Table 1. IgE levels (ELISA $A_{492} \pm SD$) in microfilaraemic patients ($n = 18$) after diethylcarbamazine treatment

	A_{492}					
	Pretreatment	Days posttreatment				
Antigen	Day 0	15	30	90	240	365
Sd_{30}	0.22 ± 0 10	0 36 ± 0 16 **	0 06 ±0 04 *	0 02 ± 0 02 *	0 05 ± 0 04 *	0 08 ± 0 06 *
W. bancrofti L_3	0.34 ± 0 10	0 40 ± 0 22 NS	0 19 ± 0 11 *	0.16 ±0.10 *	0.24 ± 0 13 *	0 30 ± 0 18 NS

**$P < 0 05$ compared to day 0; *$P < 0 01$ compared to corresponding day 0 or day 15.
NS. not significant compared to day 0

greater changes than those to *W. bancrofti* L_3 antigen. For example, the mean reductions in IgE response to Sd_{30} and L_3 antigen 3 months after treatment were 90% and 50% of pretreatment values, respectively. The lower reduction in L_3-IgE may be caused by persistent exposure to *W. bancrofti* larvae in the endemic region. It might also be possible that DEC treatment induces changes in IgE response to epitope(s) selectively present in Sd_{30} allergen. Earlier we have demonstrated that IgE response to these antigens is filariae-specific since people living in nonfilarial regions of Orissa exhibited negligible IgE levels[2 6]. IgE production is sustained in people in endemic areas by them being continuously exposed to the infection since clinically normal individuals of the endemic region have high IgE levels[2].

Considering the biologic roles of IgE antibodies in parasite infections, which vary from allergic disorders to conferring protection to hosts[7,8], the effect of drug treatment on IgE response is an interesting topic. The changes in specific IgE levels as reported here have not been characterized in human filariasis. The present report describes the response of the levels of IgE antibodies to two different antigens, L_3 stage of *W. bancrofti*, which initiates the infection, and a purified filarial allergen, Sd_{30}. Both the antigens exhibited diminished IgE production in treated patients over a course of time.

Immunologic changes following a two-year community trial of ivermectin treatment in Guatemala of patients infected with *Onchocerca volvulus* have been reported[9]. Enhanced parasite-specific IgE levels were noticed 6 months after treatment, which fell marginally below the pretreatment level by the second year. Although antiallergic action of DEC is well documented[10–12], the underlying mechanism was not known. The present results demonstrate clearly that DEC treatment diminishes the production of filarial IgE antibodies in humans and thereby contributes to decreasing allergic reactions.

The long-term monitoring of the study population will be helpful in finding out when IgE levels would be generated again in the DEC-treated patients.

1. Hussain, R., Hamilton, R. G., Kumarswami, V., Franklin, A. N. and Ottesen, Jr E. A, *J. Immunol*, 1987, **127**, 1623–1628
2 Das, M. K., Beuria, M. K. and Dash, A P, *Int. Arch. Allergy Immunol.*, 1992, **99**, 118–122.
3. Lammie, P. J., Eberhard, M L., Lowrie, R. C. and Katz, S. P., *Trans. R Soc. Trop. Med. Hyg*, 1988, **82**, 726–729.
4. Ramprasad, P, Prasad, G. B. K. S and Harinath, B. C., *Acta Tropica*, 1988, **45**, 245–255.
5. Piessens, W. F., Ratiwayanto, S., Pissens, P. W., Tuti, S., Mc Greevy, P. B., Darwis, F., Palmieri, J. R., Koiman, I. and Dennis, D. T., *Acta Tropica*, 1981, **38**, 227–234
6. Beuria, M K. and Das, M. K., *J. Biosci.*, 1992, **17**, 435–461.
7. Oglivie, B., *Nature*, 1964, **204**, 91–92
8. Hagan, P., *Parasite Immunol*, 1993, **18**, 1–4.
9. Steel, K., Lujan-Trangay, A., Gonzalez-Peralta, C., Zea-Flores, G. and Nutman, T. B., *J. Infect. Dis.*, 1991, **164**, 581–587.
10. Thiruvengadam, K. V., Subramaniam, N., Devarajan, T. V and Zachariah, M. G. M, *J. Indian Med. Assoc.*, 1974, **63**, 278–281.
11. Murthy, P. K., Katiyar, J. C., Chandra, R., George, P. A. and Sen, A. B., *Indian J. Med. Res.*, 1978, **68**, 428–434.
12. Mackenzie, C B, *Trop. Dis. Bull.*, 1985, **82**, R1–R37.

