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Distribution and Relative Activity of the Isoenzymes of Lactic Dehydrogenase in the Tissues of *Rana ridibunda*

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Lactic dehydrogenase (LDH) is found in *Rana ridibunda* in four visible molecular forms. No evidence is fortcoming of the activity proper to isoenzyme LDH-2. The isoenzymes have a very low electrophoretic velocity compared with those of the rat and of other amphibians.

Results quite recently obtained in our laboratory with other amphibians of different species suggest that amphibians also possess an electrophoretic pattern with the five isoenzymes typical of mammals.

The authoress discusses the possible influences of adaptation to the environment throughout the course of evolution as an explanation of the different activity of the isoenzymes in the heart and in other tissues in *Rana ridibunda* adults.

Lactic dehydrogenase (LDH) is an oxidoreductase which takes part in the metabolism of carbohydrates by catalysing the passage of pyruvic into lactic acid reversibly in the process of glycolysis in anaerobic conditions.

The isoenzymes of lactic dehydrogenase are tetramers resulting from the combination of two types of polypeptides designated by the initials H and M.

They are classified according to the rate of electrophoresis in descending order, as follows: LDH-1 (H_4), the most anodic, which is characteristic of the heart in mammals; LDH-2 (H_3M); LDH-3 (H_2M_2); LDH-4 (HM_3); LDH-5 (M_4), the most cathodic, which is characteristic of the mammalian skeletal muscles.

The enzyme LDH is found in the tissues of both vertebrates and invertebrates. Amongst invertebrates, special attention has been given to the mollusc and arthropod groups (1, 12, 18), while among the vertebrates particular interest attaches to the studies carried out on fish, amphibians, birds and mammals, both in adults and in the course of embryonic development (3, 4, 7, 8, 11, 13-16, 19).

In this paper, we present the results obtained by us in *Rana ridibunda* (an anurous amphibian). We have studied the distribution of the isoenzymes of lactic

dehydrogenase in the various tissues of the adult. The results we publish in this paper will form the basis for further studies now in course in our laboratory on variations in the LDH isoenzyme pattern during embryonic development and on the possible influence of environmental conditions on the activity connected with the isoenzymes.

Materials and Methods

The animals used for the experiments were adults of the species *Rana ridibunda*, from Tarragona (Spain), freshly brought from the country. The experiments were carried out in the months of October, November and December.

A glass homogenizer was used for homogenizing various tissues in Tris-ClH buffer (0.1 M, *p*H 7.4) containing 0.25 M of saccharose at a concentration of 10 mg per ml (intestine and heart) and of 20 mg per ml (ovary, brain, liver, muscle, kidney, lung and fat bodies). Centrifuging at 4,000 rpm. The supernatant is kept in freezing conditions up to the moment of use. It is used within 48 h and is thawed out only once.

Separation of the isoenzymes was carried out by electrophoresis on polyacrylamide gel with Shandon cells. The method followed was that described by DAVIS and ORSTEIN in 1964 (10, 17) with certain modifications and with a double gel (2.5 % above and 6.5 % below). The electrophoresis was carried out for 1 h and 15 min. at a constant low temperature (16° C) and a constant potential gradient and intensity. of 4 mA per tube. The LDH activity bands were developed by incubating the gels at 37° C in the dark in a water-bath and in a solution containing 12 ml of Tris-ClH buffer at *p*H 7.5 (0.05 M), 6 ml of DL sodium lactate at *p*H 7.5 (0.5 M), 7 ml of tetrazolium nitro-blue (2 mg/ml), 0.3 ml of phenazine metasulphate (2 mg per ml) and 20 mg of NAD. Incubation is carried out for a period of 6 min., which is prolonged to 12 for fat bodies. The temperature inside the electrophoretic cells is controlled. The isoenzymes are quantified with a Chromoscan-type densitometer from Joyce Loebl Co. Ltd., Gateshead, England.

Results

The electrophoretic pattern of the LDH isoenzymes from the various tissues of the adult *Rana ridibunda* is shown in figures 1 and 2. The extracts from the heart (2d) show four LDH activity bands. All the other organs show only three molecular forms of LDH.

We did not find the isoenzyme LDH-2 in any of the tissues, while LDH-1, 4 and 5 were found constantly. LDH-3 was also found in the heart.

The skeletal muscle, liver and brain (fig. 2a, 2e, 1d) possess greater relative activity in the cathodic bands (LDH-4 and 5, fig. 3 a and b). In other words, they show the type M pattern.

