References

1. COHN, E. J., and EDSALL, J. T. *Proteins, Amino Acids and Peptides.* New York: Reinhold, 370–81 (1943).
2. GOODINGS, A. C., and TURL, L. H. *J. Textile Inst.*, **31**, T69 (1940).
3. TRISTRAM, G. R. *Advances in Protein Chem.*, **5**, 83 (1949).
4. ROTHEN, A. *J. Gen. Physiol.*, **24**, 203 (1940–41).
5. KING, A. T. *J. Textile Inst.*, **17**, T53 (1926).
6. WETTER, L. R., and DEUTSCH, H. F. *J. Biol. Chem.*, **192**, 237 (1951).
7. FEVOLD, H. L. *Advances in Protein Chem.*, **6**, 187 (1951).
8. ARMSTRONG, S. H., JR., *et al. J. Am. Chem. Soc.*, **69**, 1747 (1947).
9. MCMEEKIN, T. L., GROVES, M. L., and HIPP, N. J. *Ibid.*, **71**, 3298 (1949).
10. SCHWERT, G. W. *J. Biol. Chem.*, **179**, 655 (1949).
11. TAYLOR, J. F. *Federation Proc.*, **9**, 237 (1950).
12. STEIN, W. H., and MOORE, S. *J. Biol. Chem.*, **178**, 79 (1949).
13. PEDERSEN, K. O. *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 140 (1949).
14. ADAIR, G. S., and ADAIR, M. E. *Proc. Roy. Soc. (London)*, B, **120**, 422 (1936).
15. SVEDBERG, T., and FÅHRAEUS, R. *J. Am. Chem. Soc.*, **48**, 430 (1926).
16. PEDERSEN, K. O. *Biochem. J.*, **30**, 961 (1936).
17. BRAND, E., *et al. J. Am. Chem. Soc.*, **67**, 1524 (1945).
18. PUTNAM, F. W., LAMANNA, C., and SHARP, D. G. *J. Biol. Chem.*, **176**, 401 (1948).
19. KRISHNAMURTI, K., and SVEDBERG, T. *J. Am. Chem. Soc.*, **52**, 2897 (1930).
20. SVEDBERG, T., and STAMM, A. J. *Ibid.*, **51**, 2170 (1929).
21. LINDERSTRØM-LANG, K. *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 117 (1949).

Manuscript received March 14, 1952.

A Note on the Phosphorescence of Proteins

P. Debye and John O. Edwards[1, 2]

Baker Chemical Laboratory,
Cornell University, Ithaca, New York

As the literature on the fluorescence and phosphorescence of proteins is scanty, and since it is of interest to know more about this subject and its relation to protein denaturation, an investigation of the visible light emitted by proteins under ultraviolet excitation has been carried out.

Wels (*1*) and Vlés (*2*) reported that a blue fluorescence was observed when proteins were irradiated with ultraviolet light at room temperature. The intensity of the fluorescence (which is not strong) depends on the pH and the oxygen content of the solution and on the irradiation time. It can be excited by many different wavelengths of the ultraviolet region.

With compact animal materials such as nails, tendons, and cartilage, a distinct blue phosphorescence which lasts about 0.2 sec at room temperature has been reported (*3, 4*). The globular proteins and noncompact body materials such as muscle did not exhibit this phosphorescence.

We have found that many proteins emit a brilliant blue phosphorescence at low temperatures. However, no fluorescence in the visible range has been observed in any of our experiments.

The proteins used in this study were bovine serum albumin, egg albumin, gelatin, human γ_2-globulin, zein, human fibrinogen, silk fibroin, and keratin (human nail). Material containing protein such as bacteria (*Escherichia coli*), commercial yeast, "Witte" peptone, agar, and dehydrated beef muscle show the same phosphorescent properties as the individual proteins. The emission was observed with solid protein, with suspensions, and with solutions.

