Ontogenesis of Lactate Dehydrogenase Isozyme Patterns in two Salmonids (*Salmo salar* and *S. trutta*)

Five forms of lactate dehydrogenase (LDH) exist in most higher vertebrates. These forms, called isozymes, are tetramers generated by random aggregation of two genetically and biochemically distinct subunits, A and B[1]. In addition, some birds and mammals possess a third subunit C, which is encoded in a third locus and is apparently only active in the primary spermatocyte[2].

The pattern of LDH isozymes in lower vertebrates is more complex. Teleosts have been reported to possess from 2 isozymes in some species to 22 in others[3]. Salmonids, in particular, have been extensively studied. Their zymograms reveal 18 or more bands of LDH activity[4].

Isozyme patterns of LDH are tissue specific. In adult mammals, LDH activity of the heart muscle is concentrated in the anodal tetramer (B_4) and in skeletal muscle and liver in the cathodal tetramer (A_4). Since LDH patterns of adult organs are different, it follows that these arize during development. The developing organs of *Xenopus laevis* have been studied by KUNZ[5,6] who compared the pattern changes with histological data. It was shown that in all organs tested the full number of LDH isozymes is achieved in each, once the intracellular yolk platelets have disappeared. The adult expression of the different tissue patterns, however, does not seem to be associated with comparable histological events. The LDH isozyme changes in developing teleosts have been examined by various authors, but only extracts of whole embryos, and not of single organs, were analyzed[7].

The purpose of this research was to study the ontogeny of the tissue specific LDH patterns in 2 salmonids with meroblastic development and compare it with the already established pattern changes in *Xenopus laevis*[5,6] with holoblastic development.

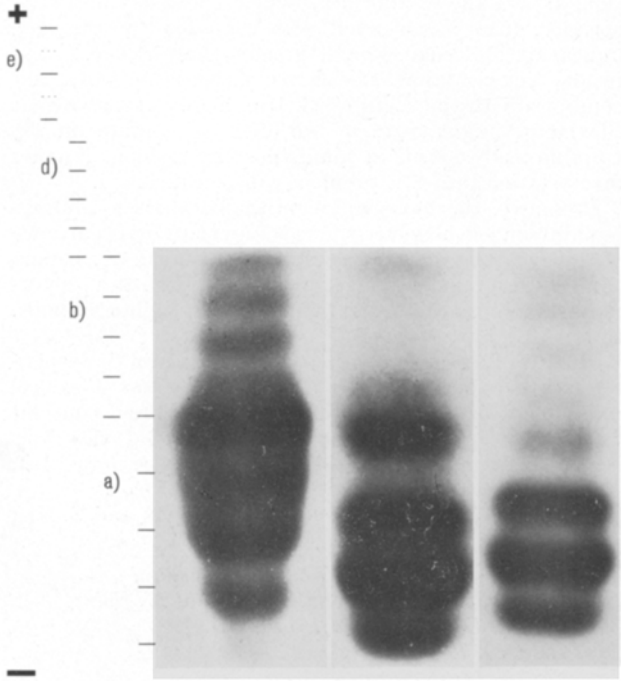

Fig. 1. LDH isozyme pattern of tail muscle of trout showing gradual development. Left, end of yolk sac phase; middle, 1 year old; right, 2 years old (prespawning). The origin is at the bottom of the gel; migration is towards the anode at the top. Designation of groups a–e after MASSARO and MARKERT[4]

Material and methods. Salmon and trout eggs were obtained locally and reared in the laboratory. The LDH isozymes were separated by starch gel electrophoresis and stained as previously described[5].

Results and discussion. Patterns of adult organs of the salmon and trout are in general agreement with the findings of MASSARO and MARKERT[4]. These authors classify the tissue specific LDH isozyme patterns of adult salmonids according to their electrophoretic mobility into 4 groups, each composed of 5 equally spaced isozymes: The most cathodic group (a) is restricted, almost exclusively, to skeletal muscle; the next group (b) comprises the major isozymes in heart and brain; this is followed, anodally, by group (d), which is the only group present in the digestive system; the most anodal group (e), of which only 3 bands are resolved, is located exclusively in the neural retina and also in the optical part of the brain[8]. This classification is used for the following description of the ontogenetic changes. It should be noted, however, that, under the experimental conditions of this study, the most anodal isozyme of group (a) and the most cathodal isozyme of group (b) overlap (Figure 1).

a) *Early ontogeny.* In the unfertilized egg of both salmon and trout, the 3 cathodic bands of group (b) are resolved, with the middle band predominant. In the fertilized egg, all 3 bands increase in intensity until the end of gastrulation. Also, at the beginning of gastrulation, a 4th (anodal) band appears while a 5th band is resolved at the end of gastrulation. HAGENMAIER[9], on the basis of biochemical and cytochemical investigations in *Salmo irideus* and *S. trutta fario*, concludes that m-RNA is stored in the yolk and gradually taken up into the embryo. Only when gastrulation has begun, the store of m-RNA is depleted and new synthesis takes place. Other workers[7] have established that no increase in levels of enzymes, in general, is observed during cleavage of teleosts; it is only at, or subsequent to, gastrulation that synthesis of new enzyme molecules occurs to a significant extent. Early ontogeny then, of the salmonids tested, is characterized by the presence exclusively of group (b) (heart bands), which reflects aerobic metabolism. Also in *Xenopus laevis*[5,6], the first isozymes to appear, during development, are those predominant in the adult heart.

