of these solutions was confirmed with the aid of a spectrophotometer. Conductance measurements on these mixtures were made in order to study the nature of this important reaction.

In Table I are tabulated the conductance results for the various quaternary salts employed in the effective conductometric titrations of a given aliquot of disodium bromophenolate blue solution. The concentration of the disodium salt of brom phenol blue was held constant at 1.5×10^{-5} M in all measurements. C refers to the total normality of the quaternary salt added. $\bar{L}_s$ is the measured specific conductivity of the solution minus the conductivity of the water. R_2D refers to the "ion-pair" compound formed between quaternary cations and divalent dye anions.

If the following metathesis occurs: 1.5×10^{-5} M $\overset{+}{Na_2}\overset{=}{D}$ + C molar $\overset{+}{R}\overset{-}{X}$ yields 1.5×10^{-5} M R_2D + 3.0×10^{-5} M $\overset{+}{Na}\overset{-}{X}$, then, $\bar{L}_s = \bar{L}_{R_2D} + \bar{L}_{NaX} + \bar{L}_{\text{excess RX}}$ assuming Kohlrausch's law of independent ion migration is applicable here. The experimental values of $\bar{L}_{R_2D}$, labelled $(\bar{L}_{R_2D})_{exp.}$, calculated on the above basis, are seen to be zero or negligible in all the cases reported. This fact substantiates the validity of the above assumption and is taken as proof of the complete formation of non-conducting ion-pairs, R_2D, in the most dilute aqueous solutions. The $\Delta\bar{L}_s/\Delta C$ *vs.* C data indicates that some of the quaternary salts show deviations from linear conductance relationships, but no critical micelle phenomenon is warranted on the basis of these results alone. The conductance results do not differentiate between unassociated R_2D and possible non-conducting associated micelle forms of the ion-pair compound.

$\bar{L}_{NaX}$ values from recognized sources[5] and Tartar's conductance values,[6] were used in calculating the experimental values of $\bar{L}_{R_2D}$ from the measured $\bar{L}_s$ values. By interpolation and extrapolation from Tartar's results on the lauryl and cetyl quaternaries, $\Lambda_{C_{14}}$, $\Lambda_{C_{16}}$ and $\Lambda_{C_{18}}$ were approximated as 20, 19 and 18. The small uncertainty in the absolute values used for Λ_{R^+} is not very significant in establishing the phenomena of ion-pair formation in these dilute aqueous solutions.

Experimental

All quaternary salts used in the conductance measurements were recrystallized three times from ethanol–benzene mixtures. The *n*-cetyltrimethylammonium bromide was obtained initially as J. T. Baker's C. P. compound. The corresponding chloride and nitrate were prepared by metathesis of the appropriate silver salt with *n*-cetyltrimethylammonium bromide in absolute methanol. *n*-Myristyl- and *n*-octadecyltrimethylammonium bromides were prepared from Eastman Kodak Co. White Label *n*-myristyl and *n*-octadecyl bromides by refluxing with a slight excess of alcoholic trimethylamine solution until a test portion showed complete solubility in water. The *n*-laurylpyridinium chloride was prepared similarly with Eastman Kodak Co. White Label *n*-lauryl chloride and alcoholic pyridine. This reaction was catalyzed by adding a few crystals of potassium iodide.

Hyamine 1622 (Röhm and Haas Co.) is 100% active *p*-tertiary octylphenoxyethoxyethyldimethylbenzylammonium chloride.

The stock solution of disodium salt of brom phenol blue was prepared from National Aniline Co. Reagent Grade brom phenol blue and C. P. sodium hydroxide.

Conductivities were measured at 1000 cycles with a Dyke–Jones bridge (Leeds and Northrup Co.) and an oscilloscope detector. A pyrex Erlenmeyer type conductance cell,[7] with a cell constant equal to 0.0552, was maintained at 25.00 ± 0.01° in an oil thermostat. The specific conductivity of the water, obtained by distillation through a Barnstead block tin still, varied only between 0.9 and 1.0×10^{-6} ohm^{-1} cm.$^{-1}$.

