nexion it is often important to know whether or not the plate contains a precipitate which is not visible either macroscopically or microscopically (cf. interference microscopy). Various fixatives have been employed in order to detect or accentuate an immunological reaction before proceeding to qualitative studies. Acetic acid and alcohol in various concentrations have been most commonly used for this purpose.

During work on an immunological system in which one could *a priori* expect a very weak precipitation reaction several fixatives were tested. These were chosen with consideration of their physical and chemical effects upon the various components in the system. It appeared that a combination of glacial acetic acid and saturated picric acid solution was preferable in those instances in which a quantitative reading of a precipitation reaction involving mainly proteins is desired.

The most satisfactory combination was found to be 6 per cent glacial acetic acid and 94 per cent saturated picric acid solution ; pH = 2·1. In these proportions the penetrating effect of acetic acid and the protein-precipitating effect of picric acid appear to advantage. These acids seem to complement each other in other respects as well. (At this pH the protein-precipitating effect of picric acid is good and pH is not appreciably altered by the precipitating reaction.)

If this method is to be used when serum forms one of the immunological components, the serum must be highly diluted. If this is not done, the gel plate must be washed before being fixed (cf. agar gel with serum added) otherwise interpretation is made impossible by the large amount of precipitate originating from the other serum proteins.

In addition to its sensitivity, the method described here offers other advantages such as a short fixation time and a result which can easily be interpreted and recorded.

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Scanning of Paper Electrophoretograms after Protein Dyeing with Bromocresol Green

THE blue colour of bromocresol green-dyed protein developed by exposure to ammonia vapour is not stable, and elution of the dyed protein has been used to obtain an electrophoretic curve from paper electrophoretograms of serum dyed with bromocresol green. One disadvantage of this is that the paper strip is destroyed when it would have been of value as a record for reference.

The use of an agent other than ammonia vapour was investigated with the view of stabilizing the blue colour of such dyed protein so that scanning could be carried out.

An organic base, with which the strip could be easily impregnated, strong enough to develop the blue colour of bromocresol green and which was miscible with the scanning oil, was looked for. Of several which were tried diethylamine was found to be suitable and the following method of use was found satisfactory.

The electrophoretograms were dried and then dyed according to the method described by Franglen and Martin[1]. The dry strips were then dipped in a solution of 10 per cent v/v solution of diethylamine in diethyl ether. The ether was allowed to evaporate and the blue colour of the bromocresol green-dyed protein developed. A series of dyed strips can be brought to this stage ready for scanning. The strips were then oiled for scanning using the mixture described by King and Wootton[2].

Scanning was carried out using an *E.E.L.* scanner with an Ilford 205 filter. After scanning, the oil and diethylamine were removed from the strips with either a 1 : 1 mixture of acetone and diethyl ether or benzene. The acetone used must be free from water otherwise diffusion of the dyed protein bands will occur. The strips were then air-dried ready for storage.

It was found that the blue colour remained stable for several weeks. This was probably due to the oil and diethylamine being removed by a non-ionic solvent leaving the bromocresol green in its blue form.

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[2] King, E. J., and Wootton, I. D. P., "Micro-analysis in Medical Biochemistry", third ed. (Churchill, 1956).

Chromatography of Xanthene Dyes

RECENTLY, Lima and Pieroni[1] have directed attention to the heterogeneous character of the dye rose bengal, which, when labelled with iodine-131, is used in tests of hepatic function[2,3]. Applying the ethanol–ammonia solvent system of Ishida *et al.*[4], they claim resolution of this dye into four components, three coloured and one colourless.

In the course of an investigation of the phloxin/tartrazine method, described by Lendrum[5] for the staining of connective tissues, I have had occasion to examine chromatographically most of the xanthene dyes in common use, and feel that my results may be of interest in relation to the observations quoted above.

A slight modification of the solvent system of Ishida *et al.*[4] was found to be the most useful ; this consisted of 0·880 ammonia, ethanol and water in the proportion 5 : 10 : 85 (by volume). Paper chromatograms were run on Whatman No. 1 paper 20 × 40 cm. by the descending method of Consden *et al.*[6] with the modification that the dye was applied as a thin line at the starting point. Circular chromatograms by a variation[7] of the method of Rutter[8] were found to be convenient for the rapid comparison of different batches and gave good separations. R_F values tended to show some variation, and the values quoted below are the mean values of several results.

Commercial halogenated fluoresceins examined included eosin *Y* ([*R*]-tetrabromofluorescein), erythrosin *R* ([*R*]-di-iodofluorescein) and erythrosin *B* ([*R*]-tetraiodofluorescein). The first of these dyes showed two coloured components, R_F values 0·16 and 0·22 ; the second gave five components, R_F values 0·08, 0·15, 0·26, 0·49 and 0·64 ; the third dye gave two components with R_F values 0·08 and 0·15.

Studies made on the partial halogenation of fluorescein with bromine and iodine in aqueous alkali showed that even at the stage where only one molecular proportion of halogen had been added, the reaction mixture could be resolved chromatographic-