The low temperature light emission properties of several metallo-derivatives of phthalocyanines, porphyrins and chlorophylls have been studied. The lowest triplet–singlet emissions of the latter two groups of molecules have been located and shown to be in accord with expected behavior based on spin-orbital perturbations introduced by heavy and paramagnetic atoms.[3,4,5] The results are summarized in Table I.

The phosphorescence of chlorophyll-*b* was reported earlier by Calvin and Dorough,[6,7] but could not be duplicated by later work.[8] Our work provides proof that the phosphorescence of chlorophyll-*b* reported earlier is a *bona fide* emission.

We observed a phosphorescence in chlorophyll-*b* with a first strong band at 8650 Å. (0,0-band at 11618 cm.$^{-1}$), using two chromatogrammed samples from different sources. Using pheophorbide-*a* as an analog of chlorophyll, we showed that its strong fluorescence (6701 Å., first band) is converted completely to phosphorescence in Cu^{++}-pheophorbide-*a*. Moreover, the phosphorescence occurs at 8675 A. (first strong band). This complete conversion in the presence of a paramagnetic ion, and the position of the emission, indicate that the phosphorescence observed in the pheophorbide and the chlorophyll are the lowest triplet → singlet emissions in these molecules.[3,5]

TABLE I

LUMINESCENCE DATA ON PORPHYRIN-LIKE MOLECULES, (ALL IN EPA GLASS[a] AT 77°K.)

Molecule	Wave length of first strong band, Å. Fluorescence	Phosphorescence
Etioporphyrin-II	6236	8060
Zn^{++}-Etioporphyrin-II	5730	7000
Cu^{++}-Etioporphyrin-II	None	6812
Ni^{++}-Etioporphyrin-II	None	6812
Phthalocyanine	6918	Not obsd.
Mg^{++}-Phthalocyanine	6705	Not obsd.
Zn^{++}-Phthalocyanine	6731	Not obsd.
Pheophorbide-*a*	6701	None found
Cu^{++}-Pheophorbide-*a*	None	8675
Chlorophyll-*a*	6645[b]	None found
Chlorophyll-*b*	6564	8650
Chlorophyll-*b*	6485[b]	..

[a] G. N. Lewis and D. Lipkin, THIS JOURNAL, **64**, 2801 (1942). [b] Room temp. in ether solution, F. Zscheile and P. Harris, *J. Phys. Chem.*, **47**, 623 (1943).

Phosphorescence was not found in chlorophyll-*a*, and that observed in chlorophyll-*b* was relatively weak, indicating a quantum efficiency of the order of magnitude of 0.1 or less under the conditions studied. However, in Cu^{++}-pheophorbide-*a* the quantum yield of phosphorescence is probably close to unity, as the emission was relatively intense. A detailed consideration of the role of chlorophyll in photosynthesis must include the possibility of strong *inter-molecular* spin-orbital perturbations. Thus, the low yield of phosphorescence in chlorophyll-*a* (*i.e.*, 0) and in chlorophyll-*b* (≤ 0.1) observed in dilute rigid glass solution at low temperatures does not rule out the lowest triplet state from participation in the photosynthetic reaction.

All observations were made using a Steinheil Universal Spectrograph GH, with f/3 coated glass optics, using three large coated glass prisms. The exciting light was the AH-6 high-pressure 1 kw. water-cooled mercury arc, monochromatized by suitable filters. Both steady excitation with filtered light, and intermittent excitation with a phosphoroscope of 10^{-4} sec. resolving time was used. In the case of Ni^{++} and Cu^{++} derivatives, only steady excitation yielded emission, indicating the shortening of T → S emission lifetime by the strong spin-orbital coupling in those compounds.

In the case of phthalocyanines, no phosphorescence was observed in the photographic infrared up to 9000 Å., it appears that the lowest triplet states of these molecules lie at longer wave lengths.

In the case of the metal derivatives of etioporphyrin-II, the strong fluorescence of the parent compound was partially quenched with consequent enhancement of phosphorescence[3,5] in the Zn^{++} derivative, while in the Ni^{++} and Cu^{++} complexes only (strong) phosphorescence was observed, as expected for strong spin-orbital coupling.