In order to find out which groups in the proteins are active, 18 amino acids were investigated. Of these, only the 3 common aromatic amino acids (tyrosine, tryptophane, and phenylalanine) gave indications of characteristic emissions. However, the remaining 15, including histidine, showed weak blue emissions which had the characteristics of those from tyrosine and tryptophane. Since it was found that as little as 10^{-9} g of tyrosine gives a discernible blue phosphorescence, it is our opinion that the blue emissions of these 15 are caused by trace amounts of the aromatic amino acids. Indeed, it seems that phosphorescence is a sensitive detector of certain impurities.

These experiments, unless stated otherwise, were carried out at the temperature of liquid nitrogen (77° K). A General Electric AH-6 mercury-vapor arc was used as the source of ultraviolet light. For the kinetic studies, an RCA 5819 multiplier phototube and either an oscillograph or a galvanometer have been employed, depending on the rate of decay. The spectra were determined with a Hilger constant deviation spectrograph and Eastman Kodak spectrographic plates.

At any particular pH, there are at least two exponential decay emissions from the majority of the proteins. Results with the oscillograph, although complicated, indicate that the lifetimes[3] are about 3 sec. Some experiments at the temperature of dry ice (193° K) were less complicated, and it was found possible to prove the monomolecular nature of the decay, the semilog plots being consistent and the decay constants being reproducible at that temperature.

The amino acid tryptophane has a bluish-white phosphorescence with a lifetime of about 3 sec at all pH values. The phosphorescence of tyrosine is brilliant and deep blue; it has a lifetime of about 3 sec in neutral and acid solutions, whereas the lifetime in alkaline media is 0.9 sec. The emission of phenylalanine also seems to be bluish-white, but its lifetime is much shorter, probably being less than 0.1 sec.

The visible spectrum of the protein phosphorescence is dependent on the pH, which fact may be attributed to the association of protons to the aromatic amino acids (*5*). In Table 1, some of the features of protein phosphorescence in alkaline media are pre-

[1] Present address: Technical Division, Electrochemicals Department, E. I. du Pont de Nemours and Company, Niagara Falls, N. Y.

[2] We gratefully acknowledge the donation of the human γ_2-globulin by J. W. Williams, of the University of Wisconsin, and also the fibrinogen and the bacteria culture by H. A. Seheraga and J. C. White, respectively, of Cornell University. Guy C. Bell, Jr., aided in some of the experimental work.

[3] The mean lifetime of an exponential decay is that amount of time necessary for the phosphorescence to fall to $1/e$ of its initial intensity.

sented. The relative intensities seem to be independent of the protein, for similar spectra were obtained with bovine serum albumin, egg albumin, and trypsin. The results in acid solution were less consistent, with the main maximum appearing at 4170 A. The phosphorescence spectra of tyrosine and tryptophane in alkaline media have been found to lie in the same wavelength region as the protein phosphorescence.

TABLE 1

PROTEIN PHOSPHORESCENCE SPECTRUM FEATURES

Wavelength (A)	Feature
4020	Marked shoulder
4180	Minor maximum
4200	Small minimum
4250	Small shoulder
4400	Broad, intense maximum
~ 4800	Cutoff

The exponential-decay phosphorescence of proteins can be attributed to tyrosine, tryptophane, and possibly phenylalanine. Since the emission is not sensitive to temperature changes, it appears to be an electronic transition. A long series of investigations was carried out by G. N. Lewis and his group on just such transitions of aromatic compounds. As the results here parallel theirs, it is assumed that the observed transitions are of the same type. The nature of this phosphorescence was reviewed by Kasha (*6*); Nauman (*5*) has studied the spectra, and McClure (*7*) investigated the transition rates. Their work indicates that the excited phosphorescent state is the lowest-lying triplet level.

