tinamide adenine dinucleotide (NAD) or the ratio of pyridine-3-aldehyde adenine dinucleotide to NAD (Table 1). The ratio of nicotinamide hypoxanthine dinucleotide to NAD decreased gradually from isozyme 1 to isozyme 5. Quite striking was the high ratio of acetylpyridine adenine dinucleotide to NAD observed for "band X."

The heat stability of the isozymic fractions was determined by heating eluates from the starch at 40, 50, and 60°C for 15 minutes. The rates of inactivation for "band X" were between those observed for isozymes 3 and 4.

Diethylaminoethyl cellulose column chromatography was performed on homogenates of testes containing "band X." The enzymes were eluted by the addition of increasing concentrations of sodium chloride in 0.01*M* sodium phosphate buffer, *p*H 7.0. Major peaks of enzymatic activity occurred at salt concentrations of 0.001, 0.075, 0.125, and 0.20*M*. The type of LDH in each of these effluents was identified by starch-gel electrophoresis. The material from the 0.001*M* concentrations gave a faint band in the isozyme-4 region, that from the 0.075*M* exhibited faint bands in the "band X" and isozyme-3 area, and the 0.125*M* and 0.20*M* developed single bands in the region of isozymes 2 and 1 respectively. Isozyme-5 activity was never detected in the effluents.

Appella and Markert (*6*) dissociated LDH into four polypeptides which could be separated into two classes on the basis of charge. From this and other evidence these investigators (*7*), and more recently Cahn, Kaplan, Levine, and Zwilling (*8*) have advanced the hypothesis that each LDH isozyme is comprised of four polypeptide subunits which are assembled from two different kinds of polypeptides (A and B) synthesized under the control of two different genes. By this hypothesis, isozymes 1 through 5 would have the following polypeptide structure: AAAA, AAAB, AABB, ABBB, and BBBB. The presence of a sixth LDH isozyme in sperm suggests that one, or possibly two different genes become active in spermatogonia at the time of puberty.

Damaged cells may release their enzymes into the circulation. Usually changes in the serum LDH isozyme pattern may be correlated with the repertory of LDH isozymes in the damaged organ (*9*). Since the new isozyme in postpubertal testis is shared by no other tissue, its presence in serum would indicate testicular pathology.

The functional and metabolic significance of the "band X" material in sperm is unknown. That it might be an index of male fertility is an interesting possibility (*10*).

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Lactic and Malic Dehydrogenases in Human Spermatozoa

Abstract. *Human spermatozoa, representing a homogeneous population of postmitotic cells, contain five electrophoretically distinct lactic dehydrogenases and two malic dehydrogenases. This indicates that molecular heterogeneity of enzymes is characteristic of the individual cell and is not a reflection of heterogeneity of cell types within a tissue.*

Lactic dehydrogenase (LDH) and malic dehydrogenase (MDH) from various species of animals and within different tissues of the same animal exist in multiple molecular forms (*1*, *2*). The term *isozyme* has been suggested to designate this heterogeneity of molecular types for a single enzyme (*2*). Since these dehydrogenase isozymes appeared in a wide variety of tissues, usually including a multiplicity of cell types, the isozyme complement of a particular tissue might be a reflection of cytologically heterogeneous material, and any particular cell type might possess only a single form of the enzyme.

Spermatozoa, postmitotic cells of known and controllable history and obtainable as a homogeneous population, were selected as the ideal material with which to attempt to resolve this question. Spermatozoa produce considerable quantities of lactic acid under aerobic as well as anaerobic conditions (*3*); this suggests, therefore, that an active LDH is present in high concentration in these cells.

This paper reports the extraction and characterization of LDH and MDH from human spermatozoa with particular reference to the occurrence of multiple molecular forms of these enzymes in these cells.

Ejaculated human spermatozoa were washed free of seminal plasma by centrifugation (2000*g* for 20 minutes) in 0.1*M* sodium phosphate buffer at *p*H 7.4. The cells in suspension were placed in a small beaker in an ice bath and disrupted by sonification for 10 minutes with a Branson model S-75 Sonifier. The suspension of disrupted cells was centrifuged at 10,000*g* for 30 minutes at 4°C, and the supernatant fluid was used for enzyme assay.

The activity of LDH was measured essentially as described by Kornberg (*4*), by following spectrophotometrically the oxidation of $NADH_2$ (reduced nicotine adenine dinucleotide) with pyruvate as substrate. Whenever possible, protein concentrations were estimated from the absorbancy values of appropriate solutions at 260 and 280 mμ (*4*).

Disc electrophoresis in a polyacrylamide gel, as described by Ornstein and Davis (*5*), was used to separate the proteins, liberated from the cells by sonification, which moved toward the anode. With this method about 20 μg of protein was resolved in 45 minutes from a 0.1 ml volume of cell suspension. Eight columns of gel, each conducting approximately 5 ma, were subjected to electrophoresis simultaneously. The columns of gel were removed from the glass supporting cylinders immersed in a dish of cold tap water by rimming with a blunt-edged dissecting needle. The columns were then fixed and stained for protein in naphthol-blue black dissolved in a 5:5:1 solution of water, methanol, and acetic acid. Excess stain was removed from the sample electrophoretically with 7½ percent acetic acid. Unfixed gels were rinsed with cold tris buffer, 0.1*M*, *p*H 8.3, and incubated at 37°C for from ½ to 1 hour in a reaction mixture comparable to that described

by Appella and Markert (*6*). The final concentration was 0.1*M* tris, 0.036*M* L(+) lactic acid sodium, or L(−) malic acid, NAD (0.3 mg/ml), nitro-blue tetrazolium (0.8 mg/ml), and phenazine metholsulfate (0.14 mg/ml), all adjusted to *p*H 8.3. In some experiments the reaction mixture described by Allen (*7*) was used with identical results.