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Biochemical basis for the differentiation of the two nonpoisonous snakes *Eryx conicus* Schneider and *Eryx johnii* Russell (family: Boidae)

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The tissue-specific patterns of lactate dehydrogenase (LDH) for two species of the genus *Eryx* are reported and characterized utilizing sodium deoxycholate. LDH-1 is predominant in *Eryx conicus*, whereas LDH-3 and LDH-2 are observed in *Eryx johnii*. Sodium deoxycholate is a selective inhibitor of LDH-5 in *Eryx johnii*. Variations are observed in all the tissues between these two species and there is a variability of tissue LDH expressions within the species.

ISOENZYMES are multiple molecular forms of enzymes[1]. They can serve in taxonomic, genetic, phylogenetic and

evolutionary studies and help to solve many biological problems[2–5]. Lactate dehydrogenase (lactate: NAD oxido reductase, EC 1.1.1.27) controls the interconversion of pyruvate and lactate in the glycolytic pathway and thereby serves as an important source of the oxidized coenzyme (NAD) during periods of transient anaerobiosis. In most of the vertebrates, the isoenzymes of LDH are formed by the random self-assembly, *in vivo* and *in vitro*, of two different polypeptides A and B to form all possible isozymes, the two homopolymers A_4 and B_4 and the three heteropolymers A_3B_1, A_2B_2 and A_1B_3. The specificity of LDH polypeptide association is brought about by a combination of noncovalent forces[6,7].

LDH constitutes an excellent model system for the analysis of the problems of polypeptide assembly. Depending upon the organism and tissue, LDH can exhibit self-assembly[8,9], epigenetic modification of assembly[10–12], genetically controlled restriction of assembly[13–17] and directed assembly.

In this paper we report our investigation of the electrophoretic characteristics of LDH from two species of nonpoisonous snakes belonging to the genera *Eryx* of the family Boidae. Our aim is to determine the tissue-specificity of LDH subunit synthesis, to look for variations at the LDH locus and to compare isoenzyme patterns from the tissues of these two species.

Two species of snakes, namely *Eryx conicus* and *Eryx johnii*, were used in this study of the LDH tissue patterns. The snakes, all adults, used in this investigation were collected from different localities of Madras.

Twelve tissues, namely eye lens, brain, trachea, lungs, heart, liver, stomach, intestine, muscle, kidney, vertebrae and skin, were dissected from fresh specimen. Care was taken to see that the eye lens was free from contamination by the vitreous humour, retinal and choroid elements. Brain, lungs, heart and liver were washed thoroughly with ice-cold phosphate buffer of pH 7.4 as to render them free from traces of blood. Stomach and intestine were washed till they were free from their contents. Other tissues were washed thoroughly with phosphate buffer of pH 7.4. All the chemicals used in this study were purchased from E. Merck (West Germany).

The tissues were homogenized in iced condition with phosphate buffer of pH 7.4. The homogenates were centrifuged at 4°C at 25,000 × *g* for 30 min. The supernatants were used for gel electrophoresis.

A 5.5% polyacrylamide gel was used[18]. The current flow was adjusted to 2.5 mA per sample and run for 45 min with Tris–glycine buffer of pH 8.3. The gels were removed and stained. Inhibition studies were carried out using sodium deoxycholate.

The main aim was to determine the tissue-specificity of the LDH subunit synthesis in order to pinpoint the species specific biochemical markers and also to compare the LDH isoenzyme patterns in these two species.