A protein phosphorescence of very long duration has been observed along with the exponential-decay phosphorescence, but the mechanism is quite different. The long-lifetime emission is highly sensitive to temperature increases, and proceeds so rapidly at the dry ice temperature that it is not observed. According to Linschitz (*8*), the spectrum probably is the same as that of the exponential-decay phosphorescence. The presence or absence of this emission is a sensitive function of the pH, for it is strong in alkaline media but absent in acid media. It has been possible to study the rate of decay of the long-lifetime phosphorescence, and a report has been made elsewhere on the observed rate law and the associated mechanism (*9*).

The long-lifetime phosphorescence can be attributed to certain forms of the aromatic amino acids. Phenylalanine does not have a long-lifetime emission; tryptophane does in neutral and alkaline media, and tyrosine has this phosphorescence only in alkaline media. Further study of this phenomenon with many organic compounds has shown that the anilinium ion and free phenol forms are not active, whereas the free aniline base and phenolate ion forms are active, at least under the present conditions.

A complete description of the long-lifetime phosphorescence is not pertinent here, but a brief mention of its nature will be given, for we feel that it is related to the denaturation of proteins by ultraviolet light. The molecule absorbs a quantum of light and an electron is photo-ejected into the surrounding solid. At 77° K, the rate of return, which we believe to be diffusion-controlled, is slow; the phosphorescence occurs when the electron returns to the protein.

Harris (*10*) observed that most proteins take up oxygen when irradiated with light from the mercury-vapor arc, and that tyrosine and tryptophane absorb oxygen at a rapid rate. Gelatin (which contains little tyrosine and no tryptophane, and whose phosphorescence is much weaker than that of most other proteins) and most amino acids do not absorb oxygen.

Whether the oxygen reacts with the dissociated electron, with the protein which has lost an electron, or with the protein containing a group which has been excited into the triplet state is a question which cannot be answered with present information. It does seem quite probable that the intermediates in phosphorescence play a large part in the photo-oxidation of proteins and in their photodenaturation. Further, tyrosine and tryptophane are undoubtedly the main contributors to protein phosphorescence, both of the exponential and of the long-lifetime type.

References

1. WELS, P. *Arch. ges. Physiol.* (*Pflügers*), **219**, 738 (1928); *Chem. Abst.*, **22**, 4138 (1928).
2. VLÉS, F. *Arch. phys. biol.*, **16**, 137 (1943).
3. HOSHIJIMA, S. *Sci. Papers Inst. Phys. Chem. Research* (*Tokyo*), **20**, 109 (1933).
4. GIESE, A. C., and LEIGHTON, P. A. *Science*, **85**, 428 (1937).
5. NAUMAN, R. V. Ph.D. thesis. Univ. California, Berkeley (1947).
6. KASHA, M. *Chem. Revs.*, **41**, 401 (1947).
7. MCCLURE, D. S. *J. Chem. Phys.*, **17**, 905 (1949).
8. LINSCHITZ, H. Personal communication (1951).
9. DEBYE, P., and EDWARDS, J. O. *J. Chem. Phys.*, **20**, 236 (1952).
10. HARRIS, D. T. *Biochem. J.*, **20**, 288 (1926).

Manuscript received May 19, 1952.

The Presence of Toxins other than DDT in the Blood of DDT-poisoned Roaches[1]

James Sternburg and C. W. Kearns[2]

Department of Entomology,
University of Illinois, Urbana

It has been reported by Bot (*1*) that roaches (*Periplaneta americana* L.) in the prostrate stage of poisoning after topical application of DDT may contain sufficient DDT in their blood to produce typical DDT-poisoning symptoms and death in flies (*Calliphora erythrocephala* Meig.) injected with a volume of 20 μl of such blood. Our first attempts (*2*) to duplicate the work of Bot resulted in failure, presumably because we bled roaches too early in the prostrate stage of poisoning or allowed them to approach too close to death. We have since been able to obtain samples of blood from prostrate roaches which produce the effects described by Bot, but we have not been able consistently to obtain samples with a level of toxicity that

[1] Supported by funds from the Rockefeller Foundation.
[2] We wish to thank C. Ladd Prosser for his helpful advice and loan of equipment.