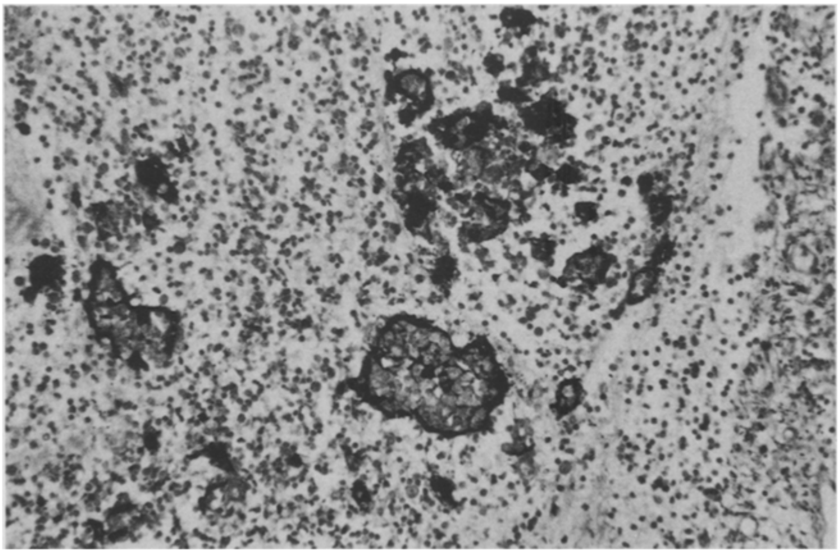


Figure 4. ACPI positive reticulum cells are surrounding lymphocyte clusters and forming irregular aggregates. ×240.

with the destructive phase in lymphoid secondary follicles with PGL remains to be elucidated. It seems to be quite analogous to the destruction of secondary follicles observed in the study of Janossy et al. which employed application of a monoclonal antibody to DRC on frozen sections; in 12 out of 23 patients with PGL this phenomenon could be found[9]. Our findings are also in line with observations by Armstrong and Horne, who, in an ultrastructural study, found discrete or semi-confluent aggregates of expanded DRC in AIDS-related lymphadenopathy[2].

1 Acknowledgments. This work has been partially supported by the grant from the Sigrid Juselius Foundation, Finland.

2 Armstrong, J. A., and Horne, P., Lancet *2* (1984) 370.
3 Tenner-Rácz, K., Rácz, P., Dietrich, M., and Kern, P., Lancet *i* (1985) 105.
4 Järvinen, M., and Rinne, A., Biochim. biophys. Acta *708* (1982) 210.
5 Rinne, A., Alavaikko, M., Järvinen, M., Martikainen, J., Karttunen, T., and Hopsu-Havu, V. K., Virchows Arch. (Cell Pathol.) *43* (1983) 121.
6 Alavaikko, M., Rinne, A., Järvinen, M., Jokinen, K., and Hopsu-Havu, V. K., Acta histochem., in press.
7 MMWR (CDC) *31* (1982) 249.
8 Sternberger, L. A., Hardy, P. H., Jr., Cuculis, J. J., and Meyer, H. C., J. Histochem. Cytochem, *18* (1970) 315.
9 Janossy, G., Pinching, A. J., Bofill, M., Weber, J., McLaughlin, J. E., Ornstein, M., Ivory, K., Harris, J. R. W., Favrot, M., and MacDonald-Burns, D. C., Clin. exp. Immunol. *59* (1985) 257.

0014-4754/85/091173-03$1.50 + 0.20/0

A testis-specific lactate dehydrogenase in the pipid frog, *Hymenochirus boettgeri*

J. Wolff and H. R. Kobel

Laboratoire de Génétique Animale et Végétale, Université de Genève, CH–1224 Chêne-Bougeries (Switzerland), 14 November 1984

Summary. Lactate dehydrogenase zymograms of mature testes of *Hymenochirus boettgeri* show in addition to the five isozymes composed of LDH-A and LDH-B subunits, a second 5-band system which is due to isozymes formed between LDH-A and a third subunit, LDH-C. These testis-specific LDH-C isozymes appear around 6 months after metamorphosis indicating that their expression is correlated with sexual maturity as is generally the case in mammals. This is the first report of a testis-specific LDH isozyme in a lower tetrapod; such isozymes have hitherto only been reported in mammals and in the pigeon.

Key words. Testis; LDH-C; *Hymenochirus boettgeri;* Pipidae.