The LDH patterns of the 12 tissues of *Eryx conicus* and *Eryx johnii* were observed and are shown in Figures 1 and 2, respectively.

The LDH isoenzymes of the snakes studied exhibited a net negative charge difference between isoenzymes. Therefore, the isoenzymes migrated more slowly towards the anode at pH 8.3.

In *Eryx conicus*, of the 12 tissues, 10 showed the predominance of only LDH-1 (B_4) isoenzyme – vertebrae and skin exhibited the four-isoenzyme pattern, LDH-5 (A_4), LDH-4 (A_3B_1), LDH-3 (A_2B_2) and LDH-2 (A_1B_3).

In *Eryx johnii*, except for lungs and vertebrae, all the other tissues analysed exhibited two isoenzyme fractions LDH-3 (A_2B_2) and LDH-2 (A_3B_1). Lungs and vertebrae showed the presence of three isoenzymes, namely LDH-4 (A_3B_1), LDH-3 (A_2B_2) and LDH-2 (A_1B_3).

The Rf values of these LDH isoenzymes were 0.18, 0.24, 0.28, 0.32 and 0.34 for LDH-5, LDH-4, LDH-3, LDH-2 and LDH-1, respectively, and are presented in Figure 3.

There was widespread resemblance among the LDH isoenzymes of these two species and a characteristic pattern of electrophoretic mobilities of LDH-5 (A_4), LDH-4 (A_3B_1), LDH-3 (A_2B_2), LDH-2 (A_1B_3) and LDH-1 (B_4). No difference was found in the LDH patterns of males and females. Each tissue exhibited a characteristic LDH pattern and the tissue-specific difference in the relative staining intensities of each LDH isoenzyme attributable to the quantitative variation in the proportion of the isoenzymes, which has been a function of a particular ratio of A and B subunits of each tissue. The staining intensity is shown in the densitometric scanning of the LDH isoenzyme of the two species, illustrated in Figures 4 and 5.

Sodium deoxycholate was used as a selective inhibitor of LDH-5 (A_4) isoenzymes to establish the subunit composition of LDH isoenzymes and to determine their genetic basis. There was a selective inhibition of A isoenzymes. The B subunits were resistant to the action of sodium deoxycholate. The characterization of the LDH isoenzymes was performed based on the literature[19].

The importance of this study lies in highlighting as to what extent the LDH isoenzymes play a role in the establishment of the basic tenets of biochemical taxonomy.

The differential regulation of gene function is a fundamental aspect of cellular differentiation. Isoenzyme (multiple molecular forms of enzymes) are ideal gene products for the analysis of differential gene function during embryogenesis and cellular differentiation[20–22].

Differences in the tissue pattern of enzyme locus expression were used to infer the extent of divergence among species. Some locus tissue expressions diverged markedly among species, while others were conserved.

Figure 1. LDH isoenzyme profile of twelve tissues of *Eryx conicus*.