Each concentration measured was prepared in a seasoned pyrex volumetric flask by adding volume aliquots of standard dye and quaternary solutions which were stored in Jena glass bottles. Then, to minimize adsorption errors, the cell was rinsed with several portions of each dye–quaternary concentration before recording the equilbrium conductance value.

(5) (a) Harned and Owen, "Physical Chemistry of Electrolytic Solutions," Reinhold Publishing Co., New York, N. Y., 1943; (b) MacDougall, "Physical Chemistry," Macmillan Co., New York, N. Y., 1936, pp. 475–479.

(6) Tartar, THIS JOURNAL, **65**, 692 (1943).

(7) Fuoss and Kraus, THIS JOURNAL, **55**, 21 (1933).

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The Decomposition of Diphosphopyridinenucleotide (DPN) and Adenosinetriphosphate (ATP) by Ultraviolet Light

BY C. E. CARTER[1]

The inactivation of cozymase (DPN) of horse red blood cells by ultraviolet light was first reported by Runnstrom, Lennerstrand and Borei.[1a] A study of the products of this photochemical reaction has not been made, although Runnstrom, *et al.*, reported that, while cozymase activity of red cells irradiated with ultraviolet light was lost, cophosphorylase activity remained, indicating that the adenylic acid moiety of the molecule was unaltered. Resolution of the products of the reactions of DPN and ATP with ultraviolet light by techniques of partition paper chromatography is reported in this paper.

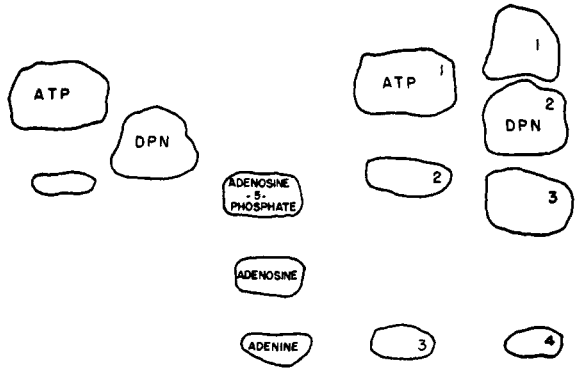
Materials and Methods

Adenosinetriphosphate (ATP) as the free acid and diphosphopyridinenucleotide (DPN) were purchased from the Schwarz Laboratories, New York. ATP when chromatographed showed one principal component and a small amount of a second component, having the distribution value (RF value) and spectrum of adenosine-5-phosphoric acid, which accounted for 12% of the total 260 mμ absorption of the mixture. DPN was homogenous when chromatographed but assayed only 38% pure by the hydrosulfite reduction procedure of Lepage.[2]

(1) Biology Division, Oak Ridge National Laboratory, operated by Carbide and Carbon Chemicals Corporation under Contract No. W-7405-eng-26 for the Atomic Energy Commission, Oak Ridge, Tennessee.

(1a) J. Runnstrom, A. Lennerstrand and H. Borei, *Biochem. Z.*, **271**, 15 (1934).

(2) G. A. Lepage, *J. Biol. Chem.*, **168**, 623 (1947).



A Chromatogram of standard solutions
B Chromatogram of products of decomposition of ATP and DPN by ultraviolet light (six hours exposure)

Fig. 1.—The products of the ultraviolet decomposition of ATP (3 mg./0.5 ml. H_2O) and DPN (3 mg./0.5 ml. H_2O), resolved by paper chromatography, compared with standard solutions of these compounds and adenylic acid, adenosine and adenine, the latter compounds employed in a concentration of 2 mg./ml. Aliquots of these solutions (0.02-cc.) were dried on the paper and chromatographed. Ascending chromatography was employed and the following *RF* values obtained: ATP, 0.83; DPN, 0.76; muscle adenylic acid, 0.71; adenosine, 0.65; adenine, 0.58. Solvent front 30.7 cm. 5% KH_2PO_4–isoamyl alcohol.

