

completely inhibited by 0.001M iodoacetate. (c) Reduction of TPN was observed when glyceraldehyde-3-phosphate was used directly as substrate.

In crude extracts TPN reduction does not require the addition of arsenate or phosphate (cf. ref. 6). In experiments with a partially purified preparation, which contains relatively greater amounts of the TPN enzyme, arsenate stimulations are observed. Nevertheless, a considerable fraction of the TPN reduction appears to be catalysed by a third (non-reversible) dehydrogenase which was also discovered originally in green leaves^{8,9}. The properties and intracellular distribution of the various dehydrogenases will be described in another communication¹⁰.

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Electrophoresis in a Thin Layer of Starch-Gel followed by a Plastifying Treatment

QUANTITATIVE analysis after starch-gel electrophoresis is usually difficult due to: (1) the diffusion of protein fractions, (2) the difference of migration according to the thickness of the gel, and (3) the fragility and opalescence of this migrating medium.

The cutting of the gels accompanied by superficial staining of the surfaces only partially resolved these difficulties. Some progress has been obtained by cutting thin slices in the median zone of the gel, which can be stained and rendered transparent after inclusion in agar and dried by heating^{1,2}. On the other hand, immersion in pure glycerol³ or transformation into 'plastic' by heat only⁴ has been tried. However, the results obtained did not permit a true quantitative analysis. The technique described here resolves these problems: (1) the stain penetrates into the gel completely, (2) the gel is transformed into a thin, transparent and supple film.

Smithies's technique⁵ is used for the preparation of the starch-gel. The gel is poured into the apparatus giving a homogeneous layer 3 mm. thick⁷. The lateral diffusion of the more concentrated proteins (for example, albumin) is limited by cutting a thin band (2 mm.) of gel between the samples. A sheet of polyethylene protects the gel from the air. The introduction of the sample is done by mixing the serum in some gel in order to obtain a continuous medium.

With this technique, the serum components give strictly parallel bands. After electrophoresis, there are two possible treatments:

(1) It is possible to transform the gel into plastic before staining. The gel is placed for 1 hr. in a bath of an aqueous solution containing 5 per cent acetic acid and 5 per cent glycerol. It is then heated with an infra-red tube of 1,200 W. for 3 hr. at a distance of

65 cm. The plastified and transparent plate is stained in aqueous dye solution during 2 hr. (naphthalene black 0.5 gm., glacial acetic acid 50 ml., distilled water 1,000 ml.). Decoloration is obtained by successive washings in 5 per cent aqueous acetic acid solution. The last bath contains 50 per cent glycerol. The gel is dried with filter paper and is heated by an infra-red lamp for 20 min.

(2) It is also possible to stain the gel directly for 4 hr., then after successive washings it is placed for 1 hr. in an aqueous solution containing 5 per cent acetic acid and 5 per cent glycerol. The gel is heated for 3 hr. with infra-red on a glass plate.

The first technique reduces the risk of deterioration during manipulation. The second results in a more supple plastic film. The transparent band can be measured by direct photometry (Fig 1).

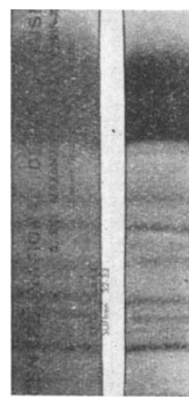


Fig. 1

In addition we noted that Poulik discontinuous buffer system⁶ has the advantage of giving a narrow zone (1 cm. wide) of albumin and clearer pre- and post-albumin zones.

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Changes in Glucose-6-phosphatase Activity in Liver and Kidney at Birth

GLUCOSE-6-PHOSPHATASE catalyses the last step in the chain of reactions by which glycogen is converted to glucose. The enzyme is found only in liver, kidney and intestinal mucosa. Its activity is low in fetal liver and increases at birth in the guinea pig¹ and the rat². Glucose-6-phosphatase has been measured in human liver only in the first half of gestation. Cori and Schulman³ found very low levels in an 18-week foetus. Villee⁴ used the production of glucose by liver slices as a measure of glucose-6-phosphatase. Activity appeared about the tenth week of gestation and had reached about half adult levels by 22 wk. Aurichio