of residual enzyme activity *vs.* protein-bound HNB is shown in Fig. 1. The titration curve of malate dehydrogenase indicates complete inhibition accompanied by binding of 2 moles reagent. The inactivation is almost certainly due to reaction with tryptophanyl residues, and the stoichiometry suggests one reactive tryptophanyl residue per subunit of enzyme. Other amino acids, such as cysteine, methionine or tyrosine, react with HNB bromide in alkaline media, but at the low pH used here, the reaction should be relatively specific for tryptophan[1-3]. The end point is in accordance with previous studies of tryptophan content[5,6], and it implies a 1:1 adduct with attachment of the methylene group of HNB to C-3 of tryptophan[5,6,9].

Since the enzyme did not react in neutral aqueous solution, but only in 8 M urea, the residues that were modified may normally exist in a sterically inacessible environment. The loss of enzyme activity on modification of tryptophanyl residues may indicate that the reactive tryptophanyl residues are essential for activity, or the activity loss may have been due to the prevention of refolding of the molecule to an active conformation.

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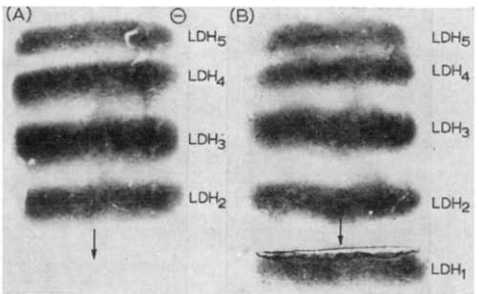
Isozymes of lactate dehydrogenase in the outer segments of the retina

The question concerning the presence of enzymes in the outer segments of the retina is of considerable interest because of the possibility of their participation in the first stage of the photoreception process.

We have shown earlier the presence of lactate dehydrogenase (EC 1.1.1.27, L-lactate–NAD oxidoreductase, LDH) activity in a fraction of bovine retina segments[1]. The activity of lactate dehydrogenase observed in this fraction was not due to the

presence of other cell structures since the specific activity of the segment extracts was higher than that of the hyaloplasma and extracts of retina mitochondria[2].

A more convincing confirmation of the presence of the lactate dehydrogenase system existing in the outer segments was obtained from a comparative investigation of the isoenzymatic composition of the enzyme system, both in these structures and in the retina hyaloplasma. Isoenzyme patterns obtained from electrophoresis in agar and polyacrylamide gels are given in Figs. 1 and 2. In these figures one can clearly



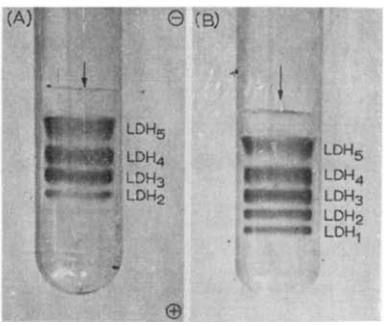


Fig. 1. High voltage electrophoresis of the isoenzymes of lactate dehydrogenase in saline extracts of the segments (A) and the retina (B). Electrophoresis was by a modified technique of WIEME[8]: 1% agar gel, 0.05 M medinal buffer (pH 8.6); current, 25 mA; field strength, 20 V/cm; separation time, 30 min; protein content in the retina extracts, 45 μg; segments extracts, 28 μg; determination of protein, by LOWRY *et al.*[9]. The extracts were prepared as mentioned in Table I. The isoenzymes were identified by the nitro-blue tetrazolium method[10].

Fig. 2. Disc electrophoresis of the isoenzymes of lactate dehydrogenase in saline extracts of the segments (A) and the retina (B). Electrophoresis was by the technique of ORNSTEIN[11] and DAVIS[12] with modifications: Spacer gel: 3% acrylamide and 0.75% *N'*,*N'*-methylenbisacrylamide; 0.5 mg% riboflavin; 10% glucose; 0.06 M Tris–HCl buffer (pH 7.4). Running gel: 5% acrylamide and 0.3% *N'*,*N'*-methylenbisacrylamide; 15% glucose, 0.07% ammonium persulfate, 0.01% dimethylaminopropionitrile; 0.5 M Tris–HCl buffer (pH 8.8). Electrode buffer: 0.05 M Tris–0.38 M glycine (pH 8.3) Samples (diluted 1:1 in 40% glucose) were placed on the top of the spacer gel; current, 10 mA ;separation time, 105 min; protein content in the retina extracts, 27 μg; segment extracts, 10 μg. The isoenzymes were identified by the nitro-blue tetrazolium method[10].

see that the extracts of the retinas exhibit 5 isoenzymatic forms (LDH_1, LDH_2, LDH_3, LDH_4, LDH_5) which correspond to those already reported[3,4]. However, in extracts of the segments using either electrophoretic media, only 4 isoenzymes (LDH_2, LDH_3, LDH_4, LDH_5) were found. As a rule we failed to detect the anodal band corresponding to LDH_1.

