Short Communications and Preliminary Notes

The irradiation of diphosphopyridine nucleotide and triphosphopyridine nucleotide by different regions of the ultraviolet light

The action of ultraviolet irradiation on diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) leads to the inactivation of these coenzymes[1,2]. The loss of activity of a $1 \cdot 10^{-4}$ *M* solution of either coenzyme is practically complete after two hours of irradiation, at which time the loss of specific absorption is not higher than 20%.

The ultraviolet irradiation results in the splitting of the molecule. About 60% of the adenine moiety of either DPN or TPN was recovered as adenosinediphosphate (ADP) and 2'-phosphoadenosinediphosphate (2'-PADP), respectively. Thus, quantitatively the most significant splitting occurs at the ester bond in 5'-position of the nicotinamide nucleotide moiety.

The source of the ultraviolet light used in our experiments was a low pressure mercury 15 W G.E. germicidal lamp. About 90% of the ultraviolet light output of these lamps is at 253.7 mμ. In view of the fact that there is some transmission below 210 mμ [3] it was of interest to determine whether the irradiation effects observed here were due to the radiations of the far ultraviolet range.

Two ml of $1 \cdot 10^{-4}$ *M* solution of either DPN or TPN was irradiated in the silica cuvettes of the Beckman DU spectrophotometer and in the Corex cuvettes for the control experiments. Five cut-off filters, obtained from Corning Glass Company, were used and the filters were placed between the cuvettes and the ultraviolet lamp. The cuvettes and the filters were fastened to the lamp with adhesive tape. The activity of DPN or TPN was measured in the Beckman DU spectrophotometer in the same silica cells in which the material was irradiated and for the control samples 2 ml aliquots were taken from the Corex cuvettes and the determinations done in the silica cuvettes. DPN was determined in the alcohol dehydrogenase system[4] and TPN in a modified glucose-6-phosphate dehydrogenase system, as described previously[2].

TABLE I

ULTRAVIOLET IRRADIATION OF DPN AND TPN WITH THE USE OF FILTERS

Filter	*Cut-off**		*Per cent* transmission at 260 mμ*	*DPN* Per cent activity	*TPN*
	Below mμ	*Above mμ*			
None**			100	3	10
None***			0	100	100
7910	210		75	4	7
9700	250		2	100	100
7740	280		0	102	103
5850	290	490	0	100	103
9863	230	420	55	19	20

* Information taken from Glass Color Filters by Corning, Corning Glass Works, 1948, pages 8 and 12.

** Irradiation in quartz cuvettes.

*** Irradiation in Corex cuvettes.

Table I shows the results obtained after two hours of ultraviolet irradiation of DPN and TPN in the silica and Corex cuvettes, with and without the filters. The action spectrum seems to follow quite closely the absorption spectrum and the inactivation occurs only when the coenzymes are exposed to high intensity of light in the region of 260 mμ wavelength. Even when all the light below 210 mμ wavelength is cut off, as with 7910 filter, almost complete inactivation takes place. Under these conditions 75% of the light of 260 mμ wavelength is available for the irradiation. However, when only 55% of the 260 mμ wavelength is available as with 9863 filter,

the inactivation is less complete reaching a value of 80%. On the other hand, when no light of 260 mμ wavelength is transmitted there is no splitting of the molecule, as with filters 9700, 7740 and 5850.

From the results shown in Table I it is clear that the ultraviolet region responsible for the splitting of DPN and TPN lies between 210 and 280 mμ. This range could be actually narrowed down, because the filter 7910 has only 25% transmission at 230, and yet there is complete inactivation of DPN and TPN. Thus, it seems reasonable to conclude that the decomposition of DPN and TPN does not necessitate the far ultraviolet radiations. The same was shown to be true for several purines and pyrimidines[3], where the loss of the specific absorption spectrum resulted from the irradiation above 210 mμ, with the possible exception of adenine and adenylic acid.

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Department of Physiology, Tufts University School of Medicine, Boston, Mass. (U.S.A.) MARIA W. SERAYDARIAN

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3-Indoleacetic acid in human urine after muscular effort

It is well known that human urine inhibits the growth and germinative power of oats when added to the soil. However, KRÁL AND SCHMID[1] observed, that urine from sportsmen after muscular effort stimulates both properties.

This observation may be explained by the presence of a growth promoting substance in urine after physical effort or by the decrease of a growth inhibiting substance.

The aim of this study was to follow up the first of the two possibilities as it is well known, since the early work of KÖGL, that human urine contains a certain amount of the powerful growth stimulator 3-indoleacetic acid (IAA).

IAA was detected in urine by paper chromatography. Aromatic compounds from a 15 ml sample of urine acidified with acetic acid are adsorbed according to DECKER[2] on 400 mg of a mixture of equal parts of charcoal and silica. The adsorbed substances are extracted with 10 ml of the following mixture: *n*-butanol, water, ammonia, methanol (2:15:2:1). The extract is evaporated to dryness and the residue dissolved in 0.5 ml water. 25 μl of this solution is applied on paper and one-dimensional chromatograms are performed in butanol-ammonia mixture (*n*-butanol, ammonia, water 15:1:4). The spots were detected by spraying the paper with a solution of $FeCl_3$ in acetic acid (1:20), after which the IAA develops a bright red colour (R_F = 0.47). The spots before and after physical exertion were compared visually and the results expressed in terms of decrease, increase or no change.

Twenty-three samples (out of 28) of urine after muscular activity showed a more intensive spot lying in the position of IAA, 2 showed a spot of similar strength and 3 showed a weaker spot then in samples before muscular activity. Specific weight of the urines was the same before and after muscular activity.

From the above results it would seem that the factor stimulating the growth of oats, found in the experiments of KRÁL AND SCHMID[1] in urine after muscular activity is IAA.

The chromatograms of each urine show a greenish-blue spot of indigo blue (R_F = 0.54) resulting from urinary indican, not only after oxidation with $FeCl_3$, but also when the paper is exposed for several hours to air. However, the intensity of this spot seems to be greater before muscular activity than afterwards. The interesting correlation between the excretion of both substances is under investigation.

Institute of Sport Medicine, Medical School, Charles University, Salmovská 5, Prague (Czechoslovakia) J. A. KRÁL, A. ŽENÍŠEK, I. STOLZ

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