directed by nucleic acids, are influenced by the photodynamic action of dyes. Phage induction can then be explained in the same way.

After the experiments described above had been finished, the paper of GEISSLER and WACKER[11] appeared. These authors observed induction of bacteriophage λ in *E. coli* K12 by means of thiopyronine only after visible light irradiation. In contrast to AO, it is well known that thiopyronine photooxidizes the guanine residues in the DNA molecule.

Zusammenfassung. Es wird über Ergebnisse bei der Induktion der Phage- und Colicinsynthese in *E. coli* (Stamm 18) durch photodynamische Wirkung des Acridinorange berichtet. Der Phagentiter wurde 11mal, der Colicintiter kaum 2mal erhöht. Acridinorange zeigte ohne Belichtung keinen deutlichen Effekt.

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[11] E. GEISSLER and A. WACKER, Acta biol. med. germ. *10*, 937 (1963).

Charge-Transfer Complexations among Biochemically Reactive Compounds[1]

The theory of charge-transfer spectra was developed by MULLIKEN[2] and a review published by ORGEL[3]. As pointed out by KOSOWER[4], a necessary consequence of the existence of electron donor-acceptor complexes stabilized by charge transfer is the ability of the ground state to absorb light, undergoing an electronic transition to an excited state for which the major contributing form is the complex.

Studies on the complexation of flavins have been reviewed by BEINERT[5]. Complexing reagents cause a decrease of intensity and a slight band shift toward longer wavelengths in both absorption and fluorescence spectra of flavins. Indeed, charge-transfer interactants in biochemistry include purine and pyrimidine derivatives, aromatic amino acids, hormones, vitamins and coenzymes, and agents which are known to uncouple electron transport from oxidative phosphorylation[6].

The present work was done to extend generally our recognition of the phenomenon of charge-transfer complexations among biochemically significant compounds, especially in the present case with complexers of the vitamin, riboflavin, which participates as coenzyme forms in oxidations catalyzed by flavoproteins, and with complexers of a recently recognized uncoupler of oxidative phosphorylation, 1,1,3-tricyano-2-amino-1-propene[7].

Experimental. Spectrophotometric measurements were made with a Beckman Model D.U. with photomultiplier tube using fused silica cuvettes at room temperature. The wavelengths selected for noting changes in optical density of any given compound were those near a characteristic chromophore where maximal decreases were seen upon complex formation.

The relative magnitude of complexation of several representative compounds with riboflavin is shown by the data in the Table. Most of the complexing reagents shown are presumed to undergo π bond interactions. Though π, π complexes probably predominate, n, π interaction is also possible in certain instances. As is true with charge-transfer complexes in general, the degree of association of these complexes is temperature dependent.

The spectral changes which accompany complexation of riboflavin with 1,1,3-tricyano-2-amino-1-propene are shown in Figure 1. As seen in part A of the Figure, ab-

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[2] R. S. MULLIKEN, J. phys. Chem. *56*, 801 (1952); J. Am. chem. Soc. *74*, 811 (1952).

[3] L. E. ORGEL, Quart. Rev. *8*, 422 (1954).

[4] E. M. KOSOWER, in P. D. BOYER, H. LARDY, and K. MYRBÄCK (Editors), *The Enzymes*, vol. 3, part B (Academic Press, New York 1960), p. 171.

[5] H. BEINERT, in P. D. BOYER, H. LARDY, and K. MYRBÄCK (Editors), *The Enzymes*, vol. 2, part A (Academic Press, New York 1960), p. 339.

[6] H. A. HARBURY and K. A. FOLEY, Proc. Nat. Acad. Sci. U.S. *44*, 662 (1958).

[7] F. S. EBERTS, JR., Biochem. biophys. Res. Comm. *3*, 107 (1960).

