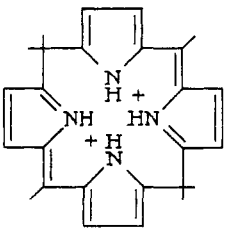
proceeds readily in deoxygenated, neutral, aqueous solution when the porphyrin is excited with light of 400, 500 or 605 mμ wave length. These wave lengths were isolated with interference filters together with suitable blocking filters from the light of a 500 watt projection lamp. Both isomer I and III uroporphyrin react similarly. The first readily detectable product of the photoreduction ($t >$ 10 seconds) is a transitory compound having intense absorption bands at 440 ($E_m \cong 10^5$) and 735 mμ ($E_m \cong 5 \times 10^4$). In the absence of light and oxygen, it is stable for at least thirty hours at neutral *p*H and room temperature. In the presence of air it has a half life of about five minutes. This autoxidation is sensitive to light. The substance also reacts rapidly with iodine. Krasnovsky and co-workers[2] have noted an absorption band at 740 mμ on illuminating hematoporphyrin in pyridine containing ascorbic acid. They favor the possibility of a radical-ion-pair, but a variety of dihydroporphyrins exclusive of structure I are also possible.

On continued illumination, an intense band at 500 mμ ($E_m = 5$–10×10^4) arises as the 440–735 mμ and the uroporphyrin absorptions fall. Over 95% of the porphyrin can be reduced, and further reduction beyond the 500 mμ stage appears to be very slow. Titration of this product with iodine reforms the porphyrin in 95 ± 5% yield. About 5 moles of iodine was required per mole of reduced porphyrin. This high value may be due to side reactions of the iodine. The reaction of iodine with tertiary amines is highly sensitive to light. The recovered porphyrin was identified by the position and by the relative ratios (6 independent values) of the intensities of its absorption bands in neutral (4 bands) and acid (3 bands) solution. Molar absorption coefficients of the reduced species were calculated from changes in the porphyrin absorption assuming only two reduced products to be present. No evidence for the presence of any colorless reduction product (*e.g.*, uroporphyrinogen) was obtained.

The photoreduced porphyrin absorbing at 500 mμ has spectral reactions characteristic of the dipyrrylmethene structure[1] of porphomethenes: (1) The absorption of porphomethenes is also at 500 mμ (with $E_m \cong 10^5$); (2) the absorption band decreases in intensity and shifts to shorter wave lengths in alkaline solution with a pK of about 9.5; and (3) the absorption vanishes in the presence of sulfite ion at *p*H 7.

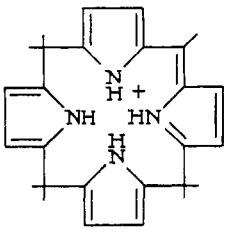
Seely and Calvin claim that the photoreduction of the zinc chelate of α,β,γ,δ-tetraphenylporphyrin by benzoin[3] forms reduced porphyrins of the chlorin and bacteriochlorin series. Under our conditions described above, the zinc chelate of uroporphyrin shows little if any reaction, and the copper chelate is not visibly affected.

Many secondary and tertiary amines will act as photoreducing agents toward a variety of dyes, *e.g.*, thiazines and flavines.[4] Ethylenediaminetetraacetic acid[5] is especially reactive. The claim is often made that the water molecule is split during the reaction[5,6] presumably with concomitant formation of the amine oxide. However, oxidation of the amine at the α carbon is more reasonable. In general the reactivity of the amine is related to its ease of oxidation at the α-carbon: *e.g.*, sparteine is very reactive, primary amines are far less reactive, and ammonium ions are unreactive. The direct isolation of the oxidized amine product is complicated by the facile autoxidation of the reduced dye and by the possible decomposition of the amine oxide if formed. In an attempt to circumvent these difficulties, the fact that small ring bridgehead amines are very resistant to oxidation at the α-carbon, yet readily form N-oxides, was used. No reasonable intermediate or transition state can be stabilized due to ring strain (Bredt's rule). In fact, the rate of photoreduction of thionine by 1,4-diazabicyclo[2,2,2]octane was found to be less than $^1/_{5000}$th that with N,N′-dimethylpiperazine. This bicyclodiamine is also vastly less susceptible to photoöxidation by iodine.



dihydroporphyrin
porphodimethene

I



tetrahydroporphyrin
porphomethene

II

Among the more interesting non-nitrogeneous photo-reducing agents we have found for these dyes are ethyl acetoacetate and 2-carbethoxycyclopentanone. Chlorophyll has a somewhat similar active "methylene" group at position 10 and possibly this molecule can act both[7] as a photo-oxidant and photo-reductant.

