system, such as blood serum, the quantitative results obtained by paper electrophoresis with LG will not be reliable.

The only advantage of LG is the absence of a coloured background.

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Nigrosine as a stain for quantitative estimation of serum proteins in paper electrophoresis

To the various dyes used for staining proteins in paper electrophoresis, another one has recently been added, namely nigrosine (NIG), which was proposed by ORTEGA in a short note[1].

The present paper deals with a study of the general properties of this dye, particularly its interaction with serum proteins on paper, and the results are compared with work carried out in recent years[2,3].

The main purpose was to check the dye-binding capacity of proteins at varying protein concentrations and surface areas on paper.

Materials and methods

Nigrosine. This dye is not a pure chemical compound, but a mixture of at least two dyes, induline violet and a yellow dye. The first is the sodium salt of a sulphonic acid derivative of the alcohol-soluble induline (indulines are phenylamine derivatives of safranines[4]). The NIG used in our experiments was a Merck product, which after qualitative chromatographic analysis appeared as a mixture of two deep blue components.

The absorption analysis of the dye in 0.01 *N* NaOH was made in a 1-cm cell, at room temperature, in the Beckman spectrophotometer model DU.

Serum proteins. Human albumin and γ-globulin fractions (by the courtesy of Istituto Sieroterapico Italiano) were used. The electrophoretic homogeneity was checked by free electrophoresis according to Tiselius. Two 5% stock solutions of the proteins were made in 0.15 *M* NaCl. The protein concentration was determined according to GORNALL *et al.*[5].

Paper. Whatman No. 1 was used in all experiments.

Estimation of dye uptake. Dilutions ranging from 0.5 to 5% were prepared from the albumin and γ-globulin stock solutions. By means of an Agla microsyringe each solution was applied, in duplicate, to paper strips 4 cm wide, as spots of 5, 10, 20, 30, 40 µl. The paper strips were divided by pencil into squares of 4 × 4 cm, and the protein solutions were applied in the center of each square. The protein spots were first dried in air, then at 105° in the oven for 15 min. Subsequently, they were placed for 10 min in a solution of NIG (1% in 1% acetic acid). The paper strips were washed in 1% acetic acid until the background paper was pale blue, after which they were allowed to dry at room temperature. The long and the short axis of the spots were accurately measured, and the surface area was calculated on the assumption that, when not perfectly circular, the spots were approximately elliptical. The paper strips were cut in square pieces (4 × 4 cm) and the dye eluted by soaking them in 10 ml of 0.01 *N* NaOH for 20–25 min.

Recovery experiments showed that the elution was almost complete. Whenever the extinction exceeded 0.800, the resulting blue solutions were diluted by adding known amounts of 0.01 *N* NaOH.

The amount of nigrosine eluted from each spot was determined spectrophotometrically at 560 mµ by comparison with a standard curve. This curve was prepared with solutions of NIG in 0.01 *N* NaOH with regularly increasing concentrations from 0 µg to 50 µg per ml; the slope of the curve was 0.0112.

Calculation of results. For each protein concentration two diagrams were made. In one the weight of the dye eluted was plotted against the protein weight and in the other against the area of each spot. The best fitting curves were drawn and the slopes measured; these represent, in the one case, the weight of dye bound to unit weight of protein, and in the other, the weight of dye distributed on the unit area of the protein spot.

Results

Absorption spectrum. Fig. 1 shows the absorption spectrum of NIG in 0.01 *N* NaOH. The spectral analysis was made within 15 min after the preparation of the dye solution and 24 h after. The absorption maximum is at 560–565 mμ and, at this wavelength, $E^{1\%}_{1\,cm}$ is 170. This latter value is very close to that of Azocarmine B. It is an overall and approximate value, comprising the various components of the mixture, and depending on the particular sample used in our experiments.

Dye uptake. Fig. 2 and Fig. 3 show the NIG uptake by albumin and γ-globulin, respectively, in terms of μg NIG/μg protein, and μg NIG/mm² spot as a function of protein concentration. The curves obtained from albumin and γ-globulin are neither linear nor completely superimposed.

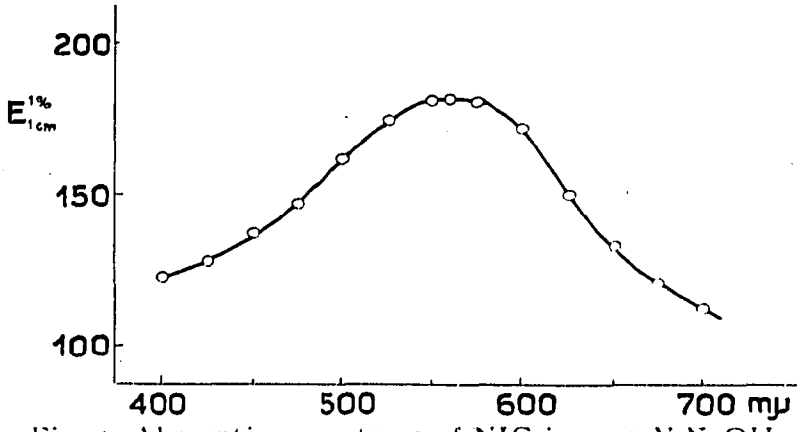

Fig. 1. Absorption spectrum of NIG in 0.01 *N* NaOH.

