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Research Note

Electrophoretic Studies on Enzymes in Ascarids
I. Lactate Dehydrogenase Isozymes in *Ascaris suum*

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Lactate dehydrogenase (LDH) has been studied in great detail from a wide variety of animal tissues (Markert, 1975). In vertebrates, two types of subunits are encoded by different two genes and are capable of association into five tetrameric isozymes (Markert, 1963). On the other hand, LDH of invertebrates differs from that of vertebrates with respects to stereospecificity and molecular configuration (Long and Kaplan, 1968; Agatsuma and Takeuchi, 1976; Agatsuma and Tsukii, 1980). Few studies are present concerning the number of forms and the nature of LDH in parasitic helminths (Nagase, 1968; Langer and Smith, 1971; Burke *et al.*, 1972). Nagase (1968) has demonstrated that the body fluid of *Ascaris suum* possessed two bands of LDH, whereas the muscle and the ovary only single band. But, Langer and Smith (1971) have shown that the whole body extracts of *A. suum* contained four isozymes of LDH with no significant sex differences.

In the present study, more detailed electrophoretic analyses of *A. suum* LDH were performed by means of polyacrylamide gel electrophoresis.

Adults of *A. suum* were obtained from intestine of naturally infected pigs at the local slaughterhouse in Kochi Prefecture in 1980. The materials were thoroughly washed with 0.85% physiological saline, and were subsequently stocked in a deep freezer at −80 C until used. Five worms of each sex were dissected into muscle, intestine, testis, seminal vesicle, ovary, uterus and body fluid. Each tissue of the organ mentioned above was homogenized in 0.1 M phosphate buffer (pH 7.5) using teflon homogenizer. The resulting homogenates were centrifuged at 1,000 g for 3 min at room temperature and the supernatants were used for electrophoresis. The precedure for vertical polyacrylamide gel electrophoresis followed essentially the method reported by Aotsuka and Asami (1979) with a slight modification. The electrophoresis was conducted at 4 C for 3 h with a constant current power at 2.0 mA/cm gel width. LDH assay was performed after electrophoresis by incubating the gel at 37 C in the same incubation mixture as those of Agatsuma and Takeuchi (1976).

Fig. 1 shows electrophoretic patterns of LDH in several different tissues of *A. suum*. No sexual difference was recognized in LDH band patterns of the muscle and the intestine extracts, and the body fluid. Muscle extract possessed one or two bands of LDH, of which slower one is designated as LDH-M. Female reproductive organs, uterus and ovary, and body fluid showed single band,

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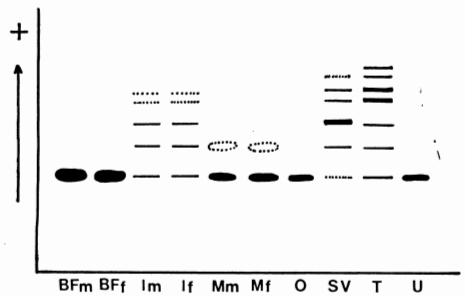

Fig. 1 Diagrammatic representation of LDH isozymes in *A. suum* tissues. BF; body fluid, I; intestine, M; muscle, O; ovary, SV; seminal vesicle, T; testis, U; uterus, m; male, f; female

having the same mobility as LDH-M. These results were, on the whole, consistent with earlier findings on *A. suum* LDH (Nagase, 1968). However, additional different types of LDH were found in other tissues used in the present investigation. That is, intestine of both sexes and male reproductive organs, testis and seminal vesicle, showed multiple banding patterns, though their mobilities were different from each other. In these patterns, most anodally migrating band was found in testis. This testis-specific band was designated as LDH-T. Testis possessed totally six or seven bands of LDH activity. Intestine and seminal vesicle LDH showed three or four bands, of which positions are between LDH-M and LDH-T.

From the results obtained, it can be assumed that LDH in *A. suum* is controlled by two loci, which encoded the isozymes, LDH-M and LDH-T. According to this hypothesis, it might be thought that the locus encoded LDH-M isozyme functions predominantly in muscles of both sexes and female reproductive organs, while both two loci function equivalently in testis, resulting in production of multiple-isozyme pattern, probably random association among each subunit produced by the two loci.

Isozyme unique to male reproductive tissue have been described for LDH in other animal species (Zinkham *et al.*, 1964, 1969). The general biological significance of this phenomenon is further emphasized by the findings of multiple forms of LDH in *A. suum* testis, though the specific metabolic roles fulfilled by these isozymes have not been ascertained.

There is little evidence to point to either a dimeric or tetrameric structure for LDH of *A. suum*. Clearly, further studies such as dissociation-association studies will necessarily be required to determine the number of polypeptides forming each isozyme.

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短　報

回虫類アイソザイムの電気泳動法による研究
I. ブタ回虫の乳酸脱水素酵素（LDH）アイソザイム

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乳酸脱水素酵素（LDH）は，嫌気的条件下でのエネリギー産生に重要な役割を果たしている酵素である．本研究では，ポリアクリルアミドゲル電気泳動法により，ブタ回虫 *A. suum* 諸器官の LDH を比較した．その結果，体腔液，筋層，雌生殖器官は，Nagase (1968) の報告と一致して，1本のバンドがみられたが，雄生殖器官や腸には，複数のバンド（5～8本）が検出された。これらの結果は，各アイソザイムが，それぞれの器官において何らかの異なった役割を果たしていることを暗示しているように思われた．