The techniques of paper chromatography of nucleotides were those previously described.[3] This method involves the localization of components on the chromatogram by transmitted fluorescent light from a Mineralight fluorescent lamp,[4] followed by elution of the compound from the paper and subsequent determination in a spectrophotometer. The chromatograms were developed by capillarity in a two-phase system consisting of isoamyl alcohol and 5% potassium dihydrogen phosphate contained in a glass vessel of sufficient dimensions so that both phases existed as shallow layers (about 1 cm.). The solution of compounds to be chromatographed was put on the filter paper strip (Whatman No. 1) about 1 cm. above the solvent line, dried and the strip then suspended from a glass rack in the top of an 18-inch glass cylinder made air-tight by closing with a desiccator lid. The rack was adjusted so that the lower end of the strip passed through both solvent phases. The chromatograms were developed for about fifteen hours and then dried in air. Mixtures of components between 5 and 100 μg. in amount each are well resolved by this technique.[3]

A high energy ultraviolet light source was constructed using 8–15 v. G. E. germicidal lamps with 95% of the ultraviolet emission shorter than 300 mμ.[5] The energy output of this source was too high to measure directly with conventional instruments. To achieve high enough concentrations of reaction products for chromatography, solutions of DPN and ATP between 0.3 and 1.0% in concentration were employed. More dilute solutions of these compounds would undoubtedly be similarly degraded by lower energy ultraviolet light sources. Solutions were exposed in quartz test-tubes which were cooled by a fan and slowly rotated by an attachment to an electric motor. Control solutions showed no spontaneous decomposition during the exposure periods employed.

