

Fig. 4. Quenching and amplification of fluorescence of rose bengal by chlorpromazine plotted against wave-length

It is known that if flavin and adenine are attached similar shifts and quenching occur in the absorption and emission spectra of the flavin<sup>8</sup>. These changes are attributed to the formation of a complex<sup>9</sup>. The complex formation between FMN and dihydrothioethyl was found to be connected to similar spectral changes<sup>10</sup>.

These changes were demonstrated as charge-transfer complex because no electron spin resonance signal was obtained<sup>10</sup>.

Therefore, we suppose that the lilac coloration of the xanthene dyes-chlorpromazine mixture is due to the formation of charge-transfer complex and that there is sufficiently strong interaction between the participating substances to abolish the photosensitization by stains<sup>7</sup>.

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<sup>1</sup> Szent-Györgyi, A., *Introduction to a Submolecular Biology* (Academic Press, New York, 1960).

<sup>2</sup> Zepalov, V. F., and Shlyapintokh, V. Ya., *Akad. Nauk. U.S.S.R.*, Ser. Chem. No. 4, 637 (1959).

<sup>3</sup> McLaren, A. D., and Shugar, D., *Photochemistry of Proteins and Nucleic Acids* (Pergamon Press, Oxford, 1964).

<sup>4</sup> Lippay, F., *Pflügers Arch. Ges. Physiol.*, **229**, 173 (1932).

<sup>5</sup> Rosenblum, W. I., *J. Cell. Comp. Physiol.*, **55**, 73 (1960).

<sup>6</sup> Lyudkovskaya, R. G., and Pevzner, L. P., *Biofizika*, **9**, 580 (1964).

<sup>7</sup> Lábos, E. (unpublished results).

<sup>8</sup> Whitby, L. G., *Biochem. J.*, **54**, 437 (1953).

<sup>9</sup> Beinert, H., *A Symposium on Light and Life*, edit. by McElroy, W. D., and Glass, Bentley, 163 (The Johns Hopkins Press, Baltimore, 1961).

<sup>10</sup> Searls, R. L., and Sanadi, D. R., *A Symposium on Light and Life*, edit. by McElroy, W. D., and Glass, Bentley, 157 (The Johns Hopkins Press, Baltimore, 1961).

<sup>11</sup> Guth, P. S., and Spirtes, M. A., *Intern. Rev. Neurobiol.*, **7**, 231 (1964).

<sup>12</sup> Szent-Györgyi, A., *Bioenergetics* (Academic Press, New York, 1957).

### Is the Antihemophilic Globulin a Protein?

THE antihemophilic globulin (AHG), in electric fields migrating in the  $\beta_2$ -fraction, and being precipitated from blood plasma by alcohol in fraction I (ref. 1) is generally considered to be a protein. Our laboratory has for a number of years been occupied with the problem of its purification<sup>2,3</sup> with the final aim of contributing to the elucidation of its structure, and, if possible, to the mechanism of its interaction with other blood-coagulation factors.

For a long time ECTEOLA-celluloses of various capacities were used in the purification experiments; later on we turned to DEAE-celluloses because of their greater protein-binding capacities. During these experiments it became clear that the higher the ion-exchange capacity, the higher the salt concentration which could be used during the absorption and washing stages of the chromatographic procedure, without the AHG leaving the column unadsorbed. In other words, AHG could still be adsorbed at relatively high salt concentrations, which permitted

most of the proteins and other impurities to flow through the column unadsorbed. A rather high ion-exchange capacity, namely, 3.2 m.equiv./g, was then found in DEAE-'Sephadex A-25' (coarse). This enabled us to work at a sodium chloride concentration of 1.6 per cent. As long as the columns were operated at about their maximum AHG-binding capacity (fraction I from 100 ml. plasma per 15 g of DEAE-'Sephadex') the preparations obtained showed absorption spectra similar to those of proteins, but lacking the peak at 280 m $\mu$  (cf. ref. 4). When the amount of plasma per column was decreased, the protein character of the absorption spectrum disappeared altogether (Fig. 1).

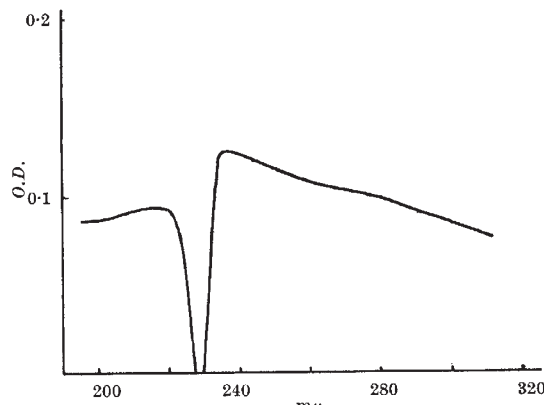


Fig. 1. Absorption spectrum of an AHG-preparation; 1.5 x normal activity

Nevertheless, such preparations had biological activities of about 150 per cent (normal plasma = 100 per cent). Some of them were hydrolysed with 5.7 N hydrochloric acid at 105°C for 18 h. Two-dimensional paper-chromatograms<sup>5</sup>, developed with ninhydrin, showed the presence of only 4 faint spots, which could not very easily be identified with any of the well-known amino-acids.

Since the homogeneity of the AHG-preparations is by no means certain, speculations about the amino-acids and the absorption spectra are not helpful; further investigations are necessary to clear up the present situation.

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<sup>1</sup> Cohn, E. J., et al., *J. Amer. Chem. Soc.*, **68**, 459 (1946).

<sup>2</sup> van Creveld, S., et al., *Thromb. et Diath. Haemorrhag.*, **6**, 282 (1961).

<sup>3</sup> Veder, H. A., *Chem. Weekbl.*, **60**, 261 (1964).

<sup>4</sup> Veder, H. A., *Maandschr. Kindergeneesk.*, **32**, 446 (1964).

<sup>5</sup> Smith, I., *Chromatographic and Electrophoretic Techniques*, **1** (London and New York, 1960).

### N-Hydroxy-2-fluorenylbenzamide, an Arylhydroxamic Acid with High Carcinogenic Activity

FOR investigations of the mechanism of action of carcinogenic arylhydroxamic acids we have prepared the new hydroxamic acid, N-hydroxy-2-fluorenylbenzamide (N-OH-2-BAF) (Ia), an analogue of the carcinogen, N-hydroxy-2-fluorenylacetamide (N-OH-2-AAF) (Ib). We have tested this compound for its carcinogenicity and