Male germ cells of mammals express a specific lactate dehydrogenase gene (Ldh-c) which is first activated in primary spermatocytes. It is presumed that this lactate dehydrogenase enzyme (LDH-C) processes the lactate present in the fluid of male and female genital tracts as a major energy source for the spermatozoon[2–4]. LDH isozymes of somatic tissues depend on two other nuclear genes, Ldh-a and Ldh-b, encoding different subunits which assemble randomly to form the active tetrameric isozymes. Variations in the amino acid sequences of the three subunits indicate that the Ldh-a and Ldh-b genes are more closely related to each other than is either to Ldh-c, and also that the gene duplications that created the different Ldh genes occurred already at an early stage of chordate phylogeny[5]. In accordance to this view is the 3-Ldh gene constitution of bony fish of the subclass Actinopterygii. While the tissue-specific expression of Ldh-a and Ldh-b is comparable in both fish and mammals, the third Ldh gene of fish has acquired either an eye or a liver specific function in the more evolved teleosts, but shows a generalized expression in more primitive fish orders[6]. In contrast, other vertebrates, i.e. amphibians, reptiles and birds seem to possess only the two Ldh-a and Ldh-b genes, with the unique exception of the pigeons, which show a LDH condition resembling that of mammals[7,8], though the homology between the testis-specific LDH-C isozymes of pigeons and mammals has not been proven. It thus appears that the expression of the Ldh-c gene has been lost in most tetrapods, despite the fact that it had seemingly been conserved throughout the main line of vertebrate evolution leading to the mammals. However, we report here the first observation of a testis-specific LDH isozyme in a lower tetrapod suggesting that the current inventory of Ldh-c in vertebrates might be incomplete.

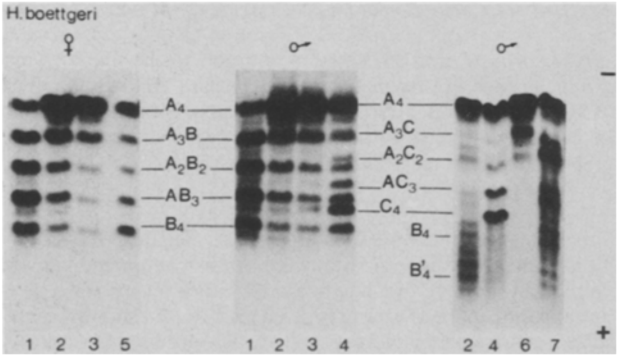
Hymenochirus belongs together with the other African genus *Xenopus* and the South American *Pipa* to the family Pipidae which appeared in the early Cretaceous and of which a variety of fossil genera are known[9, 10]. The three modern genera themselves have a rather unusually high age, since immunological distances point to a 100 million years' separation between *Hymenochirus* and *Xenopus*[11], and *Xenopus* almost identical morphologically to recent species existed already before the splitting up of Gondwana[9].

Material and methods. Two consecutive generations of laboratory bred *H. boettgeri* were used to analyze the enzyme repertories of various organs (*1* heart, *2* brain, *3* eye, *4* testis, *5* ovary, *6* skeletal muscle, *7* kidney).

Tissues were lysed in phosphate buffer (40 mM, pH 6.5) containing 2-mercaptoethanol (1 mM), EDTA (5 mM), $CaCl_3$ (3 mM) and Triton X-100 (0.2%). After centrifugation at 15,000 × g for 15 min, supernatants were extracted with CCl_4 and the emulsion cleared by a second centrifugation. Electrophoresis was performed on cellulose acetate membranes (Titan III, Helena Laboratories, Beaumont) in a pH 9.1 Tris-Glycine buffer (0.1 M) at 200 V during 40 min. Membranes were stained for LDH activity according to Harris and Hopkinson[12].

Results and discussion. LDH zymograms from various tissues of both male and female *H. boettgeri* show the well known 5-band pattern (fig.) which results from the random assembly of LDH-A and LDH-B subunits to tetrameric isozymes. Certain tissues produce unequal amounts of the different subunits giving asymmetrical zymograms with respect to LDH activity, e.g. skeletal muscle contains more LDH-A (fig., tissue 6), heart more LDH-B subunits (fig., tissue 1).

Testes of adult *H. boettgeri*, however, exhibit a second 5-band system in addition to the five A–B LDH bands (fig.). This is most clearly seen in males which are heterozygous for two alleles at the Ldh-b locus (second male in the figure), because the random combination of A, B and B′ subunits leads to 15 isozymes of different electrophoretic mobilities and hence, to less intensely stained bands. The second 5-band pattern represents therefore the five isozymes formed between LDH-A and a third subunit, LDH-C. Heterotetrameric isozymes between LDH-C and LDH-B subunits are not observed. The asymmetric distribution of activity between the various LDH bands is likely to be due to differential contributions by the various cell types, i.e. formation of the two systems A–B and A–C, and of the additional amount of A subunits takes place separated in time and/or space. This suggests that the different cell types and spermatogenetic stages in a testes probably express specific LDH repertories. Furthermore, LDH-C isozymes in *H. boettgeri* males are first detected around 6 months after metamorphosis indicating that the expression of the testis specific LDH-C is correlated with sexual maturity as is generally the case in mammals.



