Fluorescence of Amino Acids

In the December 2, 1949, issue of *Science*, A. R. Patton, Elsie M. Foreman, and Patricia C. Wilson published observations which led them to the conclusion that fluorescence by dry amino acids is "an assumption which appears unwarranted."

While we are in agreement with their observations gained with glass plates and filter paper, we wish to point out some differences of interpretation of the results. Even if one is to disregard J. de Ment's report on fluorescence of amino acids in the solid state observed at wavelengths 3650 A–3600 A (de Ment, A. and Dake, *Ultraviolet light and its application.* New York: Chemical Publishing Co., 1942.) One has to contend that more than a simple browning reaction between the amino acids and the paper is involved. Many organic substances display similar browning after prolonged heating of the paper. Assuming that it is a Maillard (browning) reaction, it must be one between the amino group of the amino acid and the paper, which results in no critical change in the amino acid molecule, as the subsequent ninhydrin reaction is strongly positive. Moreover, it has been observed that N-substituted amino acids do not fluoresce (Platner and Nager. *Helv. Chim. Acta*, 1948, **31**, 2203; Gal and Greenberg. *Proc. Soc. exp. Biol. Med.*, 1949, **71**, 88). The reasoning that extraction of the amino acid does not affect the fluorescence of the spot can be countered by the fact that neither the amino acids nor the pyrimidines or purines are 100% extractable from the paper; the losses amount to 7%–9%. This can easily be checked by radioactively labeled material. The nonextractable material might possibly be amino acids that have undergone an advanced browning reaction to the point of cyclization. In any case, in our experience, some amino pterines, and even ammonium hydroxide (prepared from ammonia gas and distilled water) leave similar fluorescent spots behind on the paper. This relationship becomes more complicated if the effect of solvents is taken into consideration. Whether this effect can be so simply described as a *sine qua non* of the filter paper is still an open question.

Another possibility is the physical aspect. It is a well-known fact that many substances that show no fluorescence in the solid state or in solution produce emission when brought into contact with a finely porous insulator, such as cellulose, cotton, or fine aluminum oxide. The insulation assures adequate isolation of radiating molecules from their neighbors so that the excitational energy cannot be dissipated. This is interpreted to mean that the main part of the substance cannot absorb energy from the activated particles, whose concentration remains at an optimum, sufficient to fluoresce. However, this possibility remains to be tested in the case of amino acid adsorption.

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The Use of a Fathometer for Surveying Shellfish Areas[1]

Although echo or sonic sounding instruments were designed primarily for navigational purposes, some of them have proved useful for hydrographic surveys and to locate schools of fish (Adams, K. T. *Hydrographic Manual*, U. S. Coast and Geodetic Survey Spec. Pub. #143, 1942; and Tester, A. L. *Bull. Fish. Res. Bd.*, Can. LXIII, 1943).

A portable depth recording instrument, Model 808-J, manufactured by the Submarine Signal Company, was recently used to obtain profiles of Pamlico Sound, North Carolina, in a survey of the bottom for shellfish beds. Several transects were made between the major shoals of the sound in depths of water varying from 10 ft to 25 ft. The different intensities of the recordings, indicating hard or soft bottom, were checked with a leadline and material was dredged with a conventional oyster dredge. In general, when hard bottom rising a few feet above the surrounding mud was located, it was found to be an oyster bed. From some fathograms it was possible to distinguish shell bottom from hard sand, although scattered oysters lying on hard sand bottom were not readily distinguishable.

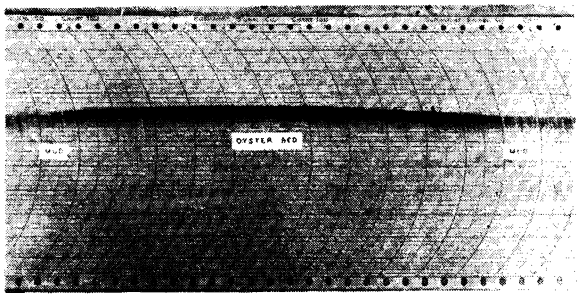

FIG. 1.

Fig. 1 is a photograph of a recording obtained while passing over an oyster bed. The depth of water overlying the bed is indicated in feet by the horizontal lines along the vertical scale A.

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[1] The Institute of Fisheries Research is indebted to the Submarine Signal Company of Boston, Massachusetts, for the loan of the fathometer.

The Chalkley Equation for Volume-Surface Ratios Applied to Open Figures

With reference to the excellent paper by Chalkley, Cornfield, and Park on the estimation of volume-surface ratios of closed three-dimensional figures (*Science*, 1949, **110**, 295), I should like to point out that the figures need not enclose finite volumes and that therefore the method has a somewhat broader field of application than the authors have indicated.

