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Note

Trypan blue staining of proteins fractionated by acrylamide disc gel electrophoresis*

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Electrophoretically separated proteins have been detected visually by binding with various dyes. The most widely used staining methods involve either Coomassie brilliant blue R250 or Amido Black. Of these, the former is known for its sensitivity since the dye can detect[1] a protein concentration down to 0.5–2 $\mu g/cm^2$. However, both of these dyes are frequently employed in both applied and fundamental research. Trypan blue is a stain which is used more often for nuclear staining than for proteins. This paper presents the results obtained by staining protein discs with Trypan blue, and they are compared with the results obtained with the conventional triphenylmethane dye, Coomassie brilliant blue R250.

EXPERIMENTAL

The sources of protein were (1) extracts from the actively growing cultures of *Aspergillus fumigatus* and *Rhizoctonia solani*; (2) the plasmodium of *Physarum* sp.; (3) rice grain albumin; (4) human serum; (5) rabbit serum.

The protein contents of the mycelial extracts, plasmodium as well as rice grains, were estimated using the method of Lowry *et al.*[2]. 50 μg of protein was used for serum samples, and 100 μg of protein was layered on top of the gel for other samples. All the samples were electrophoresed following the method of Davis[3]. In all cases a 7.5% gel was used. The following procedures were adopted. (a) Prefixation with 12.5% TCA for 1 h. (b) No prefixation —direct exposure to the dye solution. Trypan blue (Allied Chemical, N.Y., U.S.A.) was prepared in three concentrations, *viz.* 0.030%, 0.015%, and 0.010% in 25% methanol and 7% acetic acid. Of these three concentrations 0.010% was found to be ideal and hence this concentration was selected for comparison with Coomassie brilliant blue R250 (Sigma, St. Louis, Mo., U.S.A.). Coomassie brilliant blue was prepared at a concentration of 0.025% in 25% methanol and 7% acetic acid. In all the combinations gels were stored overnight and destained subsequently for 20–30 h using the same solvent system without the dyes. Then the gels were scanned, using a Joyce Loebl Chromoscan, and photographed.

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RESULTS AND DISCUSSION

The results show that Trypan blue has high affinity for proteins separated by acrylamide disc gel electrophoresis (Figs. 1–5). It binds with protein to form blue-black complexes which are quite sharp, in contrast to the blue-violet complexes formed by Coomassie brilliant blue. When the gels were exposed to staining solution without prefixation there was no loss of dye from the Trypan blue bound protein discs even after 30 h of exposure to destaining solution, indicating the stability of these complexes. On the contrary, brilliant blue diffused out of the bound proteins even after 20 h exposure to destaining solution.

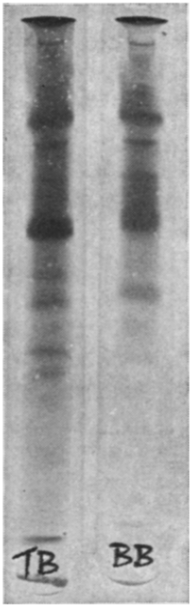
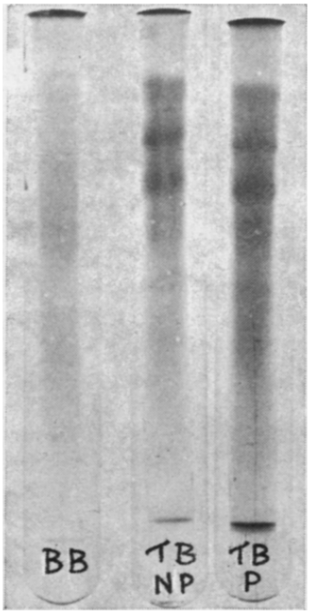


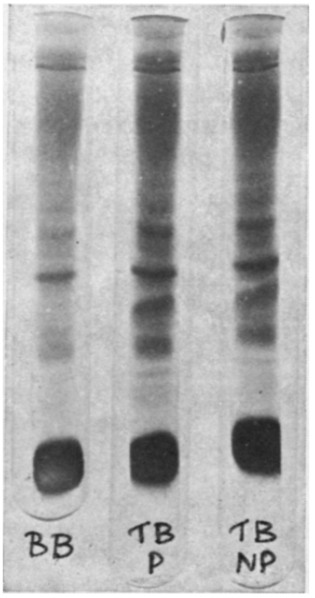
Fig. 1. Electrophoretic separation of mycelial proteins of *Rhizoctonia solani*. TB, Trypan blue; BB, Coomassie brilliant blue.

Fig. 2. Trypan blue staining of plasmodial proteins of *Physarum nicaraguense*. P, prefixation; NP, no prefixation.

