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2 February 1956

Photoreactivation of Ultraviolet-Inactivated Diphosphopyridine Nucleotide

Available information suggests that the site of photoreversible ultraviolet injury may be in a nucleotide, or in a compound containing a nucleotide, or in more than one such compound (*1*). It is further suggested that the substance is associated in some basic way with a diversity of cellular activities. The phosphopyridine nucleotide coenzymes, which are associated with a variety of hydrogen-transfer reactions, meet these specifications. The fact that a triosephosphate dehydrogenase-diphosphopyridine nucleotide complex, which loses activity in preparation, may be reactivated by light (*2*) suggests that diphosphopyridine nucleotide (DPN) may be involved. This report deals with a study of photoreactivation *in vitro* of DPN that has been partially inactivated by ultraviolet radiation (*3*). Some of the experiments discussed have been reported previously in abstract form (*4*).

Solutions of DPN (*5*) were prepared at concentrations of 1/5000 or 1/10,000 in 0.1*M* phosphate buffer at *p*H 7. The source of ultraviolet radiation (UV) was a Westinghouse Sterilamp (emitting mainly $\lambda = 2537$ A), and the source of visible light (VL) was a General Electric CH-4 spotlamp. A Corning No. 3060 glass filter, which transmits no short ultraviolet waves, and 3 in. of water were always interposed between the CH-4 lamp and solutions being exposed to VL. In typical experiments, samples were withdrawn from stock DPN solutions and were exposed to UV, to VL, or to UV and VL or were retained as unirradiated controls. Samples were irradiated in flat glass dishes with quartz covers (Vaseline sealed to prevent evaporation). Solutions were stirred with glass-enclosed magnetic fleas. The ability of DPN to accelerate the reduction of hog- or rat-liver extracts was used as the criterion of the activity of samples.

Four series of experiments are reported here. For series I, II, and III, hog liver was ground and mixed with equal parts phosphate buffer in a Waring Blendor, filtered through a clean laboratory towel, and stored frozen until used. For series IV, rat liver was prepared in the same way, except that, after being filtered through cloth, it was filtered twice through analytic-grade filter paper prior to freezing, and again after thawing, just prior to use.

In series I, irradiation periods of 2 hours were used for UV and for VL. In series II, exposure periods were 4 hours for UV and 2.5 hours for VL. The series-III samples were exposed to UV for 22 hours and to VL for 2 hours, and series-IV samples were exposed for 75 hours to UV and for 2 hours to VL. The experiment was repeated four times in series I, III, and IV and three times in series II. The direction and magnitude of result were consistent in each series of experiments.

Liver preparations were placed in Thunberg tubes. Equal amounts (1 or 2 ml) of methylene-blue solution (1/3000 in series I, II, and III; 1/5000 in series IV) and DPN solutions (variously treated) were placed in the caps. Control tubes were always run with buffer substituted for the added DPN solution. Tubes were evacuated of air, and their contents were mixed. Then the mixtures were incubated in a water bath at 30°C while achromic times were being determined. The average values for achromic times are reported in Table 1. Thunberg tubes from an experiment in series IV are shown in Fig. 1 as they appeared after 20 minutes of incubation.

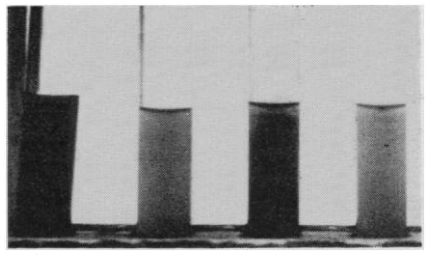

Fig. 1. A point of interest in a representative experiment. Thunberg tubes from an experiment in series IV, photographed after 20 minutes of incubation. Tubes were immediately returned to the water bath for the remainder of the incubation period. The tubes, from left to right, respectively, contain: No added DPN (achromic time, 100 min.+); untreated DPN (16 min.); UV-irradiated DPN (29 min.); DPN irradiated with UV + VL (20 min.). Photoreactivation is demonstrated by accelerated methylene-blue reduction in the fourth tube.

Liver preparations that received no added DPN reduced methylene blue slowly (series I, II, and III) or not measurably (series IV). In each experiment, the rate of decolorization was greatly accelerated by the addition of unirradiated DPN. Partial inactivation of DPN by UV was indicated by reduction of this acceleration in comparison with unirradiated controls. Visible light alone did not alter the activity of DPN. Photoreactivation of DPN samples that had been partially inactivated by ultraviolet radiation was apparent when UV treatment was followed by VL treatment. The photoreactivation of ultraviolet-inactivated diphosphopyridine nucleotide is of considerable interest as a possible model for interpretation of photoreactivation *in vivo*.

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Table 1. Data for experiments on photoreactivation of DPN *in vitro*. All values are averages of several experiments.

Experiment series	Achromic times (min)				Photoreactivation (min), UV − (UV + VL)
	Buffer only	Treatment of DPN			
		None	With VL	With UV	With UV + VL	
I	16.50	3.69	3.63	4.88	4.63	0.25
II	17.67	7.83	7.83	8.83	7.92	0.91
III	21.25	6.31	6.44	10.75	9.72	1.00
IV	78+	20.25		31.13	24.00	7.13

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1 March 1956

Serum and Liver Transaminase Activities in Experimental Virus Heptatitis in Mice

An increase (20- to 40-fold) in the aspartic-ketoglutaric and alanine-ketoglutaric transaminase activities of the serum in human epidemic virus hepatitis was found by us (*1, 2*). Later, aspartic-ketoglutaric transaminase activity in epidemic hepatitis was investigated by Wróblewski and LaDue (*3*), and comparable results were obtained.