Results

The distribution of ATP, DPN, adenosine-5-

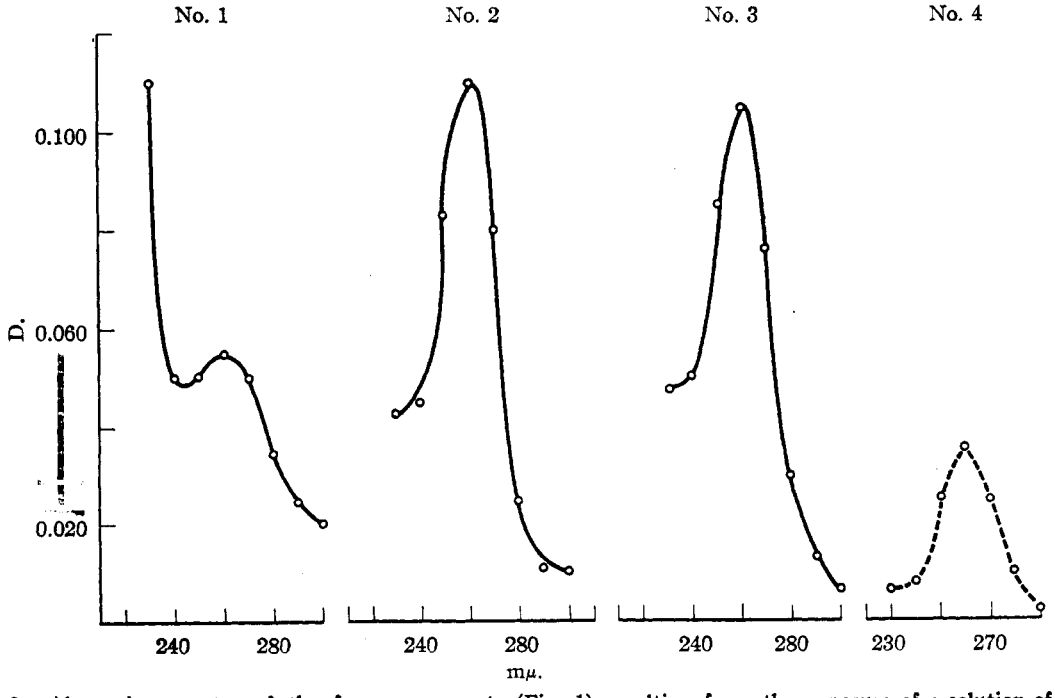
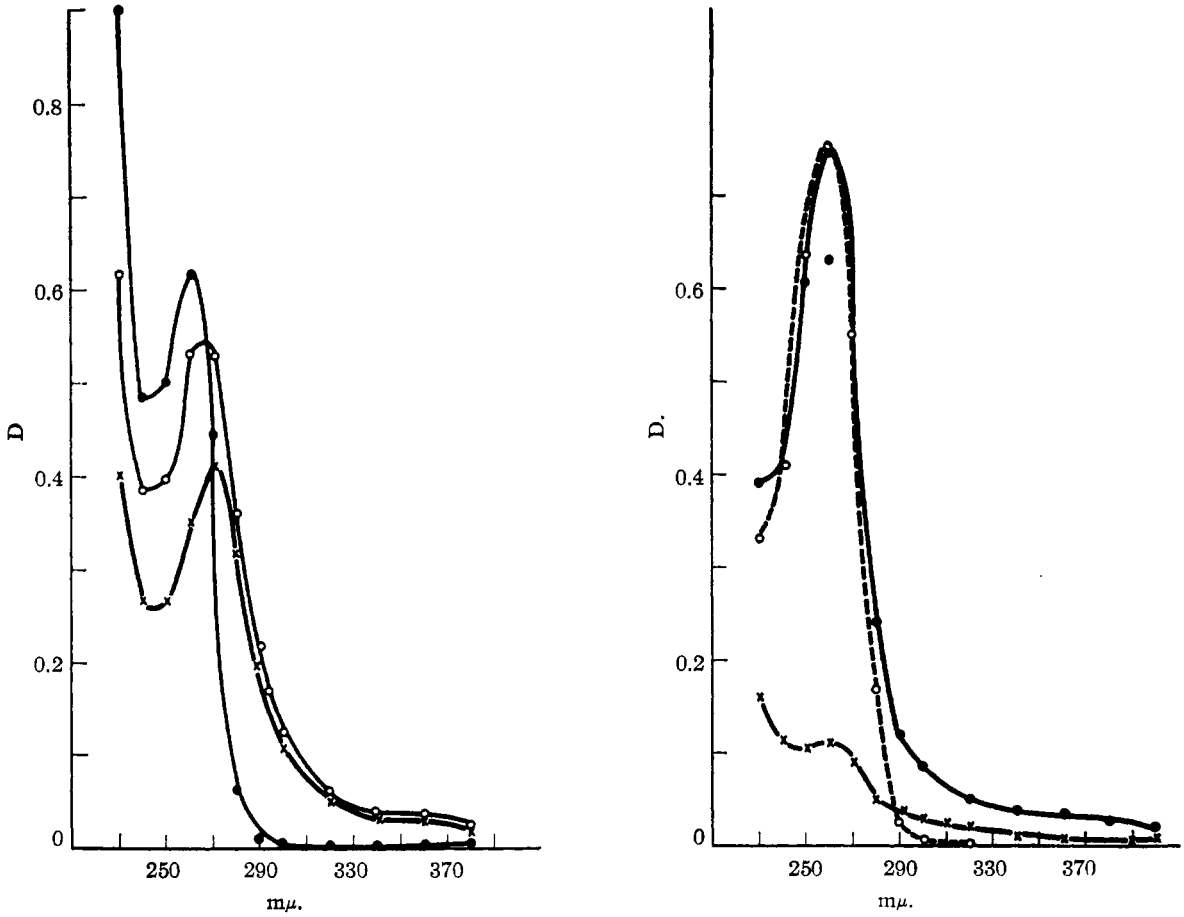


Fig. 2.—Absorption spectra of the four components (Fig. 1) resulting from the exposure of a solution of DPN (3 mg./0.5 H_2O) to ultraviolet radiation. Each spot was cut out of the chromatogram and eluted with 5 ml. of water, except the adenine spot which was eluted in 1 *N* hydrochloric acid.

(3) C. E. Carter, THIS JOURNAL, 72, 1466 (1950).