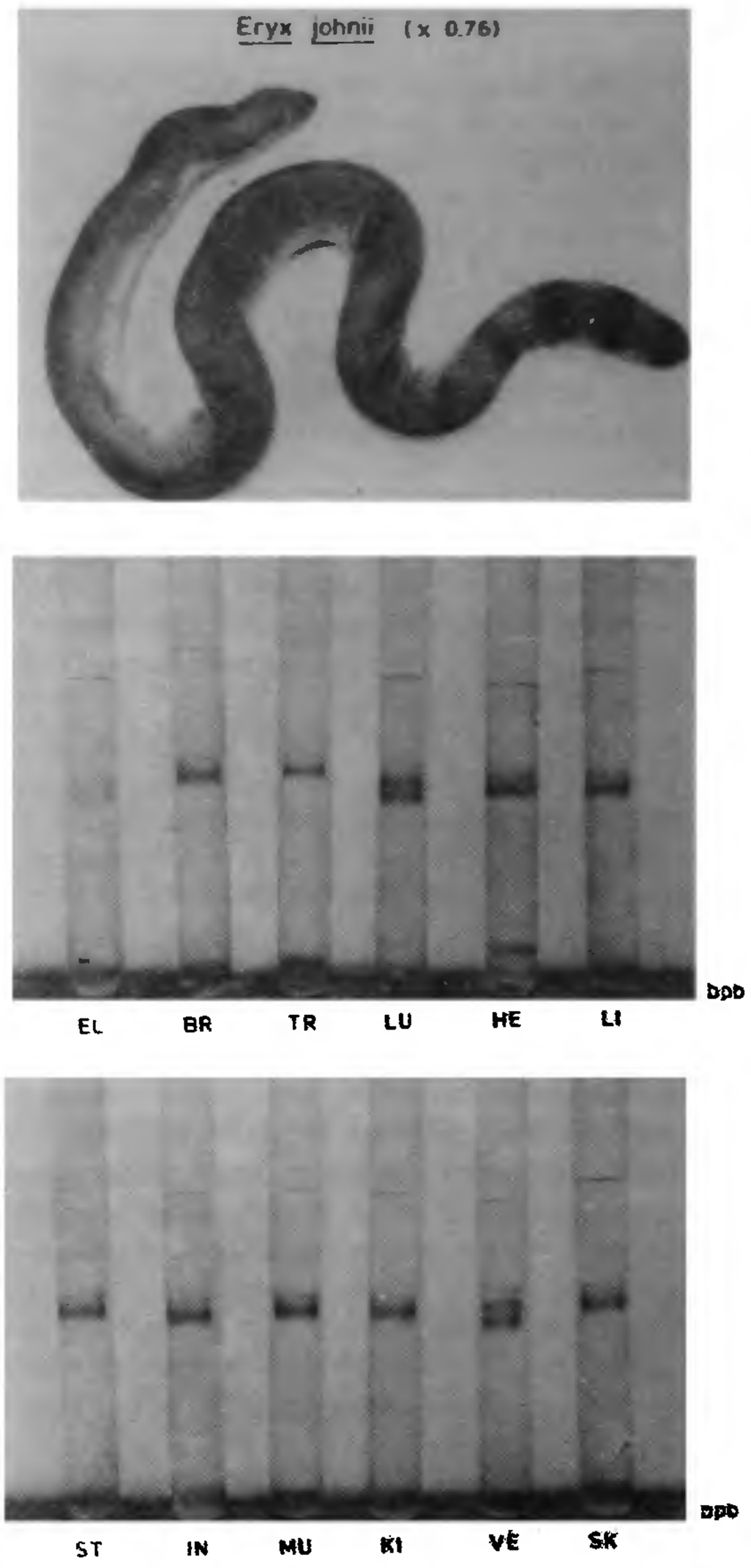

Figure 2 LDH isoenzyme profile of twelve tissues of *Eryx johnii*.

Differences in activity for a given enzyme among tissues, as well as for different enzymes within the same tissue, were sufficiently independent to permit each locus tissue expression of a species to be treated as a separate character. Statistically significant difference in levels of tissue enzyme activities among species were then used to construct a phylogeny. The phylogeny constructed using tissue enzyme expressional differences was similar to that based on enzyme structural difference and to one of morphologically based phylogenies[23].

The partial functional redundancy of L-lactate dehydrogenase (LDH) (EC 1.1.1.27) isoenzymes has permitted marked difference in the tissue regulations of their genes[24–29]. Both differential synthesis and catabolism contribute to the tissue patterns of LDH gene expression[30–34]. Tissue differences in the steady-state levels of LDH isozymes are probably due to mutations at sequences other than those coding for the respective isoenzymes. Such mutations could affect the number of enzyme molecules by altering one or more processes, ranging from transcriptions to catabolism, that is, by altering the regulations of gene expression in the broadest sense.