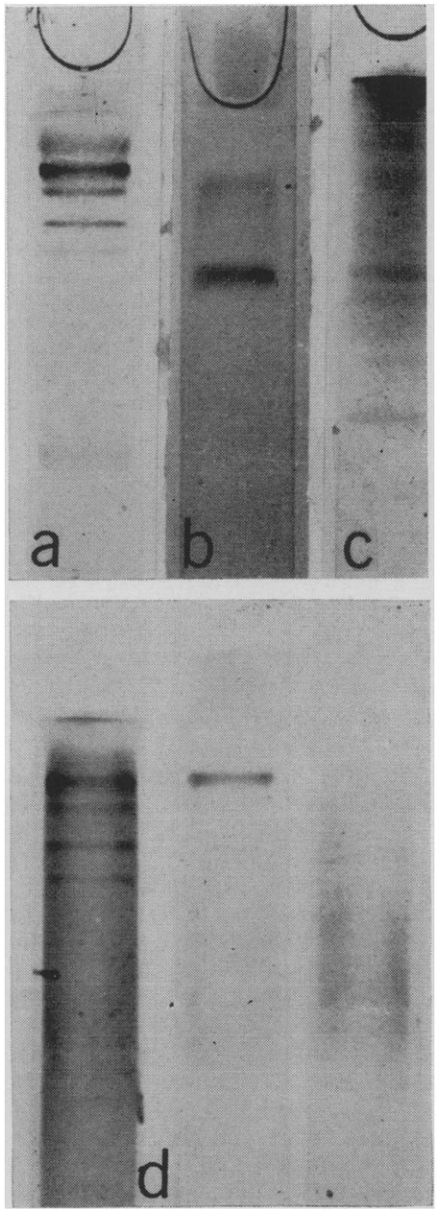

Fig. 1. Electrophoresis patterns of LDH and MDH, and protein distribution. The anode was at the bottom of the gel. (*a*) LDH pattern. (*b*) MDH pattern. (*c*) Gel stained for protein. (*d*) LDH zymogram with NAD (left), the 3-acetyl pyridine analog of NAD (center), and the pyridine-3-aldehyde analog of NAD (right) in the incubation mixture. These gels were simultaneously subjected to electrophoresis and incubated in the appropriate reaction mixture for the same length of time.

Sites of dehydrogenase activity on the gel were localized by the precipitation of the reduced tetrazolium salt which was purple in color.

Five electrophoretically distinct lactic dehydrogenases (Fig. 1*a*) and two malic dehydrogenases (Fig. 1*b*) were demonstrable in solutions obtained from the sonification of these cells. Duplicate gels stained for protein showed 16 well-defined bands separated by this procedure (Fig. 1*c*). If, for convenience, we number the sites of dehydrogenase activity from I to V in order of migration toward the anode, it appears that LDH_{IV} and MDH_{I} have the greatest activity if judged by size of band and intensity of stain: sites V, III, II, and I follow in order of decreasing activity.

These data were compared with the results of Blanco and Zinkham (*8*) on pre- and post-puberal testis. Specimens of human testis from autopsy, supplied to me by Zinkham, were assayed here by disc electrophoresis. In my system LDH_{IV} corresponds to the "band X" which they report is unique to spermatozoa. In addition, I have determined that isozymes 1, 2, and 3 from human erythrocytes and testis are identical electrophoretically to the isozymes in sperm, designated LDH_{I}, LDH_{II}, and LDH_{III}; LHD_{V} would be the same as isozyme 4 in testes, and therefore presumably in other human tissues.

When the 3-acetylpyridine or pyridine-3-aldehyde analogs of NAD were used in the incubation mixture only a single LDH band was observed on the gels; this band coincided with LDH_{IV}. These data, in Fig. 1*d*, show that there is greatest activity with NAD; the 3-acetyl analog of pyridine is capable of serving as hydrogen acceptor, and there is no reaction with the 3-aldehyde analog. This result would further support the suggestion that "band X" and LDH_{IV} are comparable, since this isozyme has a strikingly high ratio of the 3-acetyl pyridine analog of NAD to NAD (*8*). Spectrophotometric assay of the activity of LDH in the solutions from disrupted cells revealed in two experiments that the reaction, pyruvate to lactate, oxidizes $NADH_2$ at the rate of 1.22 and 1.09 μM/min per milligram of protein. These values obviously reflect the composite activity of the five molecular species of LDH.

Several studies suggest that multiple forms of enzymes occur within a single cell (*9*). Such work, however, has dealt with cells capable of division, or in different stages of division, or of different ages. The present report demonstrates conclusively that a single cell type possesses its own complement of "isozymes." A recent report describes five lactic dehydrogenases and two malic dehydrogenases in a number of human fetal tissues (*10*).

On the basis of these studies it is not possible to establish the role of molecular heterogeneity of enzymes within the metabolic framework of the cell. It seems probable that LDH_{IV} (or "band X") represents the most important isozyme for lactate metabolism and even glycolysis in the human spermatozoa.

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Ripple Marks Show That Countercurrent Exists in Florida Straits

Abstract. *About 60 percent of the area shown in photographs taken at the axis of Florida Straits exhibits well-defined current ripple marks. These ripples indicate a flow of water of at least 0.2 to 0.6 knots from the north. This current is in the opposite direction from the surface currents of 2 to 4 or more knots.*

The floor of Florida Straits is a smoothly graded valley that has its head near the northern end of the straits (*1*). The valley extends south and then west and empties at grade onto the floor of the Gulf of Mexico. In the area north