(4) This lamp is available through Fisher Scientific Company.

(5) F. W. Oliphant and A. Hollaender, *Public Health Reports*, 61, 598 (1946).



●, Nicotinamide, 30γ/cc.; O, after 1 hr. ultraviolet radiation; ×, after 2 hr. ultraviolet radiation.

O - - - O, CO-1,50 γ/cc.; ●—●, after 1 hr. ultraviolet irradiation; ×—×, after 14 hr. ultraviolet irradiation.

Fig. 3.—The effect of ultraviolet radiation on the absorption spectra of solutions of nicotinamide and DPN.

phosphoric acid, adenosine and adenine on the one-dimensional chromatogram are shown in Fig. 1a and the products of the reaction of ATP and DPN with ultraviolet light are shown in Fig. 1b. When the products of the ultraviolet degradation of ATP were quantitatively determined it was found that there was a 40% decrease in the 2600-Å. absorption of the ATP spot, that the concentration of adenosine-5-phosphoric originally present as a contaminant in the ATP solution had not changed, and that the adenine component which resulted from the reaction with ultraviolet light accounted for 21% of the original 2600-Å. absorption of the ATP solution. Similar experiments with muscle and yeast adenylic acid and adenosine showed that solutions of these compounds were not degraded by ultraviolet light in exposure periods up to ten hours in the apparatus described.

The products of the reaction of ultraviolet radiation with a solution of DPN were each dissolved in water from the chromatogram illustrated in Fig. 1 and their absorption determined in the ultraviolet spectrophotometer. As shown in Fig. 2, component no. 2, which has the *RF* value of DPN and also exhibits a spectrum characteristic of this compound, is apparently unaltered DPN. This component accounts for 26% of the original 260-mμ absorption of DPN solution. Since component no. 4 has the *RF* value and spectrum of adenine, it appears that the adenosine pyrophosphate moiety of DPN is degraded by ultraviolet radiation in a manner similar to that of the ATP reaction. The absorption spectrum of component no. 1 is that of nicotinamide but this datum does not exclude a nucleoside linkage in the compound. In the absence of an authentic sample of the nicotinamide nucleoside or nucleotide, this component is tentatively identified only as a pyridinium compound. Both components no. 1 and no. 3 exhibit whitish fluorescence on the chromatogram, a characteristic of dihydropyridine compounds.[6] Although solutions of DPN following exposure to ultraviolet radiation show increased absorption in the 290–390 mμ region (Fig. 3), they do not exhibit the 340-mμ maxima, characteristic of reduced DPN. It was also found that a 1% solution of

(6) C. Oppenheimer and K. G. Stern, "Biological Oxidations," Interscience Publishers, New York, N. Y., 1939.

DPN, following one and one-half hours exposure in the ultraviolet apparatus employed, showed an 18% decrease in 340 mμ absorption when assayed by the hydrosulfite method described by Lepage.[2] The data in Fig. 3 indicate that the spectral changes in solutions of DPN exposed to ultraviolet radiation are to a large extent accounted for by reactions of the nicotinamide moiety of the molecule. Component no. 1 (Fig. 2), which exhibits the nicotinamide spectrum and a white fluorescence, is probably a photochemically altered pyridinium compound split from the DPN molecule whereas component no. 3, which has the absorption spectrum of DPN, is believed to be DPN with a photochemically altered nicotinamide moiety.

Ultraviolet radiation of solutions of DPN did not liberate inorganic phosphorus from the molecule, whereas in the experiment illustrated in Fig. 1, 10 μg. of inorganic phosphorus per mg. of ATP was formed in the course of a six-hour exposure to ultraviolet radiation.

Discussion

The above data indicate that several reactions take place in the decomposition of DPN by ultraviolet radiation including photochemical changes in the pyridinium moiety of the molecule and rupture of the nucleoside and nucleotide linkages. The loss of coenzyme function of DPN, the ability to function in hydrogen transport mechanisms by virtue of reversible hydrogenation of the quaternary nitrogen of the pyridinium base, is probably associated with the first of these reactions.