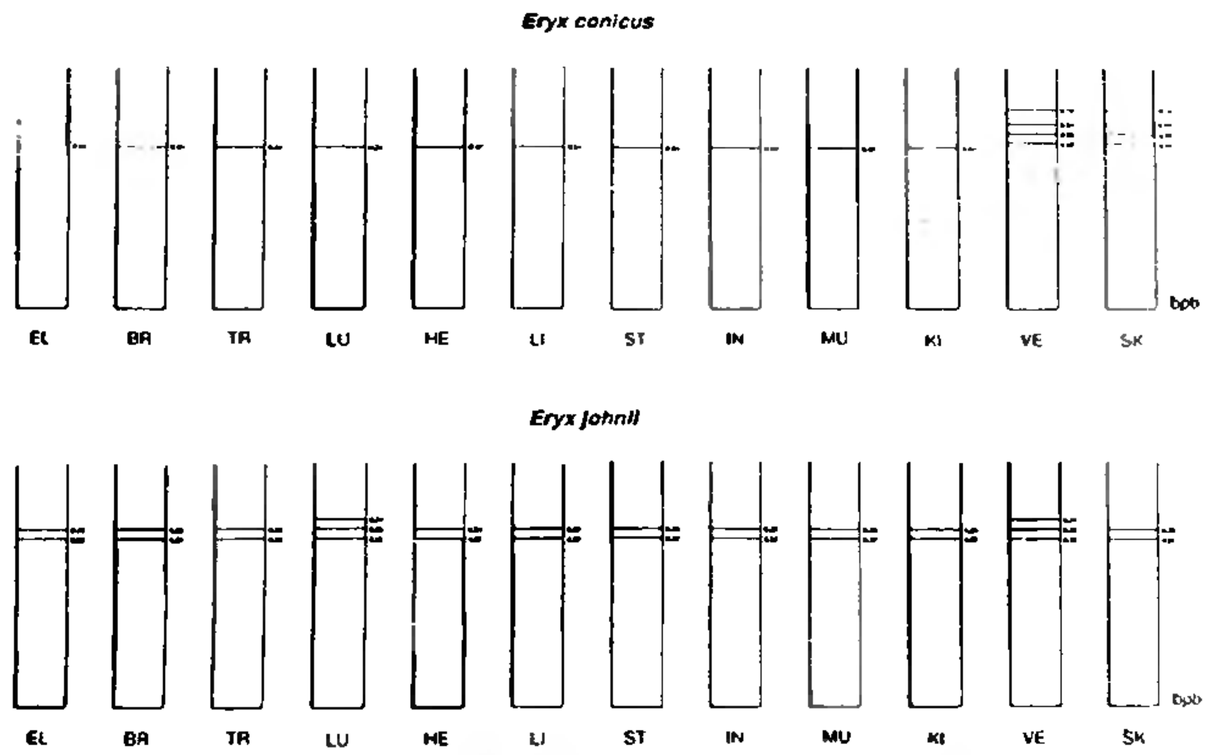

Figure 3. Rf-values of LDH isoenzymes of twelve tissues of *Eryx conicus* and *Eryx johnii*.