The heart and ovaries (fig. 4) show

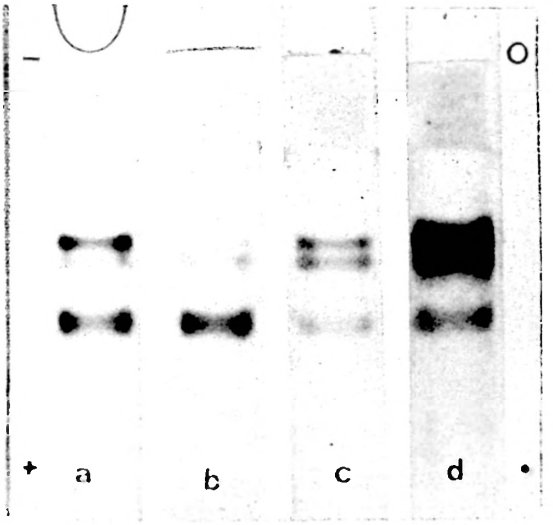

Fig. 1. *Electrophoretic pattern of LDH isoenzymes.*
Kidney (a); ovary (b); lung (c); brain (d) of the adult *Rana ridibunda*. O = origin.

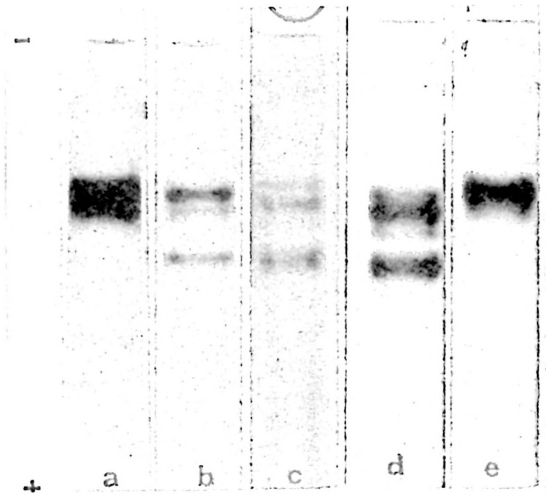

Fig. 2. *Electrophoretic pattern of LDH isoenzymes.*
Muscle (a); intestine (b); fat bodies (c); heart (d) and liver (e) of the adult *Rana ridibunda.*

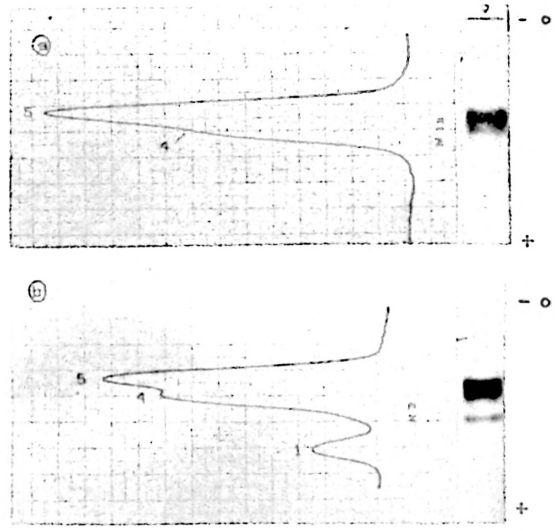

Fig. 3. *Electrophoretic pattern and zimograms of LDH isoenzymes.*
Muscle (a) and brain (b) of the adult *Rana ridibunda.*

greater relative activity of the anodic isoenzymes (LDH-1), as we find in most of the higher vertebrates studied; but in contrast to the higher vertebrates, the heart, especially, shows relatively high activity on the part of the cathodic isoenzymes (LDH-4 and 5), so that it might also be included in the intermediate type (MH).

The kidney, intestine, lung and fat bodies are included in the group of intermediate-type patterns (fig. 5 and 6).

The electrophoretic pattern of the LDH isoenzymes in the heart of *Rana ridibunda* differs very considerably from that found in other vertebrates, and also from that of other anurous amphibians such as *Xenopus laevis* and *Discoglossus pictus* (8, 2). As we can see from a rapid comparison of the isoenzymatic patterns (fig. 7), in *Discoglossus pictus* we observe a gradual decrease in the relative activity of the five isoenzymes, the highest value being the figure for the most anodic, LDH-1, which, as we know, is characteristic of highly aerobic tissues. In *Rana ridibunda*, even though the highest activity is found in the case of isoenzyme LDH-1 (zymogram for fig. 4b), we also observe a high degree of activity in the case of the isoenzyme LDH-4.

