être, soit révélées localement, soit dosées après élution de la gélose coupée en segments parallèles.

Il est enfin possible d'appliquer l'E.S. à un mélange d'antigènes préalablement soumis à une électrophorèse en gélose, l'axe du champ électrique de l'E.S. étant perpendiculaire au premier axe de progression; on obtient ainsi les résultats de l'analyse en quelques heures (les γ-globulines antigènes ne pouvant pas, évidemment, être étudiées ainsi). L'E.S. a d'ailleurs été appliquée, indépendamment[9], à l'étude de la réaction des virus avec leurs anticorps homologues, avec des résultats très encourageants.

Il semble donc que l'E.S. fournit une méthode simple et rapide d'étude des différents antigènes d'un mélange, en particulier ceux de poids moléculaire élevé. Elle permet également de suivre le comportement électrophorétique des complexes solubles formés au cours de la réaction.

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Multiple forms of lactic dehydrogenase in rabbit cornea

The cornea has been shown[1] to contain a highly active LDH. The high enzyme activity and its apparent localization in the epithelium has stimulated further interest in rabbit corneal LDH. This communication demonstrates that when soluble proteins are extracted from rabbit cornea and resolved by anion-exchange chromatography, three forms of LDH are detected.

Extracts were prepared from whole cornea, epithelium-endothelium and stroma. The latter two were obtained after denuding the stroma by scraping both sides of the cornea with a scalpel blade. The tissues were homogenized in 2 vol (w/v) 0.1 *M* phosphate buffer, pH 7.1, and centrifuged at 34,800 × *g* for 60 min. The supernatant fluids were dialyzed overnight against 0.005 *M* phosphate buffer, pH 7.1, and cleared at 34,800 × *g* for 60 min. All operations were carried out in the cold.

DEAE columns (0.9 × 10 cm) were prepared according to the methods of PETERSON AND SOBER[2]. The extract was applied to the column and eluted with a convex gradient of increasing chloride concentration (see Fig. 1). Protein content[3], pH and LDH activity[1,5] were determined in each column fraction. LDH activity in

Abbreviations: LDH, lactic dehydrogenase; DEAE, diethylaminoethylcellulose; DPNH, reduced diphosphopyridine nucleotide.

the fractions was unstable to freezing and thawing, and therefore all activity measurements were made sooner than 24 h after collection.

