SHORT COMMUNICATIONS

Unspecific staining of paraproteins by picric acid

Paraproteinaemia can often be diagnosed by means of paper electrophoretic analysis of the serum proteins. Further investigations with the ultracentrifuge and with starchgel or immuno-electrophoresis are, however, necessary sometimes. These techniques are rather specialised and beyond the possibilities of most routine laboratories.

YEOMAN[1] developed what seemed to be a useful screening method; he reported the selective staining of myeloma paraproteins with a pyronin–methyl green solution after paper electrophoresis. Other investigations showed that in some cases of non-myeloma paraproteinaemia, such as Waldenström's macroglobulinaemia, a positive staining also may be obtained, while in other cases no staining reaction can be detected[2]. For these reasons, this method is of limited value in the screening of sera for paraproteinaemia.

ŠACHMAN AND MALASKOVÁ[3] recently described a very simple staining technique with an alcoholic picric acid solution which they claimed to be specific for paraproteins. The authors did not investigate, however, whether the local high protein concentration in the paraprotein band could be responsible for the proposed specificity. In that case this method again would be of very limited value.

The results of an investigation into this possibility are described below.

MATERIAL AND METHODS

Control sera were obtained from blood donors. The sera from 24 patients used in this study all contained considerable amounts of paraprotein as verified by immunoelectrophoresis. 10 sera contained paraprotein of the β_{2A}-type, 6 of the γ-type, 6 of the β_{2M}-type and 2 of the Bence-Jones type. They were stored at -23° for 1 month to $1\frac{1}{2}$ year.

The human albumin and the human normal γ-globulin were commercial preparations*.

A purified preparation of a γ-myeloma paraprotein was obtained by chromatography on DEAE-cellulose of fresh serum from patients. Elution was carried out as described by FAHEY AND HORBETT[4] with a phosphate buffer pH 8.0 and a concentration gradient from 0.02 to 0.3 *M*. Immunoelectrophoresis revealed only the presence of a small quantity of normal γ-globulins apart from the paraprotein component.

Paper electrophoresis was performed on Whatman No. 1 paper. Electrophoresis time was 5 h, and the applied voltage 9 V/cm. The protein fractions were denatured by drying at 120° for 30 min.

Amidoblack staining was performed as described by DE JONG[5]. Picric acid staining was carried out substantially as described by ŠACHMAN AND MALASKOVÁ[3]. Picric acid was washed out with 3 subsequent batches of distilled water instead of with running tap water. This modification proved necessary to obtain reasonably reproducible results.

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The amount of dye bound by a protein fraction was found by cutting out the fraction concerned and eluting the dye with a solution which contained 500 ml of 96% ethanol, 20 g of NaOH and 500 mg of EDTA/l.

The optical density of the resulting solutions was measured at 530 mμ for amidoblack and at 460 mμ for picric acid.

RESULTS

The control sera showed after staining of the strip with the picric acid solution one yellow spot corresponding to the albumin fraction. In the sera from patients a

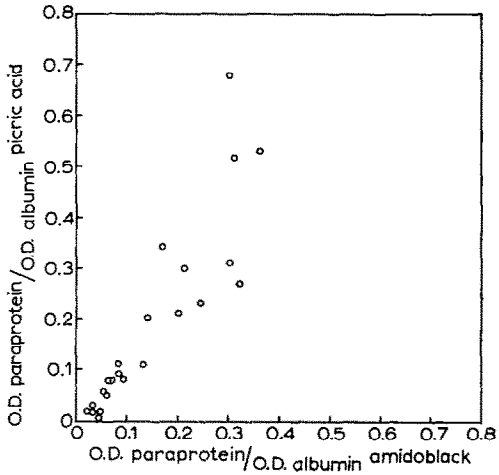

Fig. 1

second spot corresponding to the paraprotein band was found. In order to examine a possible correlation between the quantity of protein present in the fractions under consideration and the amount of bound picric acid, the strips were cut lengthwise after electrophoresis. One half was stained with amidoblack and the other half was treated with picric acid. The picric acid spots were cut out as well as the corresponding amidoblack areas. The dyes were extracted and the optical densities of the dye solutions measured.

The results are given in a scatter diagram (Fig. 1). The data are expressed as the quotient of optical densities of the paraprotein component and of the albumin fraction. A strong correlation ($r = 0.88$; $P < 0.001$) was found.

Some further experiments were carried out with human albumin, normal human γ-globulin and isolated γ-myeloma paraprotein to eliminate a possible difference in regard to their binding capacity for picric acid. Electrophoresis was omitted in these studies. The proteins were applied in different quantities on separate equal areas of the same Whatman No. 1 strips. After fixation, the paper strips were stained with the picric acid solution, the yellow spots cut out, the stain extracted and the optical density of this solution measured. The results are given in Table I. From these results it can be seen that the amount of dye bound by the protein fractions depends only on the total quantity of protein present.

Finally, strips obtained in the manner described above, were washed in distilled

water for different times, before the dye was extracted and the optical density measured. The data obtained from these experiments (Table II) clearly show that the affinity for picric acid of equal concentrations of normal and abnormal γ-globulin is exactly the same.

TABLE I

	Applied (μg)	*Concn./2.5 cm²* (μg)	*O.D. 460 mμ* $\times 10^3$
P	362	724	80
γ	362	724	91
P	181	362	43
γ	181	362	43
P	181	181	42
γ	181	181	47
P	91	181	13
γ	91	181	14
Alb	181	181	45

P, myeloma γ-globulin.
γ, normal human γ-globulin.
Alb, human albumin.

TABLE II

	Concn./2.5 cm² *in μg*	*O.D. 460 mμ* $\times 10^3$ *Elution time*	*3′*	*4′*	*5′*	*6′*	*7′*
P	181		47	26	16	9	4
γ	181		44	24	15	6	6
Alb	181		56	31	21	12	6

P, myeloma γ-globulin
γ, normal human γ-globulin
Alb, human albumin

DISCUSSION

It is obvious from the results of our experiments that paraprotein components are not specifically stained by picric acid. A positive staining reaction, which depends largely upon the length of time of the washing-out procedure, therefore only indicates an area of a certain concentration of proteins.

The application of this method has therefore no advantage over well-established protein-staining methods such as amidoblack or azocarmine.

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