Lactate dehydrogenase zymograms of organ extracts from three specimens of *Hymenochirus boettgeri*: *1* heart, *2* brain, *3* eye, *4* testis, *5* ovary, *6* skeletal muscle, *7* kidney. Somatic tissues of both sexes and of ovaries show a 5-band pattern of LDH activity, each band representing one of the five possible tetrameric combinations between LDH-A and LDH-B subunits (A_4, A_3B_1, A_2B_2, A_1B_3, B_4). Testes (organ 4) show an additional system of five bands due to the random assembly of LDH-A with third subunit, LDH-C; this is most clearly seen in males wich are heterozygous for two differnt alleles B and B′ of the Ldh-b gene (second male, organ 4).

Other tissues than testes seem not to express the Ldh-c gene; the faint bands along the A–B LDH system in zymograms of somatic tissues (fig., first male) are probably 'secondary enzymes' which appear especially under the electrophoretic conditions applied here, which allows a good resolution of the A–C LDH system.

The LDH zymograms from adult as well as from maturing testes of *H. boettgeri* resemble much those of rats, guinea pigs and certain other mammals, in which male germ cells also contain heterotetrameric isozymes composed of LDH-A and LDH-C subunits, while the expression of the Ldh-b gene is confined to the non-spermatogenic somatic elements of the testes. Other mammals, e.g. mice, dogs, etc., show no heterotetrameric isozymes of LDH-C with other LDH subunits, probably in consequence of a more pronounced temporal and spatial restriction during subunit assembly[13].

H. boettgeri thus possesses three Ldh-genes; Ldh-a and Ldh-b show the usual expression patterns in somatic tissues, whereas Ldh-c has a male germ cell specific function. Such a testis-specific LDH isozyme could not be revealed in members of the other pipid genera. *Pipa carvalhoi* and *Xenopus tropicalis* show similar zymograms in both somatic and germinal tissues displaying the common A–B isozyme system. This could be due to coincident electrophoretic mobilities of possible LDH-C isozymes; however, various electrophoresis conditions gave essentially the same results. Tetraploid *Xenopus* species, on the other hand, have three or more Ldh genes with a preference to express A–C or B–C isozymes in different tissues[14]. This 3-gene constitution may be explained by the ancient polyploid condition of these species or, alternatively, may represent another example of the presumed basic constitution with three Ldh genes being conserved. Yet, gene duplication might happen repeatedly, and the redundant gene might acquire a specific tissue expression convergent to that experienced by another taxon.

1 This study was supported by a grant from the Fond National Suisse de la Recherche Scientifique to H.R.K. We thank M. Sateur and F. Bourquin for their technical help.

2 Blanco, A., and Zinkham, W.H., Science *139* (1963) 601.
3 Goldberg, E., Science *139* (1963) 602.
4 Wheat, T.E., and Goldberg, E., in: Isozymes 7, p. 113. Eds M.C. Rattazzi, J.G. Scandalios and G.S. Whitt. Alan R. Liss, Inc., New York 1983.
5 Li, S.S.L., Fitch, W.M., Pan, Y.E., and Sharief, F.S., J. biol. Chem. *258* (1983) 7029.
6 Markert, C., Shaklee, J.B., and Whitt, G.S., Science *189* (1975) 102.
7 Fisher, S.E., Shaklee, J.B. Ferris, S.D., and Whitt, G.S., Genetica *52/53* (1980) 73.
8 Zinkham, W.H., Blanco, A., and Kupchyk, L., Science *144* (1964) 1353.
9 Estes, R.E., Herpetologica *31* (1975) 263.
10 Estes, R.E., and Reig, O.A., in. Evolutionary Biology of the Anurans, p. 11. Ed. J.L. Vial. University of Missouri Press, Columbia 1973.
11 Bisbee, C.A., Baker, M.A., Wilson, A.C., Hadji-Azimi, J., and Fischberg, M., Science *195* (1977) 785.
12 Harris, H., and Hopkinson, D.A., Handbook of Enzyme Electrophoresis in Human Genetics. North Holland, Amsterdam 1976.
13 Goldberg, E., J. exp. Zool. *186* (1973) 373.
14 Wolff, J., and Kobel, H.R., J. exp. Zool. *223* (1982) 203.

0014-4754/85/091175-02$1.50 + 0.20/0