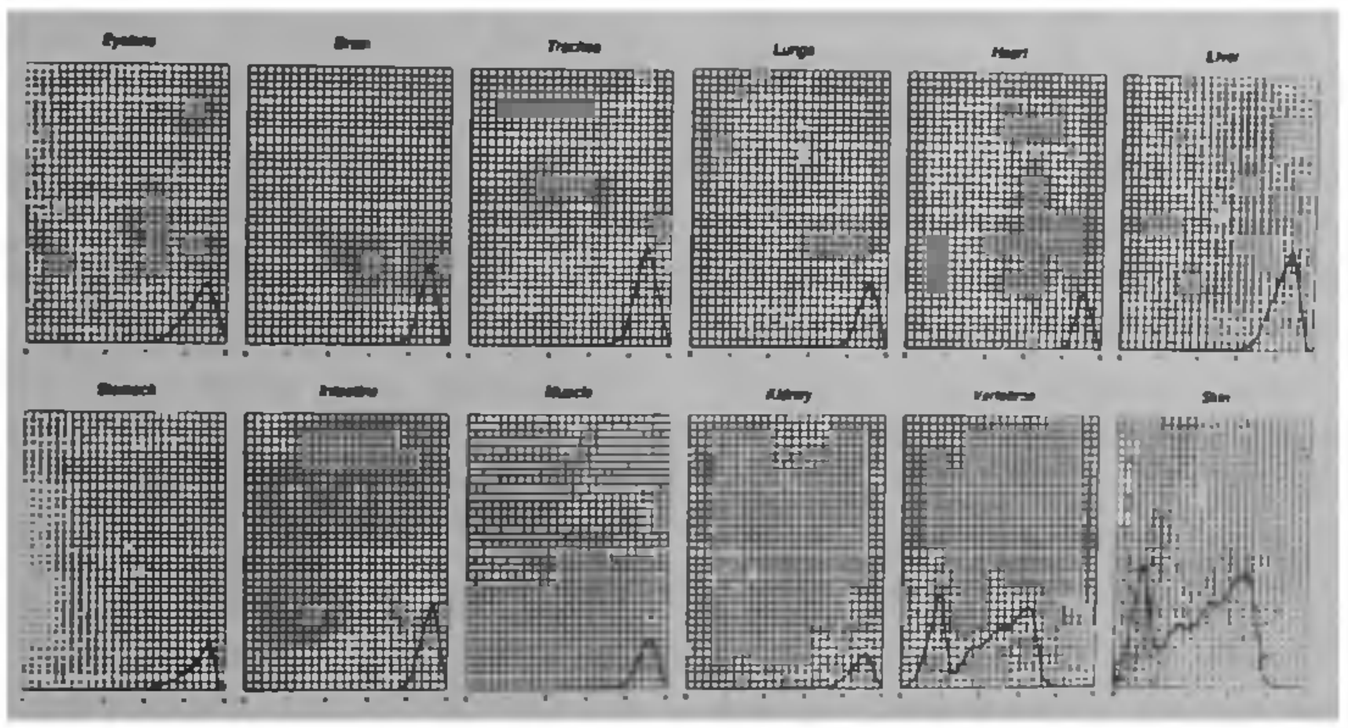

Figure 4. Densitometric scanning of LDH isoenzymes of *Eryx conicus*.