The labilization of the nucleoside linkage in ATP by the pyrophosphate group is shown by the identification of adenine as a product of the ultraviolet degradation of ATP, whereas adenosine and adenylic acid remained unchanged following exposure to ultraviolet radiation. The identification of adenine as a product of the decomposition of DPN indicates that the pyrophosphate linkage in this molecule exerts a similar effect.

OAK RIDGE, TENN. RECEIVED MARCH 3, 1949

Hydrogen-bonding in Polyacrylate Solutions[1]

BY DAVID EDELSON[2] AND RAYMOND M. FUOSS

We recently[3] reported data on the conductance and viscosity of aqueous solutions of sodium polyacrylate and of poly-4-vinyl-*n*-N-butylpyridinium bromide. While conductances at the same equivalent concentration were not greatly different for these two polyelectrolytes, a striking contrast was observed in relative viscosity η_r. For example, at c = 0.2873 monomoles per liter, the relative viscosity for the polyacrylate was 230 while that for the polybromide at c = 0.2737 was only 8.0. Further experimental work has served to suggest an explanation of the difference.

The molecular weight of the polyacrylate was not known and cannot be determined by conventional methods. We therefore decided to prepare the methyl ester.

A 50-g. sample of the 16% aqueous polyacrylic acid was evaporated to dryness under low pressure; the residue was taken up in 50 ml. of methanol. After adding 1.5 ml. of sulfuric acid as catalyst, the solution was refluxed for eight hours; to hold the polymethyl acrylate in solution, 30 ml. of acetone were added during this time. After evaporation nearly to dryness at room temperature under vacuum, the residue was taken up in methyl ethyl ketone and the ester was precipitated as a gum by pouring the solution into water. After washing to remove sulfuric acid, the ester was dissolved in dioxane and recovered by sublimation of solvent from the frozen solution.[4] The product was slightly rubbery, but hardened after several days drying under vacuum at room temperature. A weighed sample was titrated in dioxane–water solution with standard caustic; residual acid was low, and indicated at least 85% esterification.

Viscosities of the ester were measured in dioxane solution. The results are given in Table I, where C is concentration in g./100 ml., and $\eta_{sp} = (\eta - \eta_0)/\eta_0$.

TABLE I

VISCOSITIES OF POLYMETHYLACRYLATE IN DIOXANE

C	η	η_{sp}/C
0.0000	0.01195	(2.35)
.1156	.01553	2.60
.2290	.01960	2.80
.470	.03022	3.26

The reduced viscosities are linear in concentration, with k' = 0.336, and extrapolate to an intrinsic viscosity of 2.35. Comparison with data for polymethylmethacrylate in benzene[5] and for polyvinylacetate in acetone[6] indicates a degree of polymerization of the order of 6000 for our polyacrylate. Since the polybromide had a degree of polymerization of 2000, and since reduced viscosity increases more slowly than molecular weight, the much higher viscosity of the polyacrylate can hardly be ascribed to its higher molecular weight alone.

Since, therefore, the high viscosity of the sodium polyacrylate solution presumably cannot be accounted for on the basis of long chains in the polyanion, some other explanation becomes necessary. Cross-linking of polyacrylate ions due to hydrogen bonds between carboxyl ions of different chains *via* water molecules would lead to a very high macroscopic viscosity. The critical test of

(1) Part of Project NR 054-002 of the Office of Naval Research.

(2) Post-doctoral Research Fellow, Yale University 1949–1950.

(3) D. Edelson and R. M. Fuoss, THIS JOURNAL, **72**, 306 (1950).

(4) F. M. Lewis and F. R. Mayo, *Ind. Eng. Chem., Anal. Ed.*, **17**, 134 (1945).

(5) J. H. Baxendale, S. Bywater and M. G. Evans, *J. Polymer Sci.*, **1**, 237 (1946).

(6) H. Staudinger and K. Warth, *J. prakt. Chem.*, **155**, 261 (1940).