Therefore the cathodal isoenzymes were more prevalent in the segments than in preparations of the entire retina. Further support for this finding was obtained from experiments on the influence of high concentration of pyruvate on the reaction rate catalyzed by lactate dehydrogenase[5]. The inhibition by high concentrations of pyruvate of the reaction in extracts of segments was less pronounced than in extracts of the entire retina (Table I).

The role played by lactate dehydrogenase in segments is not yet clear. We have attempted to determine the influence of light on lactate dehydrogenase activity of segments. It was establisted earlier that when the segment fraction isolated in darkness was bleached, the corresponding saline extract exhibited a lower enzymatic activity

TABLE I

THE EFFECT OF HIGH CONCENTRATION OF PYRUVATE (0.1 M) ON LACTATE DEHYDROGENASE ACTIVITY OF SALINE EXTRACTS OF THE RETINA AND SEGMENTS

Isolation of segments by the procedure of SHUKOLYUKOV[6] (a combination of the methods of differential and sucrose density gradient centrifugation). Determination of lactate dehydrogenase was by the method of KORNBERG[7]. Preparation of saline extracts: 0.9% NaCl was mixed (9:1) with the segments of retina, thoroughly homogenized and centrifuged for 20 min at +3°. For each experiment 10–30 μg of protein extract was used.

	$\Delta A_{340\ nm}$ per 2 min		a/b
Pyruvate concn.:	*0.01 M (a)*	*0.1 M (b)*	
Retina	0.083	0.033	2.50
Outer segments	0.130	0.075	1.72
Retina	0.100	0.040	2.50
Outer segments	0.080	0.065	1.23
Retina	0.100	0.050	2.00
Outer segments	0.085	0.060	1.40
Retina	0.160	0.055	2.90
Outer segments	0.140	0.065	2.10

than extracts from unbleached segments[1]. It was also shown that illumination did not affect the reaction of the saline extracts themselves. However, if the segment extracts prepared in triton X-100 were exposed to light, a certain decrease (up to 20%) in both

TABLE II

EFFECT OF LIGHT ON LACTATE DEHYDROGENASE ACTIVITY OF OUTER SEGMENT EXTRACTS OBTAINED BY TRITON X-100

Preparation of triton extracts: 1% triton prepared in 0.9% NaCl was mixed (9:1) with the segments of the retina, incubated for 15 min and centrifugated at 20 000 × *g* for 20 min. The supernatant was diluted by 1% triton and adjusted to a protein concentration of 5 mg/ml. For each determination 30–50 μg protein extract was used. The mean data ± S.E. of 10 determinations are presented as μmoles of NAD oxidised per min × 10.

Enzymatic activity		*Decreasing activity (%)*
In dark	*After bleaching*	
0.39 ± 0.04	0.32 ± 0.03	18
$P < 0.01$		

the rate of reduction of pyruvate and the oxidation of lactate was observed (Table II). However further experimental work is needed to elucidate the mechanism of this inhibition.

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Polyacrylamide gel electrophoresis of DNA polymerase from Ehrlich ascites tumor cells and recovery of active enzyme

Polyacrylamide gel electrophoresis should be useful for monitoring DNA polymerase (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) preparations at different stages of purification and for identification of the band or bands representing active protein. CAVALIERI AND CARROLL[1] reported studies on the *Escherichia coli* DNA polymerase; however, considerable activity was found to be lost after recovery of the protein from the gel. NEUHOFF AND LEZIUS[2] have described two assay methods for detecting the *E. coli* enzyme in microgels. To date there has been no report of electrophoresis of mammalian DNA polymerase or of success in recovering enzyme with a high yield of activity from gels.

The maintenance of the ascites cells and the preparations of the whole cell hypotonic extract and its pH-5 precipitate was as given by ROYCHOUDHURY AND BLOCH[3]. Samples of the pH-5 precipitate, subjected to chromatography on a sequence of DEAE-cellulose, phosphocellulose, hydroxylapatite and Sephadex G-100 columns were provided by Dr. R. Roychoudhury. All enzyme samples were dialyzed overnight against 2 l of cold Buffer A (50 mM potassium phosphate, pH 7, 10 mM 2-mercaptoethanol, 1 mM EDTA, 20% ethylene glycol).

The Tris–HCl–glycine electrophoresis system[4] showed a clear protein banding pattern with hypotonic extract protein, however, DNA polymerase activity was lost in protein extracted from the gel. Three buffer systems running at pH 7–7.5 did not produce banding patterns (lysine–HCl–glycine, imidazole–asparagine, and imidazole–glycine). The following procedure was found to permit resolution of protein bands and at the same time maintain the enzyme in an active form. Stock solutions were: A (80 ml of 1 M potassium phosphate, pH 7.5, 0.23 ml *N,N,N,N'*-tetramethylethylenediamine