DIPHOSPHOPYRIDINE NUCLEOTIDE-TRYPTOPHAN INTERACTIONS

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It was previously reported (Alivisatos, 1958; Alivisatos et al., 1960a, Alivisatos et al., in press) that DPN* interacts with certain indoles, including serotonin and tryptophan. These interactions, most probably of the charge-transfer complex type, are manifested by the intense yellow coloration appearing upon admixture of the two reactants (e.g., DPN and tryptophan). Similar observations were reported by Cilento et al (1959). Certain changes in the region of the spectrum below 300 mμ., also reported earlier (Alivisatos et al., 1960a) were later recognized as artifacts arising from stray light when high concentrations of one of the reactants was present in the control (Alivisatos et al., 1960b).

In a recent report, Remily et al. (1960) suggested that our observations as well as those of Cilento et al. (vide supra) were entirely due to artifacts and that no interactions whatsoever between DPN (or, other pyridinium-bearing compounds) and tryptophan could be observed.

We deem it necessary to demonstrate conclusively that artifacts are not responsible for the observations above 300 mμ and that the conclusions based on these observations are sound.

When these phenomena were first observed in our laboratory in 1957, they became apparent by the visual appearance of a yellow color.

* Abbreviation: DPN, diphosphopyridine nucleotide.

Remily et al. (1960) suggest that DPN alone may also appear yellow in alkaline solutions. This is true for concentrated alkaline DPN solutions on relatively long standing. However, DPN - tryptophan interactions may be observed at pH values of 2.0 or below (see table 1). Furthermore as

TABLE I

ABSORBANCIES OF DPN AND TRYPTOPHAN SOLUTIONS AND OF DPN-TRYPTOPHAN MIXTURES AT CERTAIN WAVE LENGTHS

Component	Molar Concentration	Absorbancies				pH
		330 mμ	340 mμ	360 mμ	380 mμ	
DPN	5×10^{-2}	0.156	0.075	0.025	0.009	2.0
Tryptophan	5×10^{-3}	0.015	0.013	0.009	0.009	2.2
DPN plus Tryptophan	as above	0.837	0.635	0.418	0.305	2.0

Readings were taken in 1 cm. lightpath quartz cuvettes in a DU-Beckman Spectrophotometer, with water as a blank. DPN was a product of the Pabst Laboratories and the solution showed the recorded pH without any buffering. The pH of unbuffered L-tryptophan (Nutritional Biochemicals Corporation) solutions of the concentrations indicated in the table was 6.1. It was lowered to 2.2 with the aid of HCl. However, absorbancy readings of an unbuffered solution were essentially identical to those reported in the table for the acidified solution. The DPN-tryptophan mixture was unbuffered. Its pH was recorded immediately after the spectrophotometric readings were taken. My thanks are due to Mrs. Frieda Ungar for this experiment.

the data of Table 1 show the absorbancies of DPN and tryptophan alone are magnitudes below values which might lead to artifacts. The spectroscopic counterpart of the visual color appears to be a broad featureless absorption band extending from beyond 400 mμ to at least 300 mμ (transfer spectrum). However, the high absorption of both DPN and the indoles at the concentrations required for interaction (see table I) prevents extension of the curves in the region below 300 mμ (Alivisatos et al, 1960b).

It is difficult to explain the complete absence of interaction in the experiments of Remily et al (1960), although it is possible that the model compound utilized in their experiments (N-benzyl nicotinamide) was not identical with the desired 1-benzyl-3-carboxamide pyridinium (chloride).

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