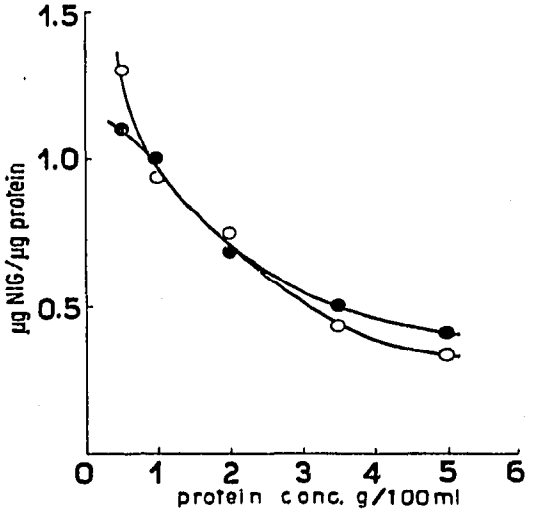

Fig. 2. Uptake of NIG by albumin (—○—○—) and by γ-globulin (—●—●—) per unit weight.

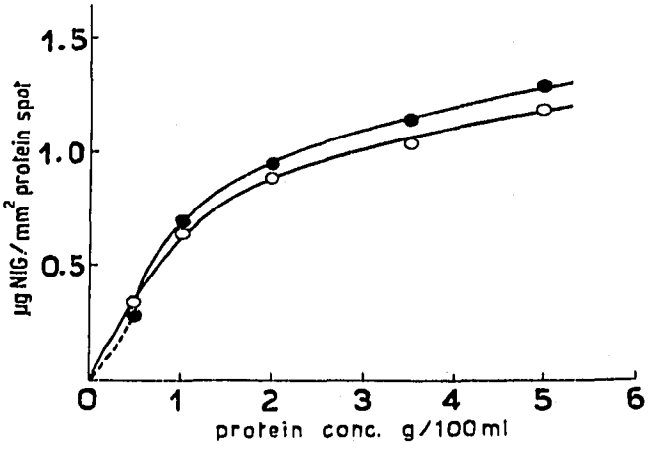

Fig. 3. Uptake of NIG by albumin (—○—○—) and by γ-globulin (—●—●—) per unit area.

The dye uptake by γ-globulin (Fig. 2) appears to be less than that of albumin at the lowest concentration; the reverse was found at higher concentrations. This is obvious also from Fig. 3.

Discussion

Our results show that the dye-binding capacity of protein for NIG is the highest so far observed among dyes used in paper electrophoresis[2,3].

The dye uptake, however, varies with protein concentration as well as from one protein to another, at least for the lowest and the highest concentrations. At the lowest concentrations, the different binding capacity of albumin and γ-globulin can most likely be ascribed to the chemical nature of the dye, whereas at the highest concentration, where the behaviour is reversed, another factor may be involved, namely the protein density on paper. This effect was previously observed in the case of bromophenol blue[2,3].

In addition to this behaviour of NIG with regard to different proteins, there are some other factors that must be considered. NIG does not satisfy the rest of the criteria of a dye suitable for routine work. Although it is cheap, easy to use and stable, it does not stain only protein, but paper as well. Furthermore, since it is a mixture of different dyes, its behaviour is subject to variation from one batch to another, a property it has in common with a great number of dyes currently used.

We are therefore of the opinion that NIG cannot be recommended as a stain for the quantitative determination of protein in paper electrophoresis.

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Chromatographic evidence for the existence of a protactinate ion

So far no evidence for the existence of a protactinate seems to exist, except for the observation that the yield of Pa_2O_5 is decreased if this substance is washed with alkali.

We wish to present here some chromatographic and electrophoretic evidence that Pa(V) may be dissolved in alkali and that such a solution has similarities to a solution of tantalate. The isotope of Pa used in these experiments was a preparation of ^{233}Pa in 6 N HCl which was shown in numerous experiments to be free from fluoride and radioactivities other than ^{233}Pa. When the 6 N HCl solution is evaporated and stirred with 5 N NaOH, a suspension is obtained, which when placed on a paper strip and developed with N NaOH stays at the point of origin (Fig. 1). Another sample

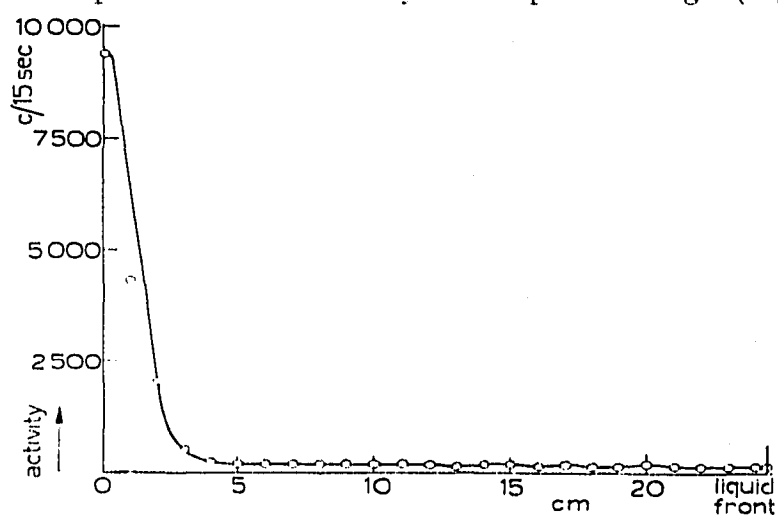

Fig. 1. Distribution of ^{233}Pa on the chromatogram when the tracer is mixed with 5 N NaOH solution. Whatman No. 1 paper. Developing solvent N NaOH.