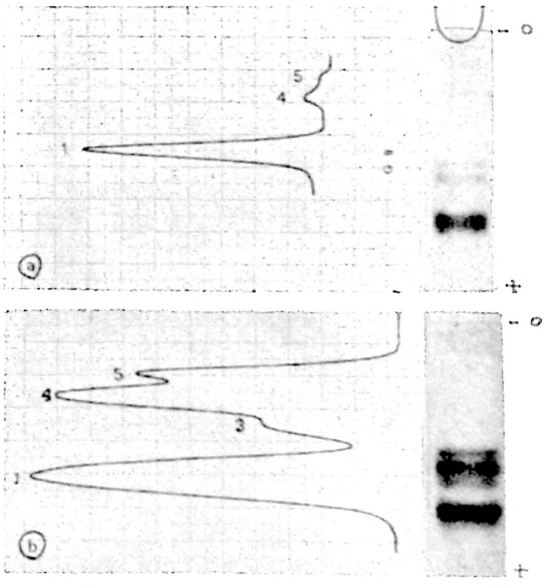

Fig. 4. *Electrophoretic pattern and zimograms of LDH isoenzymes.*
Ovary (a) and heart (b) of the adult *Rana ridibunda.*

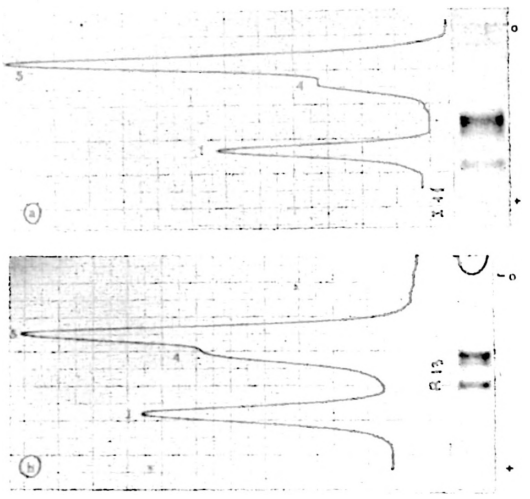

Fig. 5. *Electrophoretic pattern and zimograms of LDH isoenzymes.*
Intestine (a) and kidney (b) of adult *Rana ridibunda.*

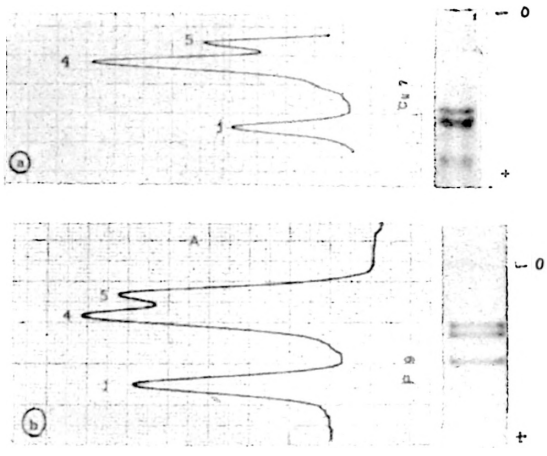

Fig. 6. *Electrophoretic pattern and zimograms of LDH isoenzymes.*
Fat bodies (a) and lung (b) of adult *Rana ridibunda.*

Comparative studies carried out in our laboratory on *Rana esculenta* show that LDH-2 is found in this species in nearly all the tissues of the adult animal. LDH-2 has not been found in *Rana ridibunda*, but the distance between the LDH-1 and

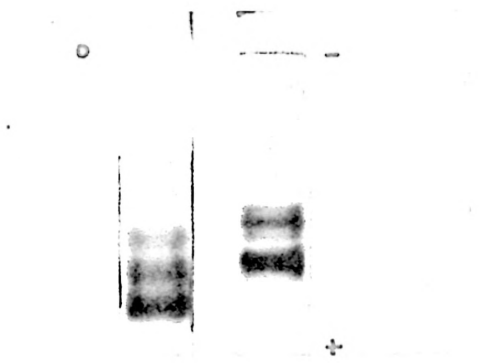

Fig. 7. *Electrophoretic pattern of LDH isoenzymes.*
Hearts of *Discoglossus pictus* (left) and *Rana ridibunda* (right).

the LDH-3 bands is double the distance between any two of the remaining isoenzymes.

Discussion

The presence of a particular number of LDH isoenzymes in the various species of mammals together with the fact that, after analysis of a great variety of tissues, the isoenzymatic pattern of each one turns out to be different, constant and characteristic, led to the conclusion that the specific nature of the isoenzyme distribution is highly significant from the biological point of view.

