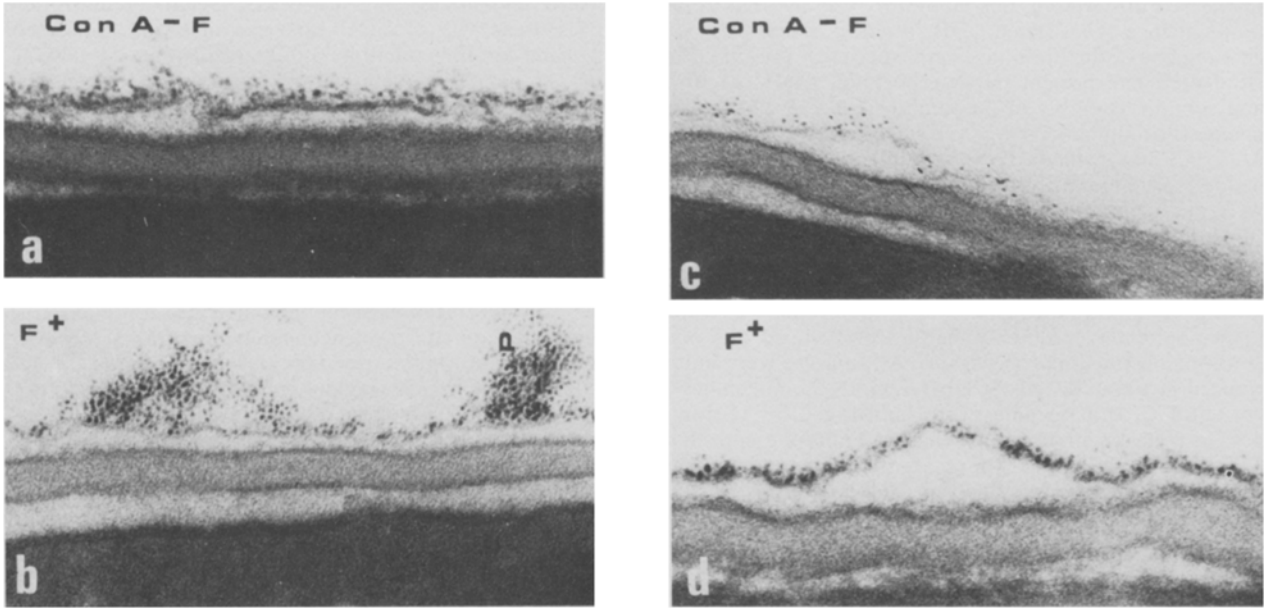


ConA-F and $F^+$ labeling distribution on head plasma membrane of PBS-washed (a and b, respectively) and urea-treated (c and d, respectively) spermatozoa. × 110,000.

inversion, using 30 µl of commercial preparations (Miles Lab.) per ml of incubation medium. Labeled cells were processed for electron microscopy and unstained sections were examined with a Philips E400 electron microscope.

*Results and discussion.* More than 80% of the PBS-washed spermatozoa demonstrated a rapid, straightforward progression, while after urea treatment this percentage was reduced to 70%. Seminal plasma was removed from the spermatozoa after circulation of 10 ml of PBS. As soon as the urea solution started to flow over the spermatozoa, a protein peak was observed in the filter effluent which reached the base-line after 6 ml of solution. Electrophoretic analysis showed that proteins collected from PBS fractions were identical to those collected from urea fractions. As far as the labeling experiments are concerned, PBS-washed spermatozoa bound ConA-F as well as $F^+$ in a patchy, outstretching pattern along the plasma membrane (fig., a and b), whereas urea-treated spermatozoa took up both the labels to a lesser degree and bound them in a more regular pattern (fig., c and d). On the basis of these results it appears that spermatozoa are easily removed from seminal fluid using synthetic membrane filters. Furthermore, it appears that the coating proteins remaining on the cell surface after PBS washing are removed by the urea treatment while cell integrity, as assessed by sperm motility analysis and by electron microscopy, remains intact. The close similarity between PBS-(seminal) and urea-(spermatozoal) released proteins suggests an external origin of the sperm coat[6]. The clear-cut differences in ConA-F and $F^+$ distribution before and after urea treatment confirmed the ability of urea to remove coating material from the sperm surface. In our hands, the method proposed here seems to have an edge on current techniques because it does not necessitate tryptic digestion or detergent incubation, thus preserving the integrity of spermatozoa. Finally, it provides both coatfree spermatozoa and relatively pure coat preparations suitable for subsequent studies.

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## Lactate dehydrogenase specificity and subunit assembly in neural tissues of the teleost *Phallichthys amates*

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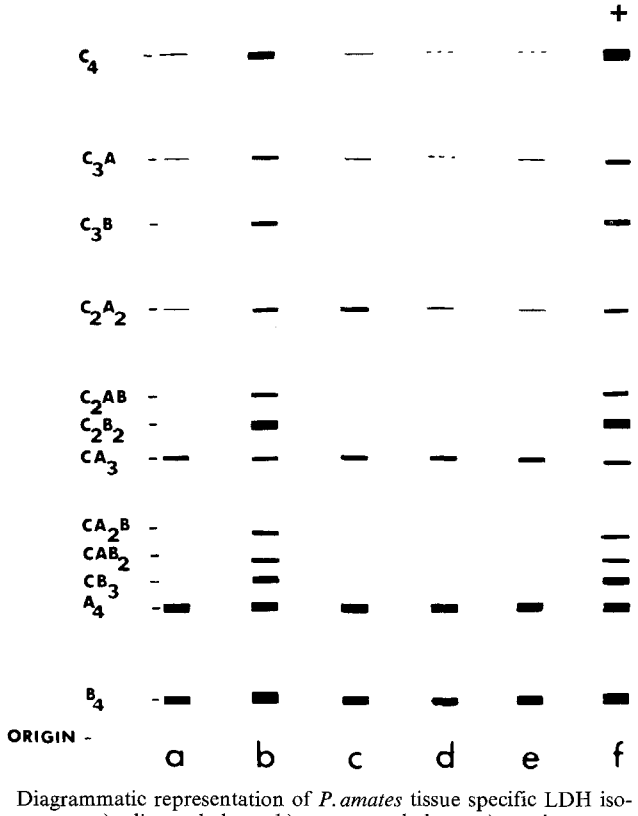
*Summary.* The tissue specificity of lactate dehydrogenase (EC 1.1.1.27) in brain and eye of the teleost *Phallichthys amates* was examined by acrylamide gel electrophoresis. It is suggested that subunit association is a function of gene product accessibility superimposed upon genetic restriction of assembly.