Complexation of various compounds with D-riboflavin[a]

Complexing compound	64 μM compound alone $\lambda m\mu$	O.D.	130 μM riboflavin added O.D.	Δ O.D.	% decrease
L-Tryptophan	275	0.36	0.03	0.33	92
p-Aminobenzoic acid	265	0.90	0.22	0.68	76
Triethylenemelamine	225	2.70	0.64	2.06	76
Flavianic acid	225	1.27	0.43	0.84	67
Picric acid	220	0.89	0.30	0.59	66
m-Dinitrobenzene	240	0.97	0.52	0.45	46
Caffeine	275	0.60	0.33	0.27	45
o-Phenylenediamine	290	0.20	0.14	0.06	31
3,5-Dinitrosalicylic acid	215	0.76	0.61	0.15	20
Anthranilic acid	210	1.45	1.16	0.29	20
Carbonylcyanide *p*-chlorophenylhydrazone	230	0.50	0.41	0.09	18
L-Phenylalanine	205	0.55	0.52	0.03	5

[a] Values for $\lambda m\mu$ and O.D. of complexing compounds were measured in 0.1 M sodium phosphate buffer, pH 7, against a buffer blank. Values for O.D. of complexing compounds in the presence of riboflavin were measured in the buffer against a buffer plus riboflavin blank.

sorbancy of the alloxanoid portion of riboflavin at 266 mμ is markedly diminished and shifted to a longer wavelength of the complex towards 280 mμ. Similarly, in part B can be seen the decrease in absorbancy of the trinitrile at 274 mμ and the shift to λ_{max} of the complex near 285 mμ. Since the complex species is examined by difference spectra, the possible slight contribution from fluorescence or light scattering of riboflavin-containing solutions is obviated.

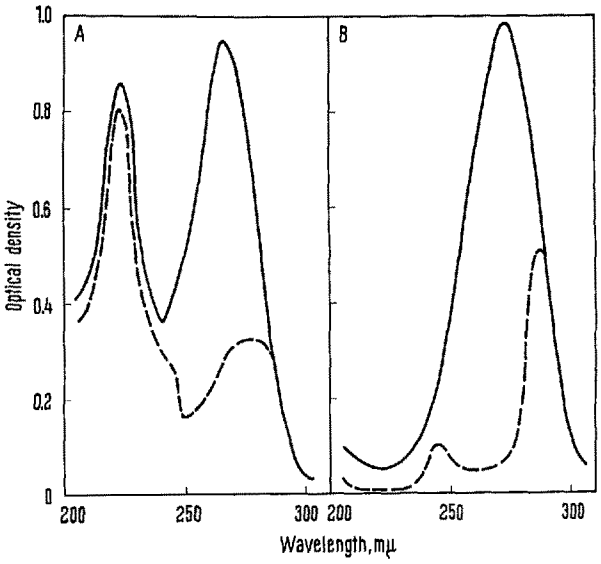

Fig. 1. Spectral changes accompanying complexation of D-riboflavin with 1,1,3-tricyano-2-amino-1-propene in 0.1 *M* sodium phosphate buffer, pH 7. In part A, the spectrum (solid line) of 30 μ*M* riboflavin in buffer against a buffer blank is compared with the difference spectrum (dashed line) of 30 μ*M* riboflavin plus 430 μ*M* trinitrile in buffer against a buffer plus trinitrile blank. In part B, the spectrum (solid line) of 64 μ*M* trinitrile in buffer against a buffer blank is compared with the difference spectrum (dashed line) of 64 μ*M* trinitrile plus 260 μ*M* riboflavin in buffer against a buffer plus riboflavin blank.

