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THE DECOMPOSITION OF DIPHOSPHOPYRIDINENUCLEOTIDE (DPN) AND
ADENOSINETRIPHOSPHATE (ATP) BY ULTRAVIOLET LIGHT

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THE DECOMPOSITION OF DIPHOSPHOPYRIDINENUCLEOTIDE (DPN) AND
ADENOSINETRIPHOSPHATE (ATP) BY ULTRAVIOLET LIGHT

C. E. Carter

The inactivation of cozymase (DPN) of horse red blood cells by ultraviolet light was first reported by Runnstrom, Lennerstrand and Borei (1). 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 m μ absorption of the mixture. DPN was homogenous when chromatographed but assayed only 38% pure by the hydrosulfite reduction procedure of Lepage (2).

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 airtight 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 μ g. in amount each are well resolved by this technique³.

A high energy ultraviolet light source was constructed using 8 - 15 volt G. E. germicidal lamps with 95% of the ultraviolet omission shorter than 300 $m\mu$. (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.

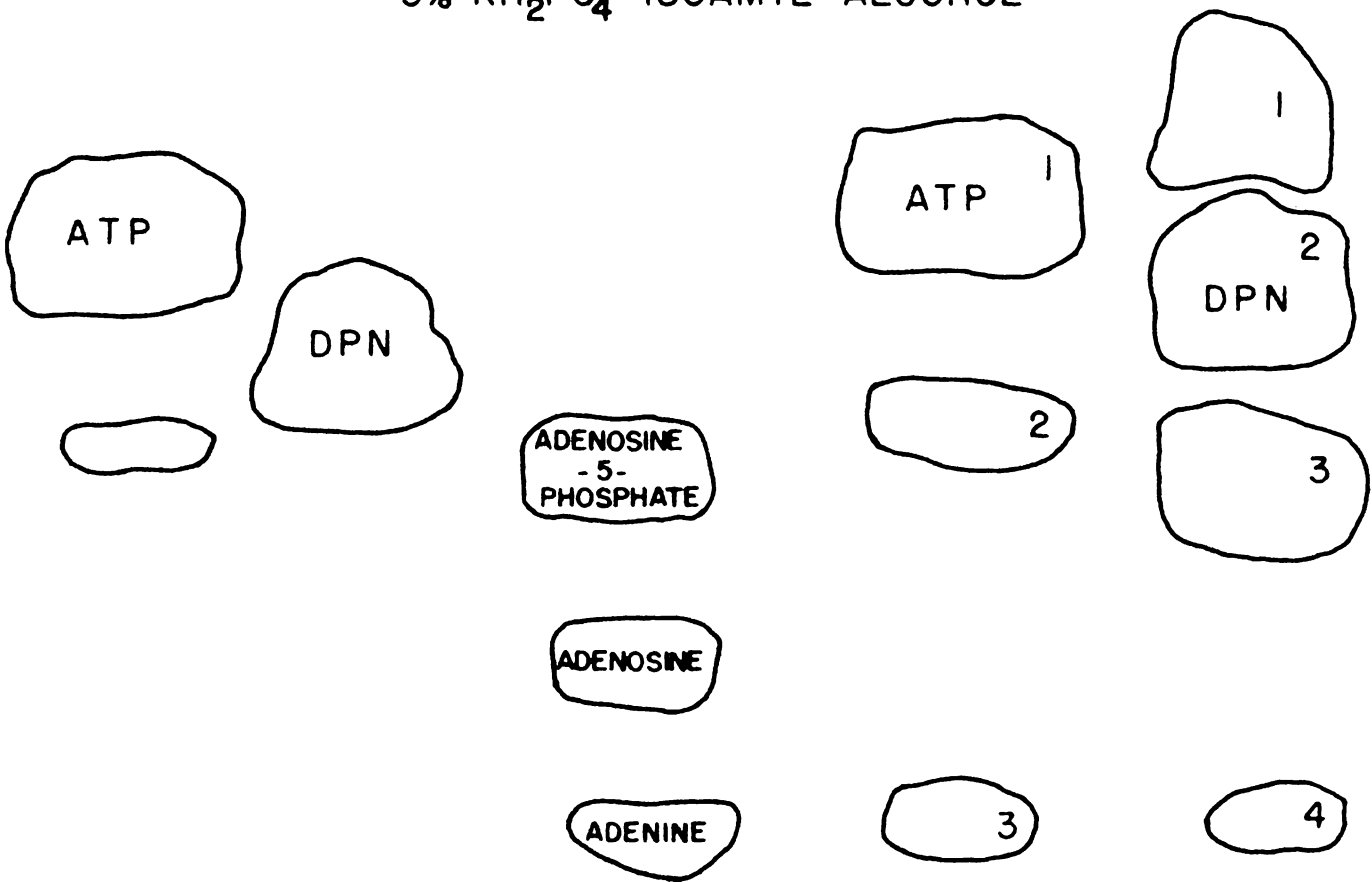
Results

The distribution of ATP, DPN, adenosine-5-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 A 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 A absorption of

Figure 1

The products of the ultraviolet decomposition of ATP (3 mg./0.5 ml. H₂O) and DPN (3mg./0.5 ml. H₂O), 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.

