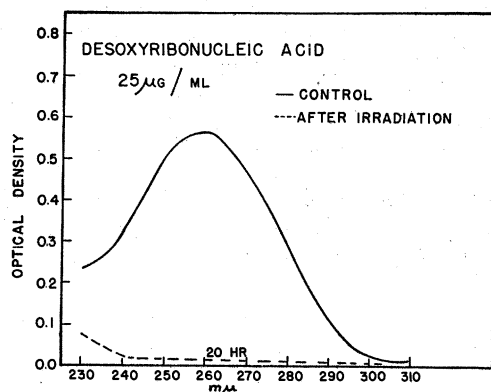


FIG. 2.

It seems to us that these conflicting findings were probably the result of differences in the conditions of experimentation, and we have investigated the possibility, with suitable dilutions and dosages, of observing consistently the complete disruption of the rings in all purine and pyrimidine compounds. We have been able to observe such effects in all substances studied.

We have irradiated sodium ribonucleate, sodium desoxyribonucleate, adenosine triphosphate, 3-adenylic acid, adenosine, adenine, xanthine, hypoxanthine, uric acid, guanine, uracil, and caffeine. The concentration of the nucleate solutions was 25 μ g/ml, except where noted. All other solutions were 5×10^{-5} M. In early experiments, the solutions were titrated almost to neutrality but were unbuffered. Later, experiments like those illustrated were buffered at pH 7.2 with phosphate. Irradiation was carried out with a low-intensity mercury discharge lamp (GE 15-w germicidal lamp), approximately 90% of whose radiation was at 253.7 m μ . About 8 ml of solutions were placed in 150-mm long, 10-mm out-

side diameter quartz and Pyrex tubes (for test and controls), which were fastened to the lamp along its longitudinal axis, thus permitting maximum intensity of irradiation. The volume of solution used did not quite fill the tubes, so that by gentle longitudinal oscillation the gas bubble stirred the contents of the tube, thus insuring complete exposure to the radiation. (In most experiments, the gas was air, but some experiments were carried out in an atmosphere of nitrogen.) It was found that the temperature of the solutions exposed to radiations (both test and control) quickly reached about 36° C, where it remained constant. The absorption spectra were determined with a Beckman spectrophotometer between 230 m μ and 310 m μ .

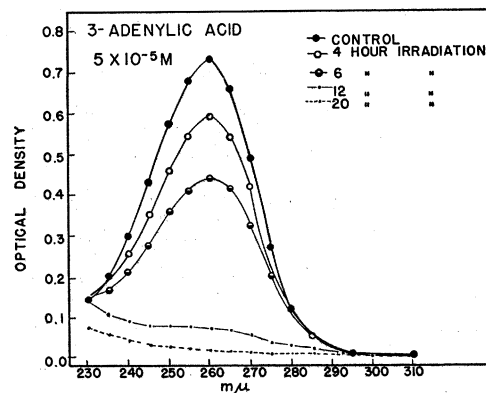


FIG. 3.

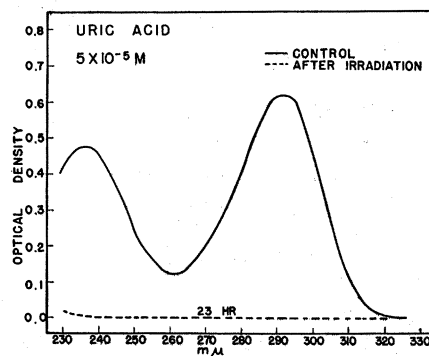


FIG. 4.

Our results indicate that in all cases irradiation for 12 hr or more of dilute solutions (concentrations suitable for spectrophotometric readings) disrupts the pyrimidine structure completely, so that the characteristic absorption spectra are lost. The effects on the absorption curves of a few of the substances studied are shown in Figs. 1-4. In these figures, the spectra of solutions before irradiation are not shown, since they practically coincide with those obtained in the control Pyrex tubes. We repeated Hollaender's experiment with .5% desoxyribonucleate and, like him, observed very little change in the absorption spectrum. However, when we irradiated a dilute solution, there was, as in the case of ribonucleate, complete abolition of ultraviolet absorption, as will be seen in Fig. 2. In other words, the failure to demonstrate an

effect in concentrated solutions appears to be due to masking by unchanged material.

