

reading, just slightly above background, after these steps were taken. It is concluded, therefore, that sucrose does not permeate the red cell wall.

Thus centrifugation of blood at top speed in a clinical laboratory centrifuge for 10 min can leave as much as 7.6 per cent of the supernatant liquid entrapped among the red blood cells. Sucrose does not permeate the red blood cell wall.

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Received August 17, 1967.

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## Isoenzymes of Phosphoglucose Isomerase in Mice

A PROCEDURE has been developed for the visualization of the glycolytic enzyme, phosphoglucose isomerase, after electrophoresis in a starch gel. The staining mixture applied to the sliced gel surface consists of fructose-6-phosphate (free of glucose-6-phosphate), glucose-6-phosphate dehydrogenase, NADP, phenazine methosulphate and the tetrazolium salt MTT. The formation of glucose-6-phosphate by the isomerase is coupled to the reduction of the tetrazolium dye, which turns blue.

No heterogeneity has yet been observed for phosphoglucose isomerase in human haemolysates, but variant isoenzymes were found when haemolysates from a number of wild mice (*Mus musculus*) were examined, and a similar type of polymorphism was also encountered in haemolysates and muscle extracts from various strains of laboratory mice. Inserts (a) and (c) in Fig. 1 are from a pair of wild mice, and it can be seen that insert (a) gave a single enzyme band migrating significantly nearer to the anode than the single more cathodically migrating band seen in insert (c). When the mice of this pair were crossed they produced progeny of both sexes, all of which gave the triplet pattern shown in insert (b); the most anodically migrating band of the triplet was indistin-

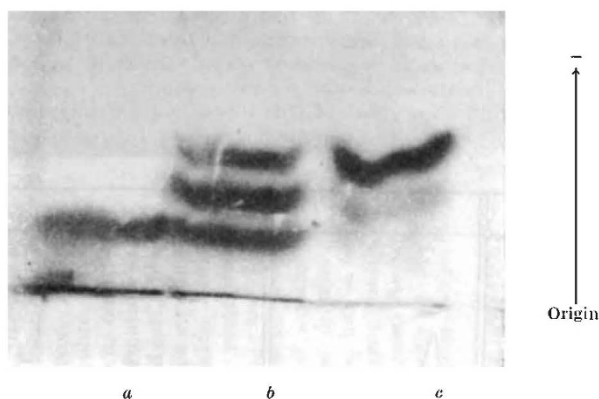


Fig. 1. Starch gel electrophoretic patterns obtained after electrophoresis of mouse haemolysates and specific staining for phosphoglucose isomerase. Haemolysates were made and electrophoresis was carried out as described before<sup>8</sup>, but metal cooling plates were used with a potential difference of 20 V/cm for a period of 4 h. The staining mixture applied to the gel surface was made by mixing 0.3 molar tris buffer at pH 8.0 (1.5 ml.), 0.1 molar magnesium chloride (0.5 ml.), 18 nmolar sodium fructose-6-phosphate (0.2 ml.), 6 mmolar NADP (0.1 ml.), purified glucose-6-phosphate dehydrogenase (0.2 ml. of a solution containing 10 U/ml.), MTT (1 mg) and phenazine methosulphate (0.5 mg), just before use. It is important for the fructose-6-phosphate to be free from glucose-6-phosphate, and in this laboratory it is prepared as needed by the acid hydrolysis of crystalline trisodium fructose diphosphate. Inserts (a) and (c) contained haemolysates from a breeding pair (see text), and insert (b) contained haemolysate from one of the progeny.

guishable in mobility from that appearing on its own at insert (a); the most cathodically migrating band was likewise indistinguishable from the one appearing on its own at insert (c), and the third band of the triplet had intermediate mobility. This situation, which is analogous to that found for the enzyme phosphoglucose dehydrogenase<sup>1-4</sup>, and for certain other enzymes<sup>5-7</sup>, is consistent with a dimeric structure for the enzyme phosphoglucose isomerase, and indicates that this enzyme in mice is likely to be controlled by an autosomally linked locus.

We thank Mr R. Redfern and his colleagues of the Ministry of Agriculture, Fisheries and Food, Tolworth, for the wild mice.

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Received August 16; revised September 22, 1967.

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## Fractionation of Protein Solutions by Membrane Partition Chromatography

HIGH flux preformed ultrafiltration membranes, serving as diffusive barriers to the transport of macromolecular species, have been found to be extremely useful for: the non-denaturant concentration of dilute protein solutions<sup>1</sup>; as a practical means of rapid dialysis and evaluation of micro-ion binding phenomena; for separating the products of proteolysis during digestion (unpublished work of Blatt, Robinson, Zipilivan and Hudson); and in the determination of lower molecular weight moieties<sup>2</sup>. These membranes are hydrous gels formed from the complex interaction product of polyanions and polycations and are marketed under the name of 'Diaflo' by the Amicon Corporation, Cambridge, Mass. The molecular weight retentive capacities of these membranes, 'UM-2' and 'UM-1', have been arbitrarily fixed at 500 and 10,000, respectively. In a recent study, a gel membrane with a more expanded structure (exclusion limit molecular weight 35,000) was investigated as a partitioning device in the removal of the albumin impurity from crystalline alpha lactalbumin<sup>3</sup>. Whereas these membranes are non-porous hydrous gels, ultrafilters with exclusion limits in excess of 40,000 require pored structures for formulation.

Five membranes (arbitrary molecular weight exclusion limits in parenthesis) were evaluated for their protein retentive properties: (a) 'XM-100' (100,000); (b) 'XM-50' (50,000); (c) 'XM-4a' (35,000); (d) 'UM-1' (10,000) and (e) 'UM-2' (500). A modified ultrafiltration cell<sup>4</sup>, similar to that used for the evaluation of proteolysis, was used (Fig. 1). In this system, a non-pulsatile buffer flow is established and the ultrafiltrate is passed through the ultraviolet monitoring system until baseline stability is achieved. The pump and ultraviolet analytical system are components of the 'Spectrochrom' apparatus, Beckman Instrument Co., Fullerton, California. After equilibration the protein solution is injected into the sampling valve and the absorbance of the ultrafiltrate recorded at 280 m $\mu$ . In all studies, 1 ml. of 1 per cent protein solutions,