

nancy, as well as the high concentration of inhibitors of fibrinolysis in the placenta²², might be responsible for the observed decrease in the blood fibrinolytic activity mainly in the last months of pregnancy with a rapid return to its nonpregnant level after delivery²³.

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Variation in the lactase dehydrogenase activity of the esophagus¹

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Summary. Quantitative assay and electrophoretic study of lactate dehydrogenase (LDH) from various tissues of the opossum esophagus were performed. On the basis of expression of the LDH isozymes, we concluded that the smooth muscle of the body of the esophagus carry on more anaerobic glycolysis than the striated muscle. The smooth muscle of the gastroesophageal junction carry on both anaerobic as well as aerobic glycolysis.

Histochemical and biochemical studies have shown that metabolism in muscle tissues varies according to muscle types and their pattern of physical activity²⁻⁵. Recent studies have shown physiological difference in the response of the different segments of esophageal muscle strips to hypoxia^{6,7}, indicating possible metabolic differences. The purpose of the present investigation was to study the glycolytic metabolism of different segments of esophageal muscle by analyzing the relative activity of the isozymes of lactate dehydrogenase (LDH).

Materials and methods. Studies were performed on both male and female opossums (*Didelphis virginiana*) due to structural and functional similarity of the esophagus in this species to that of man⁸. The animals were anesthetized by i.p. injection of 150 mg/kg of sodium barbital. Under anesthesia, the animals were sacrificed by left thoracotomy and puncture of the heart. The entire esophagus was carefully dissected out with a rim of the stomach and the whole specimen was placed in icecold 0.9% sodium chloride solution. After cleaning the outer surface, the esophagus was opened by an incision from the lesser curvature side of the stomach. Samples were obtained from the following areas: a) pure smooth muscle zone of the body of the esophagus, b) pure striated muscle zone of the body of the esophagus, c) junctional area between the striated and smooth muscle zones, and d) smooth muscle from the gastroesophageal junction. Additionally, samples were also taken from esophageal mucosa and the diaphragmatic muscle tissue. The tissues were homogenized separately in cold homogenizing buffer (0.15 M KCl, 0.25 M K₂HPO₄, pH 8.54). Crude tissue extracts were obtained by collecting

the supernatant following centrifugation at 10,000 × g in refrigerated condition. The crude extracts from these various tissues were then subjected to vertical starch gel electrophoresis. Following electrophoresis, the gel slice was stained for LDH as described by Shaw and Prasad⁹. Spectrophotometric assay of LDH was done by measuring the change in absorbance at 340 nm due to reduction of NAD⁺ in a Unicam DB spectrophotometer. The assay mixture contained 2.5 ml and 0.2 M Tris-HCl buffer pH 8.0, 77.5 mM lactate, 5.6 mM NAD⁺ and 0.1 ml of crude extract at 25 °C. 1 unit of enzyme is defined as the amount of enzyme producing the conversion of μM of NAD per min. Protein was determined according to Lowry et al.¹⁰.

Results and discussion. Electrophoretic study of muscle extracts showed that the striated muscle of the body of the esophagus, esophageal mucosa, diaphragm striated muscle and the muscle from the gastroesophageal junctional area have all 5 bands of LDH, although the esophago-gastric junctional muscle have very faint LDH-1 as compared to others (figure). The extracts of smooth muscle of the body of the esophagus and the junction of striated and smooth muscle had only 4 molecular forms of LDH. LDH-1 was absent in these 2 tissues. The quantitative measurement of LDH (table) showed that the striated muscle tissues had higher LDH activity as compared to smooth muscle areas. The enzyme LDH is involved in the glycolytic metabolism. Extensive study on this enzyme has shown that the A and B polypeptides in this enzyme are significantly different in their K_m values with respect to various NAD analogues and in thermostability¹¹. It has been also shown that subunit B of LDH is preponderant in tissues with abundant oxygen