Brilliant blue did not stain the background to the same extent as Trypan blue, hence the latter took more time (30 h) for destaining than the former (20 h). In fact, most of the available dyes did not satisfy this criterion and so such stains are not much in vogue. Be that as it may, densitometer scanning of Trypan blue stained proteins revealed sharper peaks than the conventional triphenylmethane dye (Figs. 6–9). Furthermore, the complexes of Trypan blue stained rice albumins, fungus proteins and serum samples are better suited to photographic recording since they show greater colour contrast than the brilliant blue bound proteins (Figs. 1–5).

Once this stain has been prepared, it can be used up to five times without much loss of sensitivity, which reveals another advantage over brilliant blue, which lost its





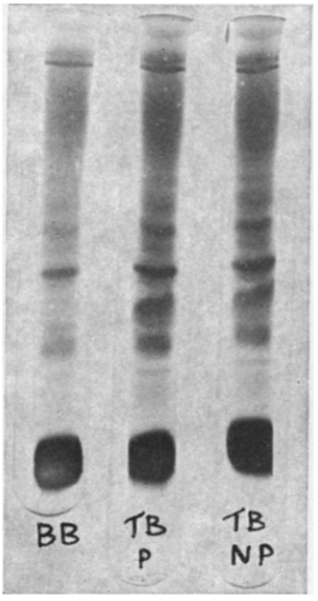


Fig. 3. Electrophoretic separation of rice albumins.

Fig. 4. Electrophoretic separation of rabbit serum proteins.

Fig. 5. Electrophoretic separation of human serum proteins.

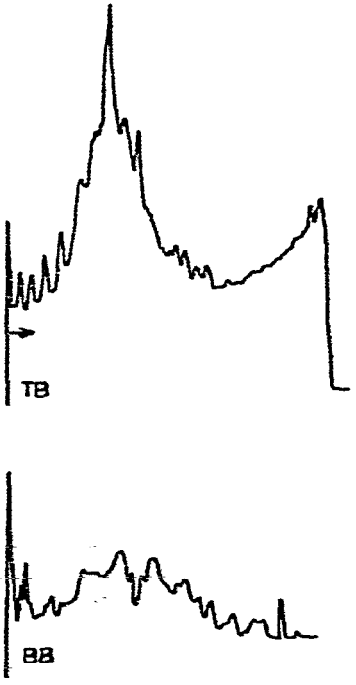


Fig. 6. Densitometer scanning of mycelial proteins of *Aspergillus fumigatus*.

Fig. 7. Densitometer scanning of mycelial proteins of *Rhizoctonia solani*.

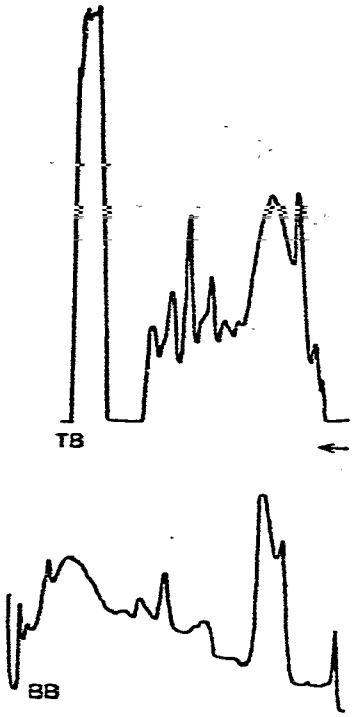


Fig. 8. Densitometer scanning of rabbit serum proteins.

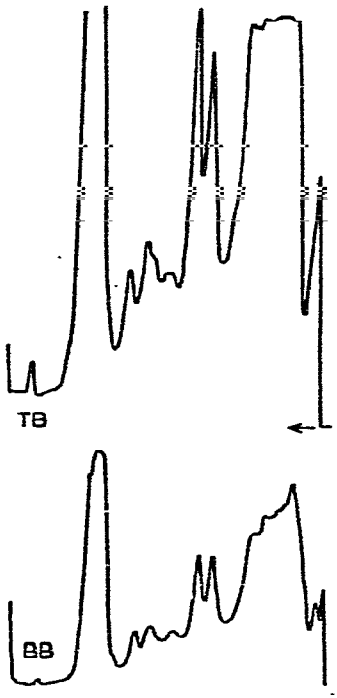


Fig. 9. Densitometer scanning of human serum proteins.

sensitivity after just two consecutive staining procedures. This attribute of Trypan blue warrants further research because it involves conservation of the dye, which is required in such a small amount.

Since fixation and staining are functions of specific chemical properties of proteins[4] it was considered that proteins prefixed with 12.5% trichloroacetic acid has an advantage over the unfixed ones. Prefixation did show sharp resolution but this step is not an absolute requirement. Nevertheless, prefixation reduced the time taken for destaining probably by forming precipitates with proteins and thereby preventing the diffusion of separated proteins into the gels. This is true for all proteins regardless of their sources.

Originally Trypan blue was used for staining nuclear materials of plants, fungal cell walls and viruses in infected plants tissues[5]. Implicit in the present finding is the fact that Trypan blue staining offers considerable promise for proteins separated by polyacrylamide disc gel electrophoresis.

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