# COMPARATIVE ACTIVITY OF SOME ENZYMES IN MUSCLE TISSUE IN VARIOUS PARTS OF THE RABBIT STOMACH

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Enzyme activity was compared in functionally different parts of the muscle tissue of the rabbit stomach. Hexokinase, cytochrome-c oxidase, total lactate dehydrogenase activity, and the relative content of LDH-1 were found to be considerably higher in the fundal portion than in the pyloric part.

KEY WORDS: gastrointestinal tract; muscular coat; enzymes; isoenzymes.

The metabolic profile of muscle tissue is closely connected with the intensity of the work performed by the muscle and the character of its contractile response. As regards skeletal muscle this problem has been studied in considerable detail [7, 12]. Meanwhile, considerably less attention has been paid to the comparative analysis of the composition and activity of enzymes in functionally different parts of the smooth muscle, with the exception of the myometrium. The muscle tissue of the stomach is an interesting object for investigations of this sort. The muscle of the fundal and pyloric parts of the stomach in fact has well-marked functional differences. Frequent tonic contractions are observed in the fundal portion, whereas in the pyloric part the movements have the typical rhythmic character [10], and the strength and amplitude of the contractions in the pyloric part are much greater than in the fundal part [6].

The object of this investigation was to compare the activity of certain enzymes of carbohydrate and energy metabolism in the muscle of the fundal and pyloric parts of the rabbit stomach. The data presented in this paper were obtained by determining the activity of hexokinase (HK: 2.7.1.1), lactate dehydrogenase (LDH: 1.1.1.27), and cytochrome-c oxidase (CCO: 1.9.3.1), and also the isoenzyme spectrum of LDH.

## EXPERIMENTAL METHOD

Experiments were carried out on male chinchilla rabbits weighing 2.5-3 kg. Soon after decapitation of the animal the stomach was freed of its contents. The muscle tissue of the fundal and pyloric parts (the boundary between them runs along the falciform fold) was quickly freed from the mucous and serous membranes and also from visible pieces of fat. To prevent the action of proteolytic enzymes of the mucous membrane these manipulations were carried out in cold 0.05 M phosphate buffer, pH 7.4. The muscle tissue thus isolated was carefully cut into pieces with scissors and then minced in a glass homogenizer with Teflon pestle (1000 rpm) for 1.5-2 min in 0.03 M phosphate buffer, pH 7.4.

After exposure for 15 min with 0.5% sodium deoxycholate (DOC) the homogenates were centrifuged at 12,000 g for 20 min at 0-4°C. Activity of HK in the resulting supernatant was determined by a spectrophotometric method [1], total LDH activity by the method of Wroblewsky and La Due [14], the LDH isoenzyme spectrum by a method modified by Markelov [5] without the addition of cyanide to the incubation medium, and CCO activity by the method of Cooperstein and Lazarow [8]. CCO activity was calculated by the formula $A = \Delta E \times 60/$ mg protein, where $\Delta E$ is the extinction at $\lambda = 550$ nm before and after enzymic oxidation of reduced cytochrome c. The protein content in the extracts was determined by Lowry's method

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TABLE 1. Isoenzyme Spectrum of LDH (in %) in Muscle of Various Parts of the Stomach (M ± m)

| Part of stomach | LDH-1 | LDH-2 | LDH-3 | LDH-4 |
|---|---|---|---|---|
| Fundal | 63,4±1,30 | 20,0±0,83 | 9,6±1,22 | 6,0±0,91 |
| Pyloric | 38,4±1,43 | 36,2±0,62 | 18,3±0,98 | 7,1±0,48 |
| P | <0,001 | <0,001 | <0,001 | <0,05 |

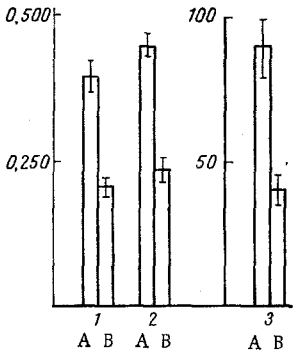


Fig. 1. Enzyme activity in fundal (A) and pyloric (B) parts of muscle tissue of the rabbit stomach: 1) HK activity (in μmoles glucose/mg protein/h); 2) total LDH activity (in μmoles pyruvate/mg protein/min); 3) CCO activity (in $\Delta E_{550}$ before and after enzymic oxidation of reduced cytochrome c/mg protein/h). Ordinate: on the left; HK and LDH activity on the right; CCO activity.

[11]. The total LDH activity and the LDH isoenzyme spectrum were determined in extracts not treated with DOC.

## EXPERIMENTAL RESULTS AND DISCUSSION

As the results in Fig. 1 show, the specific activity of the enzyme studied was much higher in the fundal than in the pyloric part of the stomach: approximately twice as high for HK and LDH and 2.5 times higher for CCO. Similar relations were found when the activity of the enzymes was expressed per gram wet weight of tissue: activity of HK 29.7 ± 0.06 and 14.4 ± 0.42 μmole glucose/h, of LDH 4.49 ± 0.18 and 2.07 ± 0.12 μmole pyruvate/min, of CCO 8,148 ± 819 and 3299 ± 468 conventional units/h for the fundal and pyloric parts, respectively (differences statistically significant – $P < 0.001$).

It is interesting to compare the results of investigation of the activity of CCO, reflecting the intensity of metabolism as a whole and the aerobic course of oxidation, with the LDH spectrum obtained in the fundal and pyloric parts. On comparison of the relative content of LDH isoenzymes in parallel experiments (Table 1) predominance of the LDH-1 isoenzyme can be seen in the fundal part, whereas in the pyloric part the content of LDH-2 and LDH-3 was somewhat higher (no LDH-5 was found in these experiments, evidently because of its small relative content). An LDH spectrum of similar character was obtained by Prochazka et al. [13] in their study of the muscle tissue of the fundal and pyloric parts of the rat stomach. According to some workers [9], LDH, consisting of four H-polypeptide chains, is synthesized intensively in tissues with a mainly aerobic type of metabolism.

Rhythmic contractions in the pyloric part of the stomach and tonic in the fundal part thus correspond to quite definite differences in tissue metabolism: the activity of the enzymes of aerobic oxidation studied was much higher in the fundal than in the pyloric portion.

A relationship of a similar character between the type of the contractile response and activity of the enzymes of anaerobic oxidation has also been found for skeletal muscle. For instance, in the slow skeletal muscles the mitochondria are more numerous and the activity of the enzymes of aerobic oxidation is higher than in fast skeletal muscles [12]. In the period of early ontogeny the motor response of the skeletal muscle of animals is tonic in character, and only with age is the typical tetanic response formed [2]. These changes in the contractile response correlate clearly with changes in the activity of some enzymes. In particular, in the initial period of individual development an extremely high level of CCO activity [3] and predominance of LDH-1 [4] are observed in skeletal muscle.

The results given in this paper are evidence of a close connection between the character of the motor response of a muscle and the direction of its metabolic processes.

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