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MEMBRANE DIFFUSION STUDIES WITH PROTEINS AND NUCLEIC ACIDS

Sir:

Previous studies[1,2] dealing with separation by differential diffusion through cellophane mem-

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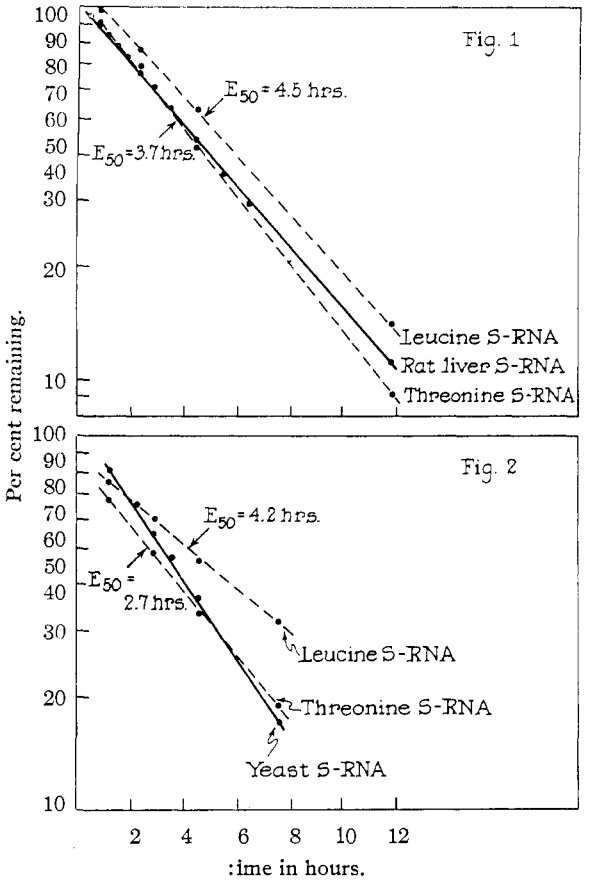

Fig. 1.—Rate of dialysis of rat liver SRNA through zinc chloride treated Visking cellophane: -·—·- measured by optical density at 260 mμ; - · - · - measured by incorporating activity in terms of counts per minute[6] for leucine SRNA and threonine SRNA.

Fig. 2.—Rate of redialysis of yeast SRNA: -·—·- O.D. at 260 mμ; · · · · · · incorporating activity for leucine SRNA and threonine SRNA.

branes have shown that the highest selectivity is obtained when the solute of interest will barely pass. A film cell providing maximum membrane area compensated for the slowness of the diffusion. A simple modification now provides stirring on both sides of the membrane, further shortening the time. A membrane of such porosity that 50% (E_{50}) of a sample of ribonuclease (mol. wt. 13,600) passes in 4 hours gave an E_{50} value of 9 hours for lysozyme (14,800).

Linear stretching of wet 20/32 Visking dialysis casing to near the break point before release gave tubing considerably smaller in size and less porous. Hydrostatic pressure applied to such tubing while it was being stretched linearly gave tubing larger and markedly more porous. Thus tubing with a ribonuclease E_{50} of a few hours could be stretched to give E_{50} values of about 6 hours for β-lactoglobulin (35,000). None of these would pass plasma albumin (67,000).[3]

Treatment[4] with $ZnCl_2$ for a short time gave a membrane with E_{50} value of a few hours for plasma albumin and longer treatment gave a similar value with the dimer of plasma albumin (134,000).

In studies with nucleotides and soluble ribonucleic acid (RNA),[5] salt solutions, contrary to our experience with proteins, have accelerated diffusion. Rat liver "soluble" RNA (SRNA) has not thus far passed untreated membranes under any conditions. After maximum stretching an E_{50} value of 18 hours was obtained with a 0.4% solution of SRNA in 1 molar NaCl. A membrane treated with $ZnCl_2$ to give an E_{50} value (no salt) of 7.5 hours with plasma albumin gave an E_{50} value (by optical density of 260 mμ) with both rat liver SRNA and yeast SRNA of nearly 4 hours in the salt solution (Fig. 1 and 2). Assay[6] for specific leucine and threonine incorporating activity was consistent with complete recovery. Re-dialysis of mid-fractions (Fig. 1 and 2) after recovery gave essentially the original escape patterns.

The data afford preliminary evidence for a degree of uniformity with respect to size for leucine and threonine incorporating RNA but detectable differences between them with the leucine one from both liver and yeast passing more slowly (Fig. 1 and 2). Equilibrium ultracentrifugation[7] with the RNA which passed through the membrane gave a Z average molecular weight of 30,000 ± 4,000.

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(3) This investigation was supported in part by a research grant, A-2493 B.B.C., from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

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(7) Performed by Dr. David Yphantis of the Rockefeller Institute.

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