SOLVENT FRONT 30.7 cm.
5% KH_2PO_4 -ISOAMYL ALCOHOL



A

CHROMATOGRAM OF
STANDARD SOLUTIONS

B

CHROMATOGRAM OF PRODUCTS
OF DECOMPOSITION OF ATP
AND DPN BY ULTRA VIOLET
LIGHT (SIX HOURS EXPOSURE)

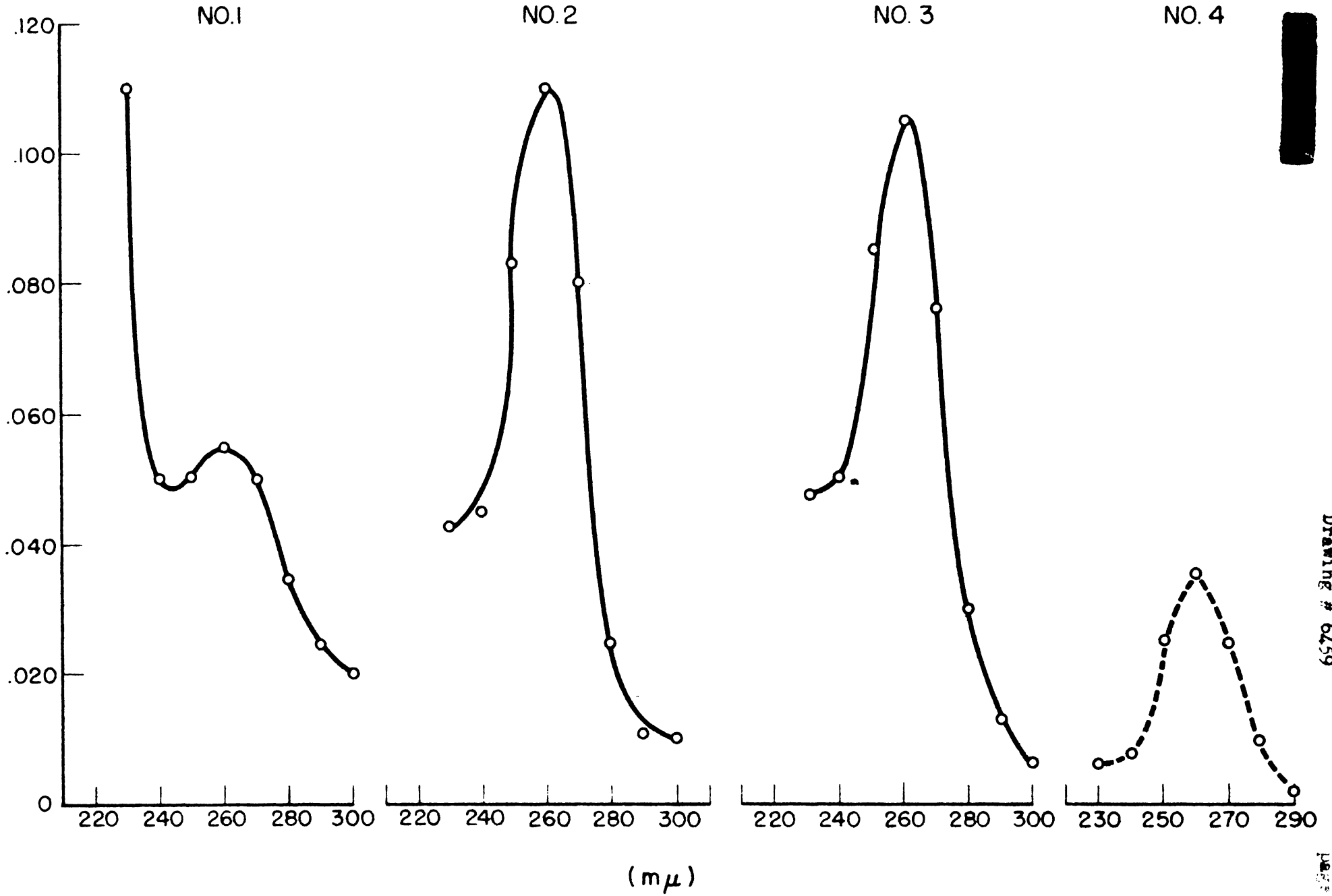
the ATP solution. Similar experiments with muscle and yeast adenylic acid and adenosine showed that solutions of these compounds were not degraded by ultraviolet light in exposure periods up to ten hours in the apparatus described.