Fig. 3 shows the time course for one of our tested substances, adenylic acid. Exposure to ultraviolet light for 4 hr results in approximately 20% reduction in absorption at 260 m μ , whereas for 6 hr the reduction is approximately 40%. After 12 hr exposure, the selective absorption spectrum disappears. Uric acid, which has two peaks, one at 235 m μ and the other at 290 m μ , also behaves as do all the other nucleic acid derivatives. Complete obliteration of its absorption curve is observed in Fig. 4.

The curves showing the effect on the other substances studied are omitted for lack of space. It suffices to say that in all cases the curves are essentially similar and reproducible.

References

1. CASPERSSON, T. *Skand. Arch. Physiol.*, 1936, **73**, Suppl. 8, 1.
2. HEYROTH, F. F., and LOOFBOUROW, J. R. *J. Am. Chem. Soc.*, 1931, **53**, 3441.
3. HOLLAENDER, A. *J. Nat. Cancer Inst.*, 1941, **2**, 23.
4. KUNITZ, M. *J. biol. Chem.*, 1946, **164**, 563.
5. OSTER, G., and GRIMSSON, H. *Arch. Biochem.*, 1949, **24**, 119.
6. OSTER, G., and McLAREN, A. D. *J. gen. Physiol.*, 1950, **33**, 215.
7. SINSHEIMER, R. L., and HASTINGS, R. *Science*, 1949, **110**, 525.
8. UBER, F. M., and VERBRUGGE, F. *J. biol. Chem.*, 1940, **134**, 273.

Contact Potentials of Evaporated Iron Films in Air and in Nitrogen at Low Pressure

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Curves showing the variation of contact potential with time for metal surfaces prepared by the evaporation process have been obtained *in vacuo* at room temperature using the method of Zisman (1). A platinum plate is used as the standard of reference.

The change in potential of an iron surface amounts to several tenths of a volt and is due largely to the sorption of oxygen. The rate is pressure-sensitive between 0.01 and 10 μ , being very low and very high, respectively. The curves at 0.1 μ are characterized by a sharp rise, followed by a rounded peak, which falls to a level about 0.3 v below the maximum.

From between 0.01 μ and 0.1 μ to the higher pressures, the potential change is reversible upon alternating the pressure from high to low values. The trend of the variations indicates that irreversible sorption is continually taking place, also, and at a rate that is more or less dependent on the average pressure. The desorption rate is lower than the sorption rate.

When pure, dry nitrogen is introduced into the vacuum chamber, no appreciable change in surface potential with

time is observed, and pulsing of the pressure does not produce corresponding alternation in potential. This is assumed to indicate that most of the observed change is caused by oxygen.

Electrical resistance variations of similar evaporated films in the same vacuum range show that gross penetration of the oxygen and its combination with the iron are negligibly slow in comparison with the surface sorption effects, because the resistance increases at a negligible rate at pressures of 0.1 μ , whereas the surface potential curve reaches its maximum within a minute. A slight reduction in resistance of the iron films occurs within a few seconds after they are deposited and may be due to atomic rearrangement or to a drop in temperature.

Exposure to pressures of 10–100 μ resulted in slow and probably incomplete resistance changes, which are not reversible with reduction in pressure. At atmospheric pressure and humidity, evaporated iron layers up to 30 Å in thickness show little change in resistance after 30 min, but the resistance gradually increases over a period of hours at a rate depending on the thickness of the film.

In connection with these studies, experiments have been conducted in which evaporated metal films were produced within an electron microscope column. Electron diffraction patterns of these films were observed continuously from the time of deposition. It was possible to follow the formation of an oxide pattern over that of the metal as a function of time and pressure. For example, the first perceptible change at a pressure of 27 μ requires 1 1/2 min for iron, whereas 27 min were required for nickel.

Studies of the type described for iron have been made on a number of other metals, with results which vary considerably from metal to metal. Details will shortly be available.

Reference

1. ZISMAN, W. A. *Rev. sci. Instruments*, 1932, **3**, 367.

Improved Technique for Weighing Tissues with the Cartesian Diver

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In the course of experiments on respiration of embryonic chick hearts using the Cartesian diver technique, it became necessary to weigh the hearts. Ordinary methods with the analytic balance proved impractical. Zeuthen (1) describes a method for weighing tissues and cells in the Cartesian diver and presents equation (1) for calculation of weights:

$$RW_x = RW_{st} \left(\frac{1 - \frac{B}{B - p_x}}{1 - \frac{B}{B - p_{st}}} \right) \quad (1)$$

In this equation RW_x is the buoyed weight of the tissue, RW_{st} the buoyed weight of the standard, B is atmos-