The tissue specific patterns of lactate dehydrogenase (lactate: NAD oxidoreductase, EC 1.1.1.27) have been investigated in numerous fish species[1–5]. In teleosts, 3 gene loci, the Ldh-A, -B, and -C loci, have been identified by immunological and biochemical techniques[6–8]. Most teleostean species exhibit high C polypeptide synthesis in derivatives of neural ectoderm, primarily in the neural retina and to a lesser extent in mesencephalon, diencephalon and optic

nerve[9,10]. Furthermore, this laboratory was the first to present evidence of restricted LDH subunit association in specific regions of the brain in several species of *Poecilia* (Poeciliidae)[11]. The present paper reports on LDH specificity and subunit assembly in the neural tissues of *Phallichthys amates* (Poeciliidae).

*Materials and methods.* Healthy adults of *P. amates* (merry widow) were obtained from local retail distributors and were maintained in stock tanks at 26 °C. Brain and eye tissues were excised from ice-immobilized fishes, washed in fish Ringer's solution[12], blotted and rinsed in deionized water. Whole brain was subsequently dissected into the following regions: diencephalon, mesencephalon (optic lobes), optic nerve, metencephalon (cerebellum), and myelencephalon (medulla oblongata). All samples were homogenized in 100 mM Tris-HCl, pH 7.5 (50% homogenates by vol.) at 4 °C. Homogenates were centrifuged for 15 min at 4 °C in a Beckman 152 Microfuge at 15,000 × g and about 8 µl of the resulting clear supernatant was immediately subjected to electrophoretic analysis. Electrophoresis was carried out employing an acrylamide gel vertical slab system (E.C. Apparatus Co.), maintained at 4 °C utilizing a recirculating water bath (Lauda K-2/R, Brinkman Instruments). For each tissue region, 20 different samples were electrophoresed in replicates of 3. Samples were run on 6% gels at 300 V for 3 h using the 0.155 M Tris-citrate buffer system (pH 6.8) of Shaw and Prasad[13]. After electrophoresis, gels were stained with an incubation medium from Shaw and Koen[14]. Subunit composition of individual isozymes was designated based upon their relative electrophoretic mobility and staining intensity on the gels. In vitro molecular hybridization was conducted employing the procedure of Markert[15], as modified by Frankel[11].




Diagrammatic representation of *P. amates* tissue specific LDH isozymes. a) diencephalon; b) mesencephalon; c) optic nerve; d) metencephalon; e) myelencephalon; f) eye. Zones with heavy shading exhibit high activity; moderate zones - moderate activity; thin zones - low activity; dotted zones - trace of activity; blank spaces indicate no detectable activity.

*Results and discussion.* The tissue specific patterns and levels of activity of LDH isozymes in brain and eye of *P. amates* are illustrated diagrammatically (fig.). Relatively high $A_4$ and $B_4$ isozyme activity is exhibited for all tissue regions, whereas the activity of the $C_4$ homopolymer predominates in extracts of mesencephalon and eye. These observations are consistent with previous reports of high Ldh-C locus activity in those regions of the nervous system associated with vision[10,16]. In addition, the number and polypeptide composition of C subunit-containing isozymes differs markedly between the regions assayed. While all regions lack isozymes of AB subunit composition ($A_3B$, $A_2B_2$ and $AB_3$ isozymes), extracts of diencephalon, optic nerve, metencephalon and myelencephalon contain heteropolymers of CA composition, exclusively. The absence of CB and CAB isozymes in these regions is most probably due to partial or complete isolation of C and B subunit synthesis, reflecting temporal and/or spatial dissociation of LDH gene function[1,11]. In contrast, extracts of mesencephalon and eye exhibit 12 of the 15 expected zones of LDH activity, indicative of the presence of the 3 homopolymeric isozymes, as well as all heterotetramers of C subunit composition (CA, CB, and CAB). While heteropolymers of AB subunit composition are absent from all tissue extracts, these subunits are present simultaneously in the same cells and are accessible to each other, since the $C_2AB$, $CA_2B$, and $CAB_2$ isozymes are present in mesencephalon and eye. It has been suggested that the A and B subunits of certain teleosts do not associate in vivo or in vitro as a result of genetically controlled restrictions on their assembly[7,17,18]. This is apparently the case in *P. amates*, as heteropolymers of AB subunit composition are not detected in any of the tissues assayed, either before or after molecular hybridization. Indeed, their assembly into heteropolymers of CAB composition appears to be facilitated by the presence of the C subunit in the tetramer[1,11], since all CAB heterotetramers are found after molecular hybridization of isozymes from diencephalon, optic nerve, metencephalon and myelencephalon.

In conclusion, the association of LDH subunits in the neural tissues of *Phallichthys* is apparently controlled by temporal and/or spatial isolation of gene function superimposed upon genetic restriction of assembly.

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