A complete discussion of the above results and the publication of the spectra obtained will be presented in a forthcoming paper.

The recent observations by Livingston, *et al.*,[9] on the transient absorption originating in excited chlorophyll molecules is thus most probably the triplet–triplet absorption, in accordance with similar observations made on other molecules.[10]

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(8) R. Livingston, "The Photochemistry of Chlorophyll," p. 179–196, in "Photosynthesis in Plants," edited by J. Franck and W. E. Loomis, Iowa State College Press, Ames, Iowa, 1949.
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(10) G. Porter and M. W. Windsor, *J. Chem. Phys.*, **21**, 2088 (1953).

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A WATER SOLUBLE SYSTEMIC INSECTICIDE O,O-DIETHYL *S*-2-ETHYLMERCAPTOETHYL PHOSPHOROTHIOLATE METHOSULFATE

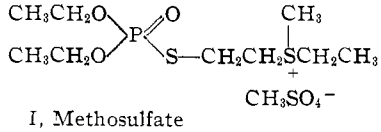
Sir:

In connection with studies concerning the chemistry and toxicology of the thiono- and thiol-isomers of Systox,[1] we have prepared and measured the insecticidal activity of O,O-diethyl S-2-ethylmercaptoethyl phosphorothiolate methosulfate (methosulfate of the thiol isomer). The product was obtained by heating equimolar amounts of O,O-diethyl S-2-ethylmercaptoethyl phosphorothiolate,[2] b.p. 106–108° (0.3 mm.), $n^{26}D$ 1.4926, and freshly distilled methyl sulfate on the steam-bath for one hour.[3] The phosphorothiolate

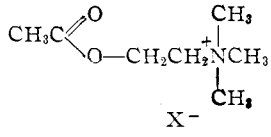
(1) Systox is the trade name given by the Chemagro Corp., New York, N. Y., for a technical mixture of O,O-diethyl O-2-ethylmercaptoethyl phosphorothionate and O,O-diethyl S-2-ethylmercaptoethyl phosphorothiolate.
(2) G. Schrader, "Die Entwicklung neure Insektizide auf Grundlage organischer Fluor und Phosphor-Verbindungen," Monograph No. 62, *Angewandte Chemie*, 1952.
(3) I. A. Usov, M. Z. Finkelshtein and V. N. Belov, *J. Gen. Chem. (U.S.S.R.)*, **17**, 2253 (1947).

methosulfate, $n^{29}D$ 1.5023, a thick viscous liquid, could not be induced to crystallize and was purified by repeated precipitations by dissolving it in chloroform and adding to a 10-fold excess of ether; calculated for $C_{10}H_{25}O_7PS_3$; P, 8.1; found, P, 8.3. The infrared spectrum[4] in chloroform solution showed strong absorptions for the P=O and P–O–C moieties at 1250 and 1015 cm.$^{-1}$, respectively, and a weak shoulder for the P–O–CH_2CH_3 moiety at 1160 cm.$^{-1}$.

The formation of the sulfonium salt increased the anticholinesterase activity by a factor of about one hundred fold: from a 50% fly-brain cholinesterase inhibition concentration of 3×10^{-6} M for the O,O-diethyl S-2-mercaptoethyl phosphorothiolate to a 3.3×10^{-8} M for the methosulfate. It is of interest to note the similarity in structure of the sulfonium ion (I) to the natural cholinesterase enzyme substrate acetylcholine (II).



I, Methosulfate



II, Acetylcholine

The product is extremely soluble in water and showed excellent systemic activity in the cotton plant. For the determination of the systemic action, the bases of young cotton plants (6-leaf stage) were treated with 5λ of the methosulfate. After various intervals leaves were picked, and contact toxicity in Munger cells and house fly-head cholinesterase inhibition by the leaf homogenates were determined.[5] The results are shown in Table I.