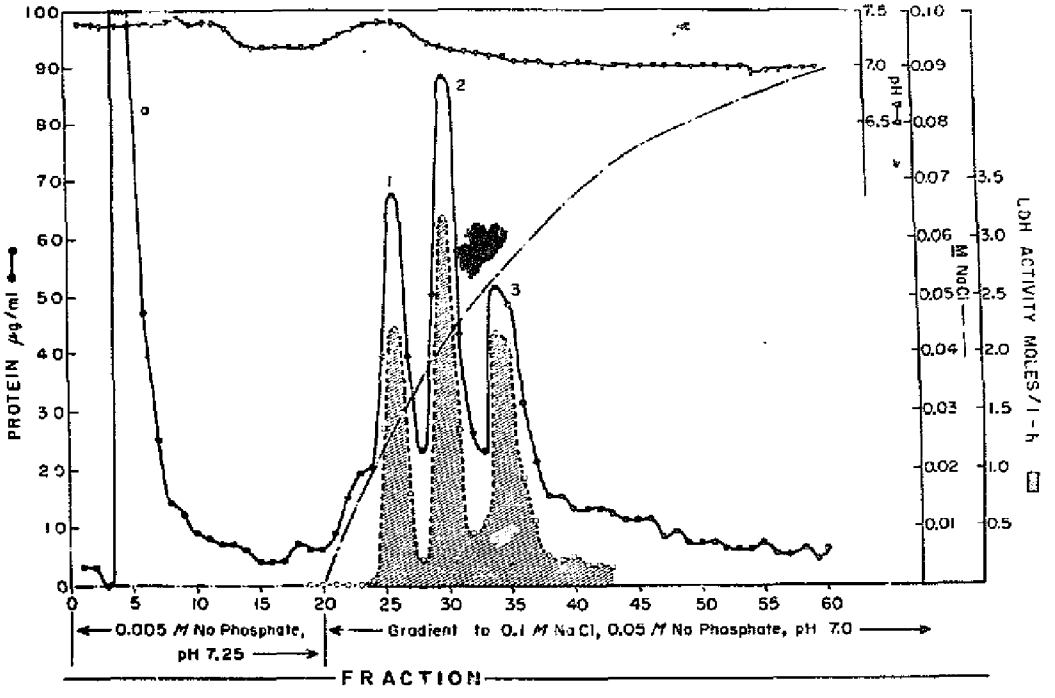

Fig. 1. Chromatography of rabbit cornea epithelial-endothelial extract on DEAE. 1 ml extract (8.8 mg protein) was applied and eluted at a flow rate of about 10 ml/h.

A large amount of non-LDH protein found in the whole corneal preparation was derived mostly from stroma with less non-LDH protein in the epithelial-endothelial preparation. The chromatograms of whole cornea and stroma were otherwise similar to that of epithelium-endothelium, and so only the latter is shown in this report. The gradient to 0.1 *M* chloride eluted four major protein peaks (*a*, 1, 2, and 3 in Fig. 1).

TABLE I

LACTIC DEHYDROGENASE ACTIVITIES IN RABBIT CORNEAL EXTRACTS AFTER RESOLUTION ON DEAE COLUMNS

Values are expressed as μmoles DPNH oxidized/min/mg protein at 38°. Numbers in parentheses denote the column fraction numbers at which the LDH activities are maximal.

Whole cornea	*Epithelium-Endothelium*	*Stroma*
Starting extract		
69.5	121.0	33.0
Chromatograph fractions		
(28) 455	(26) 550	(32) 273
(32) 528	(30) 600	(41) 308
(36) 378	(34) 708	(46) 212

LDH activity was found in peaks 1, 2, and 3; peak *a* contained less than 1% of the LDH activity in peak 1. The LDH peaks eluted at approx. 0.03, 0.04, and 0.05 *M* chloride for all extracts. The recoveries of LDH activity from the columns were 96, 93, and 75% for whole cornea, epithelium-endothelium, and stroma, respectively. The purification of LDH ranged from 4- to 9-fold, dependent upon the extract and peak considered (see Table I).

When measured in the direction of DPNH oxidized at pH 7.0 and 25°, the activity of crystalline beef-heart LDH is 300 μmoles/min/mg protein[6]. If it is assumed that the activity of crystalline LDH is doubled by increasing the temperature from 25° to 37°, then the column-purified corneal LDH would have about the same activity as crystalline beef-heart LDH[7].

The possibility of contamination of the stroma by epithelial and endothelial cells and/or fluids does exist. It is not certain that the three forms of LDH found in the stromal extracts are native to that part of the cornea, although it has been reported that rat and rabbit stroma do contain LDH activity[1].

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Sur la différence de stéréospécificité entre la déshydrogénase lactique extraite de la levure anaérobie et celle extraite de la levure aérobie

On sait que l'équipement enzymatique de la levure de boulangerie (*Saccharomyces cerevisiae*) subit des modifications très importantes au cours de l'adaptation respiratoire, c'est-à-dire au cours du passage de la vie anaérobie à la vie aérobie (*cf.* ref. 1). Il a été montré récemment[2] qu'à ces deux conditions correspondent deux lacticodéshydrogénases différentes: l'une, celle de la levure aérobie (enzyme *O*) est classique et a déjà été étudiée par plusieurs auteurs[3–7], l'autre (enzyme *N*) n'avait pas eté mise en évidence auparavant. Elle se distingue de la première par un certain nombre de propriétés physicochimiques et cinétiques, en particulier, elle ne réduit pas le cytochrome *c*.