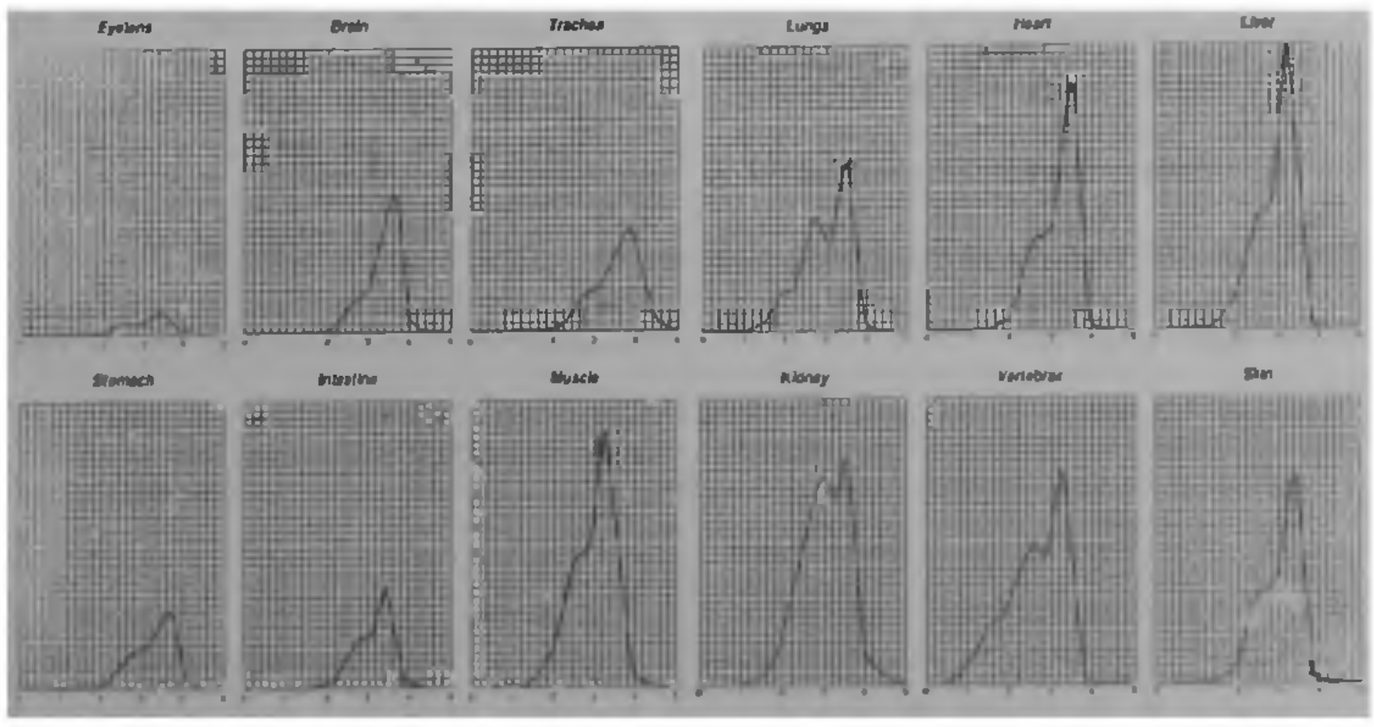

Figure 5. Densitometric scanning of LDH isoenzymes of *Eryx johnii*.

In several vertebrate species, regulatory genes that control the tissue expressions of the LDH-A and LDH-B genes have been detected[35-37]. These observations provide a basis for proposing that the species difference in the tissue patterns of LDH gene expression result from mutational changes at such regulatory genes.

Vertebrate LDH isoenzymes show one- to five-isoenzyme pattern and particularly the two-gene–five-isoenzyme model found in most of the mammals is the full array of LDH-A and LDH-B combination.

All tissues studied contained detectable amount of at least one of the five isoenzymes. Since there seems to be a homology between tissue distribution of isoenzymes in snakes and in other vertebrates, the same subunit designations A and B are used as applied to other vertebrates.

The predominance of LDH-1 (B_4) isoenzyme in all the tissues except vertebrae and skin in *Eryx conicus*, which was not observed in *Eryx johnii*, provides us a clue for identification of *Eryx conicus*.

The predominance of LDH-3 (A_2B_2) and LDH-2 (A_1B_3) in all the tissues except for lungs and vertebrae in *Eryx johnii*, which were not observed in *Eryx conicus*, gives a clear demarcation in establishing species identity.

Here with this authentic electrophoretic evidence, the LDH profile can be used as a specific biochemical marker for establishing species identity in the evolutionary scale of organisms at different levels of biological organization.

Lactate dehydrogenase of rat tissues was selectively inactivated by sodium deoxycholate[38]. Yet another highlight of this investigation is the discovery of sodium deoxycholate as specific inactivator of LDH-5 (A_4) isoenzyme in snake tissues.