TABLE I

SYSTEMIC ACTIVITY OF O,O-DIETHYL S-2-ETHYLMERCAPTOETHYL PHOSPHOROTHIOLATE METHOSULFATE

Time after treatment	House fly-head cholinestearase % inhibition 1	0.1[a]	0.01[a]	Per cent. mortality in Munger cells after 48 hours *Heliothrips haemorrhoidalis*	*Tetranychus bimaculatus*	*Aphis gossypii*
24 hr.	71	51		0	90	100
48 hr.	96	93				90
1 week	100	100	93	95	90	100
2 weeks	100	100	100	80	70	100
3 weeks	100	100	100	20	90	100
6 weeks	97	97	48	5	80	100

[a] Leaf homogenate diluted 1 to 10 and 1 to 100 with water.

Evaluation of contact toxicity of the methosulfate to the house fly, thrips, and mosquito larvae, gave LC_{50} values of 0.4%, 0.01%, 0.001%, respectively. The intraperitoneal LD_{50} to the mouse was between 1 and 5 mg. per kg.

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(4) A Perkin-Elmer model 21 self-recording infrareds pectrophotometer was used in this work.

(5) R. L. Metcalf, R. B. March, T. R. Fukuto and Marion Maxon, *J. Econ. Ent.*, **47**, 1045 (1954).

FRACTIONATION OF DEOXYRIBONUCLEIC ACID (DNA) BY ION EXCHANGE[1]

Sir:

Recent evidence[2–6] clearly indicates that the total DNA of the cell is heterogeneous. Further exploration of this finding has been aided by the development of new fractionation procedures which afford greater resolution and are applicable to small quantities of material.

Highly polymerized calf thymus DNA[7] (*ca.* 1 mg./ml., *p*H 7) was applied to columns of ion-exchangers. The adsorbed DNA could not be eluted with concentrated NaCl solution at neutral *p*H from the strong-base anion-exchanger Dowex-1 Cl^-. It was eluted from the weak-base anion-exchanger Amberlite IR-4B OH^- with stepwise increase in NaCl concentration (0.1–3.0 M) yielding some 16–20 fractions, but this resin had several disadvantages. Of the substituted-cellulose derivatives[8] tested, the weak-acid cation-exchanger CM-cellulose had no affinity for DNA, but the weak-base anion-exchangers DEAE-cellulose[8] and ECTEOLA-cellulose[9] adsorbed it quantitatively from solution.

Because of its favorable capacity, low shedding blank, the essential reproducibility of elution pattern and high recovery of DNA obtained with neutral eluting solutions, ECTEOLA-cellulose appears to be the most promising chromatographic medium. Most of the calf thymus DNA adsorbed on it was eluted by either discontinuous or continuous change of concentration of sodium chloride solution (Figs. 1 and 2). DNA from pneumococcus possessing transforming activities[10] gave patterns similar to Fig. 2. The fractionation appears to depend, in part, on the molecular size or state of aggregation of the polynucleotide components of the nucleic acids. A mixture of mono-deoxyribonucleotides was completely eluted with 0.01 M phosphate, *p*H 7, devoid of sodium chloride, whereas a deoxyribonuclease digest of calf thymus DNA containing a large proportion of oligonucleotides required increases in sodium chloride concentration up to 0.22 M for quantitative elution (*cf.* Fig. 2). A highly polymeric ribonucleic acid (RNA) from pneumococcus exhibited the same heterogeneous type of behavior as did DNA. After treatment with ribonuclease, the remaining RNA fragments were also

(1) This investigation was supported by grants from the United States Public Health Service, and from the Aromic Energy Commission, Contract No. AT(30-1)-910.

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(8) H. A. Sober and E. A. Peterson, THIS JOURNAL, **76**, 1711 (1954).

(9) Peterson and Sober have prepared ECTEOLA-cellulose by treating alkaline cellulose with epichlorohydrin and triethanolamine (unpublished). The batch of exchanger used in these studies had a capacity of 7.2 mg. DNA per g. We are indebted to Drs. Peterson and Sober for their generous gifts of these exchangers, and to Dr. G. B. Brown for bringing their previously unpublished observations to our attention.

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