however, the counting rate was affected by several conditions, such as an uneven distribution and the surface roughness of samples.

Through all experiments, no memory effect was observed. A memory effect would hardly be anticipated from the low volatility of octadecane. The present results suggest an applicability of the solid-counting method to soft β-rays of tritium-tagged substances, if the sample thickness is chosen to be in a proportional region, as shown in Fig. 1.

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References and Notes

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Electron Transfer and Absorption Spectra of Complexes

Abstract. Occurrence of light saturation in the reaction of thionine (T) with Fe^{2+} suggests the existence of $\mathrm{T}\cdot\mathrm{Fe}^{2+}$ complexes practically indistinguishable by their absorption spectra from free thionine; photoreduction seems to occur by electron transfer in such complexes, delayed by more than 10^{-5} second after light absorption. Both phenomena must be of general significance.

It has been customary to use absorption spectra to decide whether two reagents in solution form a complex or not. It was assumed that the absorption spectrum of the complex must be different from that of its components, at least to the extent to which the spectrum of a dissolved molecule usually is affected by the solvent.

However, Watson (*1*) had to postulate the occurrence of phenylhydrazine complexes of chlorophyll to account for the effect of this compound on the fluorescence of chlorophyll; and yet no change in the absorption spectrum of chlorophyll could be noted upon the addition of small amounts of phenylhydrazine. Bannister (*2*) had to make a similar assumption in the case of chlorophyll and ascorbic acid to explain the kinetics of the photoreduction of chlorophyll by this compound. Weber (*3*) found that enzymatic removal of the nicotinamide nucleus from diphosphopyridine nucleotide does not affect the absorption spectrum; and the absorption spectrum of ethyl chlorophyllide was found, by Holt and Jacobs (*4*), to be practically identical with that of chlorophyll (phytyl chlorophyllide); so it was shown that even chemical bonding sometimes leaves the absorption spectrum practically unchanged.

One of us (S.A.) arrived at a similar conclusion in a systematic study of the kinetics of photoreduction of thionine (T) by ferrous ions. The phenomenon of light saturation, unusual in simple photochemical reactions in vitro, which he observed in concentrated ferrous solutions (10^{-3} mole/liter or more) was most easily explained by postulating, as the main or only photosensitive species, a $\mathrm{T}\cdot\mathrm{Fe}^{2+}$ complex with a finite velocity of formation in the dark (see reaction 1, where $\mathrm{T_r}$ stands for reduced thionine):

$$ {}^{1}\mathrm{T} + \mathrm{Fe}^{2+} \overset{\mathrm{K}}{\rightleftharpoons} {}^{1}\mathrm{T}\cdot\mathrm{Fe}^{2+} \xrightarrow{+\mathrm{h}\nu} {}^{1}\mathrm{T}\overset{*}{\cdot}\mathrm{Fe}^{2+} \rightarrow {}^{3}\mathrm{T}\cdot\mathrm{Fe}^{2+} \rightarrow \mathrm{T_r} + \mathrm{Fe}^{3+} \qquad (1) $$

Light saturation is reached, in system I, when the rate of complex formation limits the rate of the photochemical reaction.

Yet, measurements of the absorption spectrum of thionine solutions in the presence of ferrous ions show no evidence of complexing. In fact, Hardwick (*5*), in a recent study of the same reaction, used this fact to exclude a complex mechanism and to treat the reactions as due to encounters of metastable thionine molecules with free ferrous ions. Schlag (*6*) also disregarded complex formation in his study of the thionine-iron reaction. Havemann and his co-workers (*7*), on the other hand, did inquire into the possible existence of complexes, confirmed their existence by measurements of the effect of Fe^{2+} ions on fluorescence, and assumed complexes to be the photosensitive species in the interpretation of kinetic data. They derived a value of $K = 30$ for the complex formation constant.

The kinetic data obtained in our laboratory, and presented in detail in a paper by Ainsworth (*8*), led to a somewhat higher complexing constant, $K = 200$.

In the present report we would like to point out the apparently widespread existence of dye complexes more or less completely indistinguishable from free dye molecules by their absorption spectra. In the case of the system $\mathrm{T} + \mathrm{Fe}^{2+}$ (as well as in that of the system chlorophyll + ascorbate), this behavior suggests that electron transfer can occur, in an excited complex, not as an immediate result of light absorption—in which case a new "electron-transfer" band could be expected to appear in the absorption spectrum (*9*)—but as a delayed effect. As suggested in reaction 1, the dye molecule in the excited complex may be first transferred into a metastable triplet state; this transfer is so effectively catalyzed by the paramagnetic Fe^{2+} ions as to quench fluorescence completely. An electron is transferred to the triplet dye from the associated Fe^{2+} ion after an average life-time of at least 10^{-5} second; this is indicated by the observation that the reaction is quenched by ferric ions [as well as by thionine molecules (see *10*)] in concentrations as low as 10^{-5} mole/liter. (Even resonance energy migration could not account for significant quenching of a *singlet* excited state by a quencher present in such low concentrations.) Another consequence of this mechanism is the probability of a primary back reaction within the complex; this may explain why the highest quantum yield of the $\mathrm{T} + \mathrm{Fe}^{2+}$ reaction that we were able to obtain was about 0.3.

The two conclusions—that spectroscopically unrecognizable complexes exist, and that delayed electron transfer occurs in such complexes after electronic excitation of one of the components—appear to be of some general interest.

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