

Table 1. EFFECTS OF DIPHENYLAMINE ON THE GROWTH OF *Rhodotorula gracilis*

Mgm. diphenylamine in 1,000 ml. medium	Dry weight of cells in 10 ml. medium
0	71.2
5	70.5
10	70.8
20	71.0
30	70.2
40	69.8
45	69.5
50	1.2

Table 2. FAT CONTENT OF CELLS OF *Rhodotorula gracilis*, GROWN IN THE PRESENCE OF DIPHENYLAMINE

Mgm. diphenylamine in 1,000 ml. medium	Per cent fat in the dry weight of the cells
0	60.5
5	61.0
30	59.8
45	57.0

carotenoids. In these conditions, however, the fat synthesis is not impaired, as shown by fat content determinations (cf. Table 2). Even with the highest concentration of diphenylamine, namely, 45 mgm. in 1,000 ml., the fat content of the cells is only insignificantly decreased.

In agreement with Goodwin<sup>2</sup>, we found the inhibitory action of diphenylamine towards carotenogenesis to be irreversible in a certain sense. When the cells of *R. gracilis* are grown in a medium containing diphenylamine and then, after centrifugation and washing, are transferred into a medium which contains neither diphenylamine nor a nitrogen source, where under normal conditions carotenoid synthesis can take place, the cells remain colourless. If, however, the cells treated with diphenylamine are transferred into a medium containing a nitrogen source and thus are allowed to proliferate, they gradually recover their capacity of carotenoid synthesis.

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<sup>1</sup> Turian, G., *Helv. Chim. Acta*, **33**, 1988 (1950).

<sup>2</sup> Goodwin, T. W., Jamikorn, M., and Willmer, J. S., *Biochem. J.*, **53**, 531 (1953).

<sup>3</sup> Kleinzeller, A., Málek, J., Praus, R., and Škoda, J., *Chem. Listy*, **46**, 470 (1952).

### Preparation of Transparent Starch Gels after Electrophoresis

IN the method of electrophoresis on starch gel recommended by Smithies<sup>1</sup>, the gels obtained after the electrophoresis and staining of proteins are opaque, difficult to preserve and not suitable for photometric evaluation. For this reason we have devised a technique for preparing starch gels which remain transparent after staining.

Since a protein dye in alcoholic solution, for example, a saturated solution of amidoschwarz in 10 per cent acetic acid in methanol, results in a permanently opaque gel, we prefer to use the dye in aqueous solution; 1 per cent amidoschwarz in a sodium acetate/acetic acid buffer (sodium acetate 0.1 M, 450 ml., acetic acid M, 450 ml., amidoschwarz, 1 gm.), being used for staining in agar electrophoresis<sup>2</sup>.

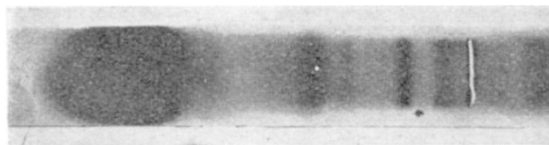


Fig. 1. Transparent starch gel after electrophoresis (human serum)

After fixing for one night in 5 per cent aqueous acetic acid the bands are stained for 10 min. and decolorized by successive washings in 5 per cent aqueous acetic acid. This washing should be prolonged (nearly 48 hr.).

A completely transparent starch gel can be obtained in the following way. A 1-mm. transverse section of stained starch gel is placed on a plate of photographic glass which has been covered with a film of melted agar and then dried in an oven. The glass plate is placed in a rectangular basin and a 1.50 per cent solution of melted agar is poured over it. After the agar has solidified the glass plate carrying the starch electrophoresis included in agar is withdrawn and a sheet of paper (Arches 302 or Whatman No. 1), is applied to the surface so as to regularize the evaporation and to absorb the non-volatile mineral salts. It is left to evaporate in an oven at 37° C. After 48 hr. a transparent film is obtained permitting the photometric evaluation of the different protein fractions of the serum (Fig. 1).

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<sup>1</sup> Smithies, O., *Biochem. J.*, **61**, 629 (1955).

<sup>2</sup> Uriel, J., and Grabar, P., *Ann. Inst. Pasteur*, **90**, 427 (1956).

### Reversal of Thymine Xyloside Inhibition in *Escherichia coli* by Glutathione

AN unexpected competitive relationship between thymine xyloside and glutathione has been found. Thymine xyloside inhibits the growth of *Escherichia coli* in a synthetic medium. This inhibition is competitively reversed by glutathione.

A wild-type *E. coli* (ATCC 26) was grown in brain-heart infusion broth for 6 hr. at 37° C., then diluted to 1/50,000 in a synthetic medium of the following composition: sodium phosphate 0.6 per cent, acid sodium phosphate 0.3 per cent, ammonium chloride 0.1 per cent, sodium chloride 0.1 per cent, magnesium sulphate 0.01 per cent, potassium chloride 0.3 per cent, glucose (autoclaved separately) 0.4 per cent. The size of the inoculum in these conditions was about  $5 \times 10^4$  cells per ml. The inoculated medium was pipetted into sterile 13 mm.  $\times$  100 mm. test tubes and the thymine xyloside or reversing agents added in seeded synthetic medium. The tubes were incubated overnight at 37° C., and the presence or absence of growth was determined by visual inspection. The minimum concentration of inhibitor to prevent growth completely was recorded.

The minimum inhibitory concentration of thymine xyloside varied with the preparation used. The best preparation gave an end-point of 1  $\mu$ gm./ml. in these conditions. However, the reversal experiments were carried out with a preparation of inhibitor which gave an end-point of 6.4  $\mu$ gm./ml. and were confirmed with the more active material.