The properties of isoenzyme LDH-1 (H_1), which is easily inhibited by pyruvate, render it particularly useful in the most aerobic organs, which eliminate the lactate in the circulation and oxidize it to pyruvate, this process of oxidation being completed in the mitochondria. On the other hand, isoenzyme LDH-5 is not inhibited with pyruvate and proves useful in the case of the organs involved in the task of anaerobic glycolysis on a large scale.

The enzymatic activity of the LDH isoenzymes in the heart of *Rana ridibunda* is greatest in the case of LDH-1, but we also observe considerable activity in that

of LDH-4, which is useful in anaerobic tissues.

If the various molecular forms of LDH play an important functional part, as has been suggested, the change-over to a more anaerobic form of metabolism in the heart of the frog might be essential during hibernation and winter lethargy. The amphibian genera *Xenopus* and *Discoglossus*, which possess an electrophoretic model similar to that of mammals as far as the heart is concerned, have been included in a primitive group of amphibians (9). Although *Discoglossus* lives in the immediate neighbourhood of stagnant water, where the supply of oxygen cannot be very large, it nevertheless possesses great vital activity, extraordinary agility and several spawning periods in a year, and its winter lethargy period is probably not so prolonged as the *Rana*. The electrophoretic model of the *Rana* heart, and the fact that most of its tissues show an intermediate type of relative electrophoretic activity of the isoenzymes, probably reflect a form of adaptation on the part of the more highly evolved amphibians to adverse environmental conditions, in particular to prolonged cold weather, during the period of hibernation.

Studies carried out by BURLINGTON and SAMPSON (6) prove that during the squirrel's period of hibernation the proportion of type M subunits in the tissues of the heart increases significantly, and they think that the distribution of the LDH isoenzymes and the enzymatic activity seem to be associated with adaptive metabolic changes during hibernation. This, therefore, would be a case of molecular adaptation to a stimulus in the environment (5).

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References

1. ACHAZI, R. K. and DUSPIVA, F.: *Wilhem Roux Archiv.*, 168, 195, 1971.
2. ALONSO BEDATE, M.: (En preparación.)
3. BOULEKBACHE, M. M. H., DEVILLERS, C., ROSEMBERG, A. J. and JOLY, C.: *C. R. Acad. Sc. Paris*, 272, 114, 1971.
4. BOULEKBACHE, M. M. H., ROSEMBERG, A. J. and JOLY, H.: *C. R. Acad. Sci. Paris*, 271, 2414, 1970.
5. BRUSH, A. H.: *Comp. Biochem. Physiol.*, 27, 113, 1968.
6. BURLINGTON, R. F. and SAMPOON, J. H.: *Comp. Biochem. Physiol.*, 25, 185, 1968.
7. CHEN, P. S.: *J. Exp. Zool.*, 168, 337, 1968.
8. CLAYCOMB, W. C. and VILLEE, C. A.: *Develops. Biol.*, 24, 413, 1971.
9. COHEN, N.: *American Zoologist.*, 11, 193, 1971.
10. DAVIS, B.: *Ann. N. Y. Acad. Sci.*, 121, 404, 1964.
11. DÍAZ-YUBERO, M. A.: Doctoral Tesis. Faculty of Veterinaria. Madrid, 1972.
12. GLEASON, F. H., PRICE, J. S., MANN, R. A. and STUART, T. D.: *Comp. Biochem. Physiol.*, 40, 387, 1971.
13. JOHNSON, K. E. and CHAPMAN, V. M.: *J. Exp. Zool.*, 178, 319, 1971.
14. LEVY, P. L., KAPLAN, R. H., WEIKSNORA, P. J., DREWRY, G. E. and SALTHE, S. N.: *Comp. Biochem. Physiol.*, 39 B, 1053, 1971.
15. LEVY, P. L. and SALTHE, S. N.: *Comp. Biochem. Physiol.*, 39 B, 343, 1971.
16. MARKERT, C. L.: In «Cytodifferentiation and Macromolecular Synthesis» (M. Locke, Ed.). Academic Press, New York, 1963, pp. 65.
17. ORSTEIN, L.: *Ann. N. Y. Acad. Sci.*, 121, 321, 1964.
18. RODRICK, G. E., PAPPAS, P. W. and SMITH, E. C.: *Comp. Biochem. Physiol.*, 40 B, 433, 1971.
19. WRIGHT, D. A. and MOYER, F. H.: *J. Exp. Zool.*, 163, 215, 1966.