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 μ 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 data 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 μ region, (Fig. 3), they do not exhibit the 340 μ maxima, characteristic of reduced DPN. It was also found that a 1% solution of DPN, following one and one-half hours exposure in the ultraviolet apparatus employed, showed an 18% decrease in 340 μ absorption when assayed by the hydrosulfite method described by Lepage². 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.

Figure 2

Absorption spectra of the four components (Fig. 1) resulting from the exposure of a solution of DPN (3 mg./0.5 H₂O) 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.

FIG. 2



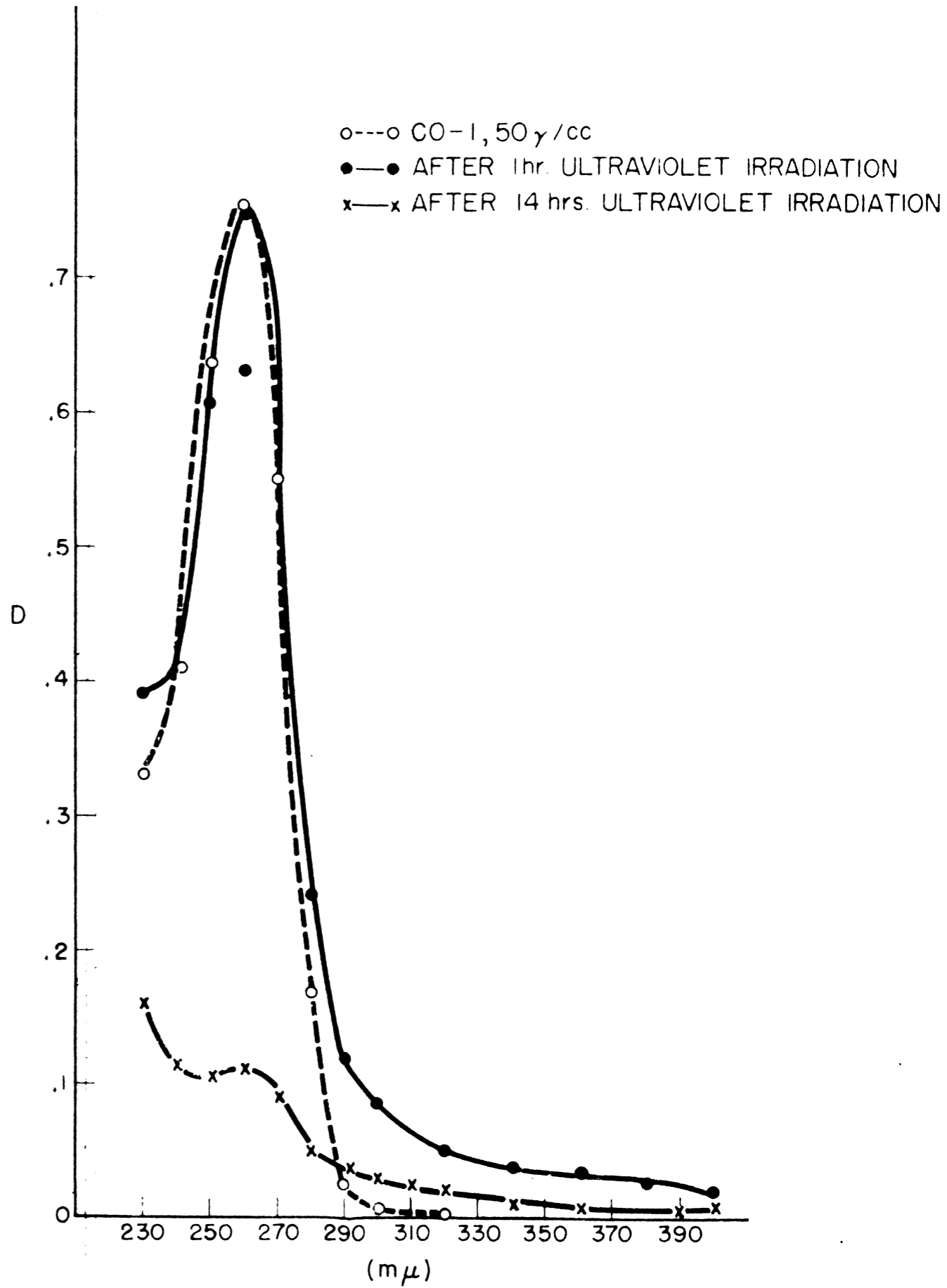
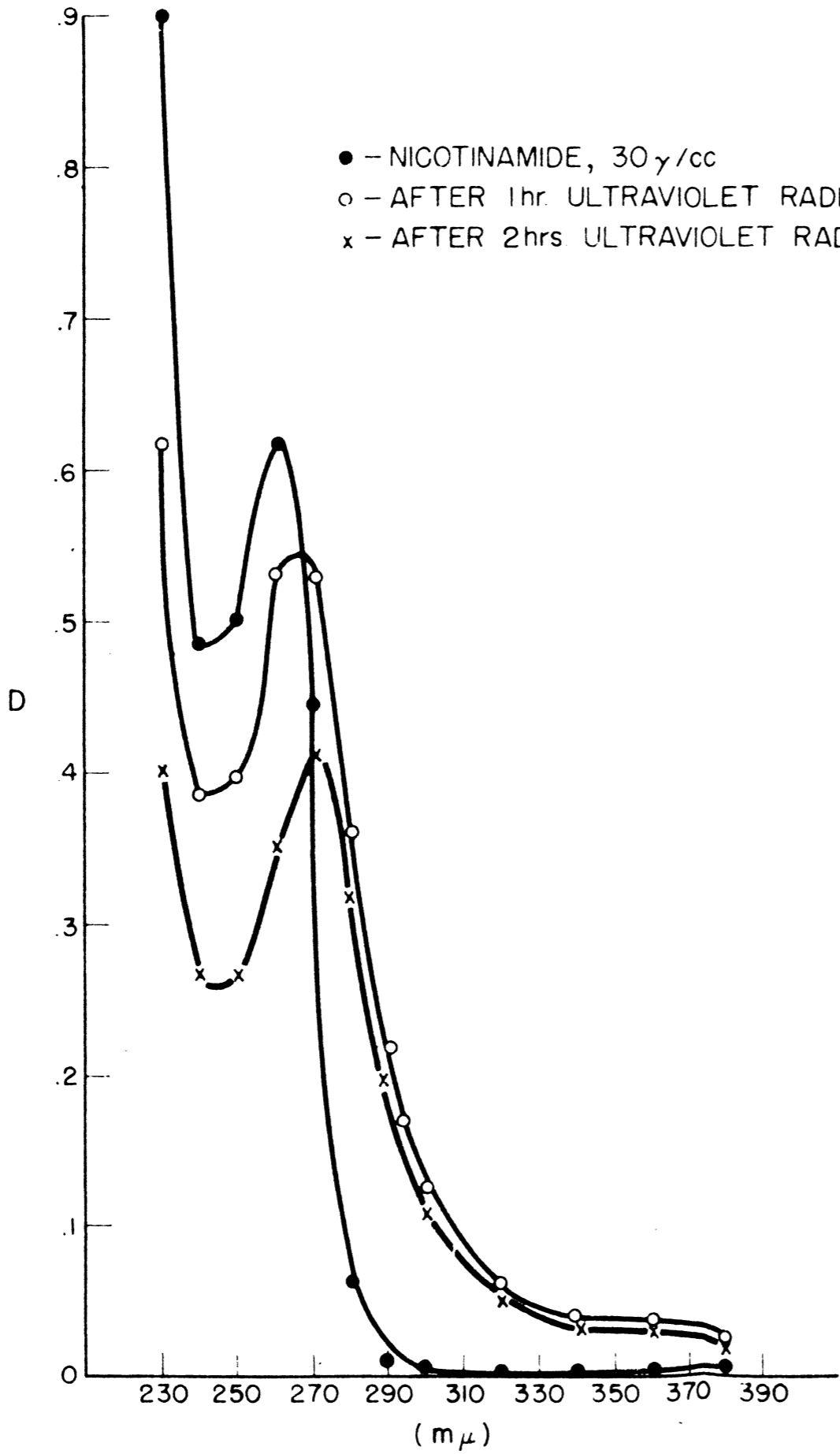
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Figure 3

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

FIG. 3



Ultraviolet radiation of solutions of DPN did not liberate inorganic phosphorus from the molecule, whereas in the experiment illustrated in Fig. 1, 10 μ g. 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.

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