1. Markert, C. L. and Moller, F., *Proc. Natl Acad Sci.*, 1959, **45**, 753–763.
2. Fisher, S. E., Shaklee, J. B., Ferris, S D. and Whitt, G. S., *Genetica*, 1980, **52/53**, 73–85
3 Callegarini, C. and Basaglia, F., *Boll Zool*, 1978, **45**, 35–40.
4. Whitt, G. S., *Isoenzymes Current Topics in Biological and Medical Research* (eds Rattazzi, M. C., Scandalios, J G. and Whitt, G S), Alan R. Liss, New York, 1987, vol. 15, pp 1–26.
5. Basaglia, F. and Callegarini, C, *Comp. Biochem. Physiol*, 1988, **B89**, 731–736
6. Appella, E. and Markert, C. L, *Biochem. Biophys Res. Commun*, 1961, **6**, 171–176.
7. Market, C. L., *Science*, 1963, **140**, 1329–1330.
8 Markert, C. L., Sixth Int. Congr Biochem. Abstracts, 1964, **IV-104**, 320
9. Epstein, C J, Carter, M M. and Goldberger, R. F, *Biochem Biophys. Acta*, 1964, **92**, 391–394
10 Markert, C. L, *Ann. NY Acad Sci.*, 1968, **151**, 14–40
11. Markert, C. L., *J. Cell Physiol.*, 1968, **72**, 213–230.
12. Markert, C. L. and Whitt, G. S, *Experientia*, 1968, **24**, 977–991.
13. Markert, C. L. and Faulhaber, I., *J. Exp. Zool.*, 1965, **159**, 319–332.
14. Salthe, S. N, Chilson, O. P. and Kaplan, N. O., *Nature*, 1965, **207**, 723–726.
15 Odense, P. H, Allen, T. M. and Leung, T. C., *Can. J. Biochem*, 1966, **44**, 1319–1326.
16. Markert, C. L., Seventh Int. Congr. Biochem Abstracts, 1967, p. 828
17. Whitt, G. S, *Arch. Biochem. Biophys.*, 1970, **138**, 352–354.
18. Dietz, A A, Lubrano, T and Rubinstein, H. M., in *Standard Methods in Clinical Chemistry*, Academic Press, New York, 1972, vol 7, p. 49
19. Schwantes, M. L. B, *J. Exp. Zool*, 1973, **185**, 311–316.
20. Markert, C. L, in *Hereditary Developmental and Immunologic Aspects of Kidney Disease* (ed Metcoff, J), Northwestern University Press, 1962.
21 Markert, C L., in *The Harvey Lectures*, Series 59, Academic Press, New York, 1965, pp. 187–218
22. Markert, C. L and Ursprung, H, *Dev. Biol*, 1962, **5**, 363–381
23. Kettler, M . K., Ghent, A. W. and Whitt, G. S, *Mol Biol. Evol*, 1986, **3**, 485–498
24 Markert, C. L., Shaklee, J. B. and Whitt, G. S., *Science*, 1975, **189**, 102–114.
25. Ferris, S D and Whitt, G. S., *Nature*, 1977, **265**, 258–260
26. Zuckerandl, E., *J. Mol Evol.*, 1978, **12**, 57–89.
27. Whitt, G. S, in *Proceedings of the Second International Congress of Systematic and Evolutionary Biology* (eds Scudder, G G. E and Revel, J L), Hunt Institute for Botanical Documentation, Pittsburgh, 1981, p. 271.
28 Whitt, G S, *Isoenzymes. Current Topics in Biological and Medical Research* (eds Rattazzi, M. C., Scandalios, J. G. and Whitt, G S), Allan R. Liss, New York, 1983, vol. 10, p. 1.
29. Whitt, G. S, *Cell Biochem Funct.*, 1984, **2**, 134–139
30. Don, M. and Masters, C. J, *Int. J Biochem*, 1976, **7**, 215–220.
31 Nadal-Ginard, B., *Proc. Natl Acad Sci.*, 1976, **73**, 3618–3622.
32. Nadal-Ginard, B, *J. Biol Chem*, 1978, **253**, 170–177.
33. Fritz, P J. and Pruitt, K. M, in *Isozymes: Current topics in Biological and Medical Research* (eds Rattazzi, M. C, Scandalios, J G. and Whitt, G. S), Alan R. Liss, New York, 1977, vol. 1, p 125
34 Masters, C. J., *J Biochem*, 1982, **14**, 685–689.
35 Shows, T B. and Ruddle, F H, *Proc. Natl. Acad. Sci*, 1968, **61**, 574–581.
36 Khlebodarova, T M. and Serov, O L, *Biochem. Genet*, 1980, **18**, 1027–1039.
37 Stock, V. and Gill, T. J., *Biochem Genet*, 1983, **21**, 933–941
38 Lehnert, T and Berlet, H. H, *Biochem J*, 1979, **177**, 813–818

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