To show this, let V_x represent the volume enclosed by a surface of area S_x, which itself is contained in a substance y of volume V_y, and let h_x and h_y represent the hits recorded in V_x and V_y respectively. The equation developed by Chalkley *et al.* is then $V_x/S_x = rh_x/4c$, in which r is the length of the needle or test line and c is the number of cuts recorded. But $V_x/V_y = h_x/h_y$. Hence $V_y/S_x = rh_xh_y/4h_xc = rh_y/4c$, which is the ratio of the volume of y to its internal surface area. Obviously, V_x and hence h_x may approach arbitrarily close to zero, so that this expression is valid for finite surfaces enclosing infinitesimal or zero volumes. This being true, the expression is also valid for open three-dimensional figures.

An example of a case to which this equation might be applied is the determination of the internal surface area of an incipiently fractured material by means of measurements on random polished sections.

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The Structure of the Acropeptides

In the 1949 edition of R. A. Gortner and W. A. Gortner's *Outlines of Biochemistry* (New York: John Wiley & Sons, 1949) reference is made on page 366 to our work on the nature of the acropeptides, viz., the products of the nonhydrolytic breakdown of various proteins (Fodor, A. and Kuk, S. *Biochem. Z.*, 1931, **240**, 123; 1932, **245**, 350). Our claim that they are closed polypeptide rings with neither *terminal* amino nor carboxyl groups is reported to have been disproved by T. S. Reid (Ph.D. thesis, Univ. of Minnesota, 1943), who insists that they are products of an alcoholysis brought about by resorcinol. We therefore wish to state the following facts:

(1) We have noticed that heating proteins in media such as ß-naphthol or resorcinol results in products which contain as a maximum 2% of these substances. Use of these media was therefore abandoned in favor of dry glycerol.

(2) We were able to prove the ring structure of the acropeptides by means of a simple calculation, based on experimental data: The number of the hexone bases—histidine, arginine, and lysine—was estimated quantitatively in acropeptides from casein. Each of these amino acids possesses, in addition to the α-carboxyl amino group which partakes in the peptide linkages and therefore cannot be estimated by titration, one more group of amino or imino nitrogen which *is* titrable by Linderstrom-Lang's titration method. The various fractions obtained from the crude breakdown product, however, gave a considerable titer in acetone, which equaled exactly the sum total of the number of (amino + imino)-nitrogen groups located in the side chains of histidine (imidazolyl ring), arginine (guanidino group), and lysine (ε-amino group) estimated quantitatively. This, in our opinion, is the most conclusive evidence for the absence of either terminal amino or imino groups in those products. If strictly nonhydrolytic conditions were observed the lowest molecular weights of our various fractions were never below 900 (Fodor, A., Fodor, P. J., and Kuk-Meiri, S. *Enzymologia*, 1947, **12**, 101).

(3) It cannot be discussed here whether the acropeptides are already preformed in the proteins or whether they are formed in the process of heating by virtue of the existence of juxtaposed carboxyl and α-amino groups, a possibility which has been envisaged before (Fodor, A. *Enzymologia*, 1939, **6**, 207). It should be mentioned in this respect only that enzymatically their behavior is in good agreement with what is known today on the correlation between the occurrence of certain amino acid residues in proteins and synthetic peptides and the specific action of proteinases.

(4) The review in Gortner's book on protein structure refers in general to the more recent publications but those of our papers that he mentions date back to 1936, although we made a comprehensive report as late as 1947, which we have already cited.

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The Conversion of Phosphorylase A to Phosphorylase B by Blood Tryptase

Cori and Green (Cori, G. T. and Green, H. A. *J. biol. Chem.*, 1943, **151**, 31) have shown that the proteolytic enzyme found in muscle and spleen converts phosphorylase A to phosphorylase B. They observed also that trypsin, acting at pH 6.0–6.2 brings about this change. I have demonstrated that the blood protease known as "tryptase" and also called "plasmin" and "fibrinolysin" (Ferguson, J. H. *Science*, 1947, **105**, 488) forms phosphorylase B from phosphorylase A. I have made quantitative determinations of phosphorylase A according to Cori and Cori and Green (*J. biol. Chem.*, 1943, **151**) and of phosphorylase B after addition of adenylic acid. The tryptase was a lyophilized preparation obtained from the Parke, Davis and Company.

It is already known that both plant and animal substances can inhibit tryptase (Christensen, L. R. and MacLeod, C. M. *J. gen. Physiol.*, 1945, **28**, 559; Mirsky, I. A. *Science*, 1944, **100**, 198) and I have found that soybean antitrypsin is very effective in preventing tryptase from converting phosphorylase A into phosphorylase B. Glycogen also inhibits this tryptase action but not completely.

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