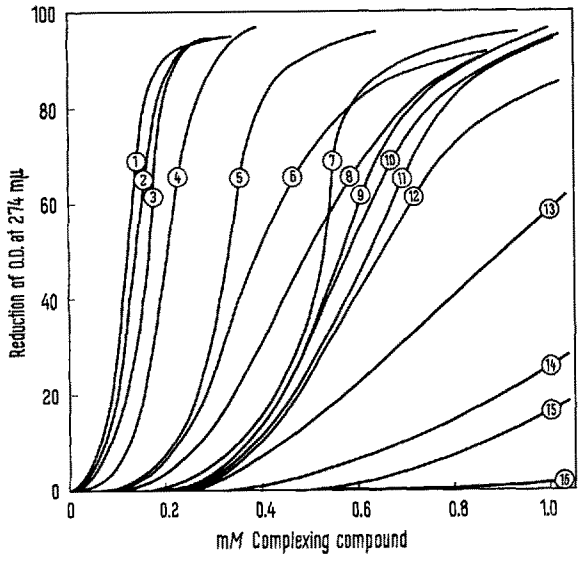

Fig. 2. Complexation of various compounds with 1,1,3-tricyano-2-amino-1-propene in 0.1 *M* sodium phosphate buffer, pH 7. Decreases in O.D. of buffered solutions of trinitrile were measured in the presence of varying concentrations of complexing compounds against blanks of buffer plus complexing compounds. The same relative decreases were found for trinitrile concentrations as high as 100 μ*M* below which Beer's law is obeyed. The compounds shown are: 1, flavin mononucleotide; 2, flavin adenine dinucleotide; 3, folic acid; 4, leucovorin; 5, caffeine; 6, diphosphopyridine nucleotide; 7, L-tryptophan; 8, 5,6-dimethylbenzimidazole; 9, thiamine; 10, orotic acid; 11, *thymine*; 12, cytidylic acid; 13, uracil; 14, 6-azauracil; 15, 6-mercaptopurine; 16, barbituric acid.

Complexation of several biochemicals with the trinitrile is shown by the data in Figure 2. Flavin and pteridine derivatives are very reactive as complex formers. Caffeine and tryptophan, which are good complexers with flavins, also are fairly reactive as are diphosphopyridine nucleotide, thiamine, and dimethylbenzimidazole found in vitamin and coenzyme forms of B_{12}. Pyrimidine analogues are somewhat less avid complexing reagents. Several other compounds examined do not complex significantly with the trinitrile under these conditions. These include histidine, imidazole, cysteine, nicotinamide, dihydrouracil, allantoin, urea, and cytochrome c.

Discussion. The ubiquity of charge-transfer reactions among biochemical compounds tends to lend some difficulty to a clear understanding of any specific role many such complexes play. However, the natural occurrence and function of certain charge-transfer complexes is well recognized. Among the latter may be mentioned the intramolecular reaction of isoalloxazine and purine moieties in flavin adenine dinucleotide as studied in some detail by WEBER[8]. There is also sufficient information on pyridinium systems to consider the role of charge transfers in di- and triphosphopyridine nucleotides[4]. The intermolecular reactions of the pyridine nucleotide coenzymes with the flavin coenzymes may be partially understood from the standpoint of their charge-transfer complexations which allow these coenzymes to interact in the transfer of hydrogen and electrons in biological systems. The role which charge-transfer interaction and resonance coupling may play in the functions of prosthetic groups of flavoproteins and other oxidative enzymes has received some study[9-12], and certainly more investigations on these systems should be forthcoming. The suggestion has been made that interesting possibilities exist in relation to electron transport-coupled phenomena such as oxidative phosphorylation[6]. Although much speculation on this point would be premature, it is perhaps noteworthy that many of the uncouplers of oxidative phosphorylation, such as some of the phenols and nitriles studied herein, are readily able to form charge-transfer complexes with the initial components, pyridine nucleotides and flavins, in the electron to oxygen transport system of living organisms.

Zusammenfassung. Bei vielen biochemischen Stoffen wird die mit Ladungstransfer verbundene Komplexbildung durch Veränderungen im gemessenen Absorptionsspektrum angezeigt. Die mögliche Bedeutung einer solchen Komplexbildung, insbesondere bei Wasserstoff- und Elektronentransport innerhalb biologischer Systeme, wird diskutiert.

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Graduate School of Nutrition, Cornell University, Ithaca (New York USA), March 17, 1964.

[8] G. WEBER, Biochem. J. *47*, 114 (1950).
[9] T. FÖRSTER, *Fluorescenz organischer Verbindungen* (Vandenhoeck and Ruprecht, Göttingen 1951).
[10] F. W. J. TEALE and G. WEBER, Biochem. J. *65*, 476 (1957).
[11] G. KARREMAN and R. H. STEELE, Biochem. biophys. Acta *25*, 280 (1957).
[12] G. KARREMAN, R. H. STEELE, and A. SZENT-GYÖRGI, Proc. Nat. Acad. Sci. U.S. *44*, 140 (1958).