Activity of lactate dehydrogenase in different tissue of esophagus

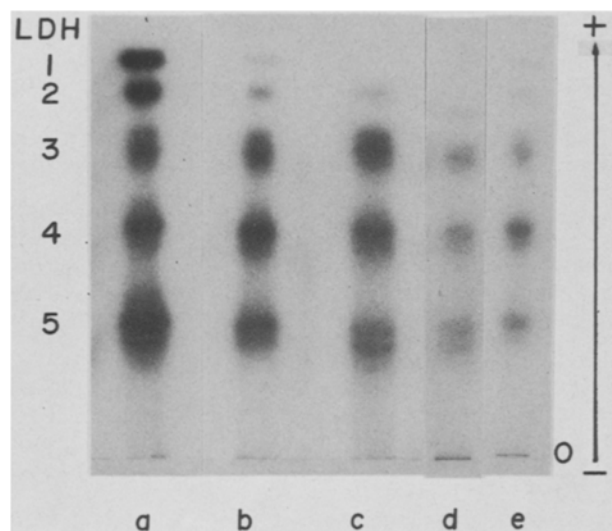
Type of tissues	Protein (mg/ml)	Specific activity	Presence of LDH forms		LDH-3	LDH-4	LDH-5
			LDH-1	LDH-2			
Striated muscle of the body of the esophagus	9.4	0.41	+	+	+	+	+
Junction of striated and smooth muscle	9.4	0.27	—	+	+	+	+
Smooth muscle from the body of the esophagus	10.6	0.24	—	+	+	+	+
Smooth muscle from the GE junction	8.7	0.30	F	+	+	+	+
Esophageal mucosa	8.7	0.15	+	+	+	+	+
Diaphragmatic (striated) muscle	14.9	0.61	+	+	+	+	+

+, present; —, absent; F, faint.

supply, whereas subunit A is associated with anaerobic glycolysis⁵.

In contrast to the striated muscle area which had all 5 LDH isozymes, the smooth muscle of the body of the esophagus lacks in LDH-1 activity suggesting that the smooth muscle in this area carry on more anaerobic glycolysis than the striated muscle segment. In this respect the smooth muscle of the body of the esophagus is similar to intestinal smooth

muscle¹². The smooth muscle from the esophago-gastric junction, on the other hand, revealed some LDH-1 activity indicating that the muscle of the esophago-gastric junction carry on both anaerobic as well as aerobic metabolism. Synthesis of the anaerobic LDH fraction is stimulated by a low oxygen tension and its biological function is to maintain activity even with an excess of lactate¹³. This metabolic concept is compatible with the idea that the lower esophageal sphincter in the gastroesophageal junction is designed to maintain a constant state of activity in contrast to the body of the esophagus which has no tone under basal conditions. The present study lends support to the observations made by others^{6,7} that the myogenic active tension of the lower esophageal sphincter is more dependent on aerobic metabolism as compared to the body of the esophagus.



Starch gel electrophoretic patterns of LDH isozymes in different tissues of opossum's esophagus (O, origin). Samples are *a* diaphragm striated muscle, *b* smooth muscle of GE junction muscle, *c* smooth muscle of the body of the esophagus, *d* junction of striated and smooth muscle, and *e* esophageal mucosa.

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Ultrastructural correlation of water reabsorption in isolated rat cauda epididymidis¹

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Summary. Electron microscopic study was made on the water reabsorption of the epithelial cells of the rat cauda epididymidis. It was shown that when the epididymal duct was reabsorbing water at a maximal rate, widely dilated intercellular spaces were seen. It is suggested that the standing gradient model of water reabsorption first proposed for the gall bladder may also operate in the cauda epididymidis.

There is evidence that fluid reabsorption takes place in the rat cauda epididymidis *in vivo*²⁻⁵ and *in vitro*^{6,7}. Fluid reabsorption is secondary to a net transepithelial transport of sodium ions, which is an energy requiring process. Like

many epididymal functions, the process is androgen-dependent, since castration in rats diminished the ability of the cauda epididymidis to reabsorb fluid⁸. In this study, an attempt has been made to show by electron microscopy the