No such increase has been found in any other type of icteric or anicteric liver diseases that were investigated by Wróblewski and LaDue (*3*) and by us (*1, 2*), with the exception of myocardial (precocious) infarction (*4*). Furthermore, these authors found an increase in the aspartic-ketoglutaric transaminase activity of serum both in cases of carbon tetrachloride poisoning in human beings and in cases of experimental carbon tetrachloride poisoning in mice (*3*).

In epidemic hepatitis, the increase in alanine-ketoglutaric transaminase activity is more consistent than the increase in aspartic-ketoglutaric activity. Consequently, the ratio of aspartic-ketoglutaric transaminase activity to alanine-ketoglutaric transaminase activity (As-K/Al-K) falls from the normal (mean) value of 1.3 to 0.64 in acute epidemic hepatitis (*1, 2*). Such a pattern is retained by the serum of a patient during his convalescence, when the absolute values of the enzymatic activities of the patient's serum are no longer diagnostically significant. We have suggested that such enzymatic determinations could be of use as a diagnostic test for human epidemic hepatitis (*1*). Our later experiments (63 cases of epidemic hepatitis) fully confirm our first results (*5*).

Questions have arisen with regard to the following: (i) whether the increase of the enzymatic activities is really related to the destruction of parenchymal liver cells as suggested by us and others (*1–5*); (ii) whether such enzymatic variations are common features of all human (epidemic, yellow fever, infectious mononucleosis, and so on) and animal virus hepatitides; (iii) whether enzymatic variations that are observed in serums are associated with variations in the same activities in liver tissue; (iv) whether the possible enzymatic variations in liver tissue are analogous or opposite to those in serums from the same subjects.

The present results (Table 1) concern two enzymatic activities of the livers and serums of mice infected with 1000 LD_{50} of hepatitis virus (MHV-3 Craig, of Gledhill and Andrewes, *6*) and killed on the fourth day of the disease (*7*). Determinations were made according to Tonhazy, White, and Umbreit (*8*).

Determinations were made of enzymatic activity in homogenates of liver tissue from each of 15 infected mice and each of 15 normal mice and in five pools of serums from normal mice and five from infected mice. Each pool contained a mixture of serums from three animals. The results obtained led us to the following conclusions:

1) An increase is shown in the investigated transaminase activities in the serums of the animals experimentally infected with MHV-strain virus. This increase is comparable to that observed in human beings with epidemic hepatitis. Furthermore, the afore-mentioned decrease in the value of the As-K/Al-K ratio is found in the serum both in cases of human hepatitis and cases of experimental hepatitis in mice.

2) The variation in either of the transaminase activities in the liver (both decrease) of an animal is opposite to the variation in the analogous transaminase activity in the serum of the same animal but roughly proportional to the latter in magnitude. The decrease in the alanine-ketoglutaric transaminase activity in livers and the increase in this activity in serums are more pronounced than the changes in activities of aspartic-ketoglutaric transaminase in serums and livers.

These conclusions support the hypothesis that the enzymatic variations observed are not limited to human virus hepatitis (*9*). Furthermore, the necrosis of hepatic cells seems to play an important role in the pathogenesis of the phenomenon (passage from the liver to the blood of enzymatic metabolites).

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Table 1. Transaminase activities at 37°C of livers and serums of mice. Aspartic-ketoglutaric transaminase activity is measured in micromoles of oxalacetate formed, and alanine-ketoglutaric transaminase activity is measured in micromoles of pyruvate formed.

Item	Normal mice	Infected mice	± %	Student's *t*
*Liver**				
Oxalacetate	115.1	86.6	− 25	4.31†
Pyruvate	111.6	58.8	− 48	5.93†
As-K/Al-K	1.03	1.46	+ 41	3.70†
Serums‡				
Oxalacetate	2.98	31.7	+ 999	3.98§
Pyruvate	0.95	37.7	+3867	4.44§
As-K/Al-K	3.04	0.84	− 75	4.42§

* Averages of 15, 100 mg of tissue for 10 minutes. † t significant > 2.763 ($P = 0.01$), $G = 28$. ‡ Averages of five pools, each from three of the same mice, 1 ml for 15 minutes. § t significant > 3.355 ($P = 0.01$), $G = 8$.

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5 March 1956

Consistent Biochemical Pattern in Malignant Tumors

In a previous study (*1*), a variety of normal and malignant tissues were subjected to cell fractionation by differential centrifugation. In all of the malignant tissues studied, the apportionment of protein among the various cell fractions showed a remarkably consistent pattern in which the nuclear and final supernatant-fluid fractions contained most of the protein and in which the mitochondrial and microsome fractions contained relatively little. The original series included 15 fractionations of nine malignant animal tumors, but only two human tumors.

Recently it has become possible to extend the observations to four more malignant human tumors (*2*). These included a lymph node affected by Hodgkin's disease, an inguinal lymph node containing an epidermoid carcinoma metastatic from the penis, a fibrosarcoma superficial to the posterior costal margin, and a cer-