Tests of postgastrulae of salmon and trout show that at the eye pigmentation stage the second band of group (a) (muscle bands) is resolved and shortly after hatching the full complement of group (a) is achieved. Development of the muscle bands coincides with the beginning of motility in the embryo. This is in accordance with observations on *Xenopus laevis*[5,6].

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[8] (a) is composed of subunits A and A′, (b) of B and B′, (d) of D and D′ and (e) of E and E′.
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Soon after hatching, also, the 3 characteristic e-bands make their appearance. This may be connected with the differentiation of the photoreceptors of the retina, since according to RINGGENBERG[10] the retina of the trout is fully differentiated, over most of its area, shortly after hatching. In *Lebistes reticulatus*, where the eyes of all embryonic stages were tested for e-bands as well as histologically, the appearance of the e-group coincides with the differentiation of rods and cones[11].

When the embryo and yolk sac of both salmon and trout are tested separately, the most anodal isozymes of group (b) move more rapidly in the latter. This may be due to a possible binding of yolk material to the enzyme in a somewhat analogous fashion to the binding of NAD to alcohol dehydrogenase[12]. The pattern of the yolk sac – with increasing activity from the cathodal to anodal end of group (b) and a faint smear anodally to it – remains the same until the last stage.

b) *Development of organ patterns*. 1. Tail muscle. When the parr marks appear on the postembryo, the full adult complement of bands in groups (a) and (b) is present, with group (b) staining less intensely. However, the relative activity within group (a) is different from the adult. In the postembryo, the anodal bands are more pronounced, whereas in the adult the cathodal bands stain more intensely. Only in the smolt stage of the salmon and the prespawning stage of the trout, the muscle pattern acquires adult expression (Figure 1).

2. Eye. Group (b) and group (e) develop shortly after hatching (group (e) moves faster in the salmon than in the trout). The relative intensities within both groups are the same as for the adult. 2 bands of group (d) appear towards the end of yolk sac resorption. Thus, by the end of the postembryonic phase, the eyes of both salmon and trout possess the adult number and distribution of LDH activity (Figure 2). The pattern remains the same during subsequent development.

3. Heart. Heart extract of both salmon and trout fry (1 week after yolk resorption) yield group (b), but with the exception of the second most rapidly moving band.

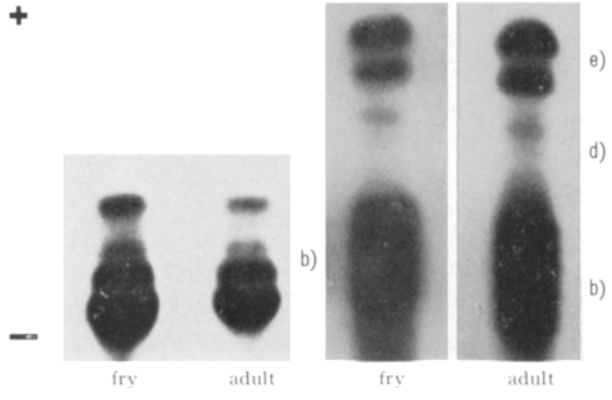

Fig. 2. LDH isozyme pattern of trout heart (left) and salmon eye (right) showing that both organs have acquired an adult pattern alreday at the fry stage.

The number of bands and distribution of activity are the same as in the adult fish (Figure 2). The pattern remains unchanged in all stages of the subsequent growth period.

4. Brain. 5 bands of equal intensity (group (b)) are resolved in the fry of salmon and trout. Faint and diffuse activity in the region of group (a) and group (d) is also observed. The pattern remains the same throughout the growth period into adulthood.

5. Liver. Liver extracts of salmon and trout fry exhibit, with increasing activity from the cathodal to the anodal end, all 5 bands of group (b). At the position of group (d) diffuse activity is sometimes evident. This early liver pattern resembles closely the isozyme pattern for the yolk sac. Morphological and histological observations show that in teleosts the liver is in close contact with the yolk: in salmonids the contact is particularly close, which makes some authors suggest that the liver is a resorptive organ for yolk[13]. The similarity of both early liver and yolk patterns may reflect the presence of yolk material in the liver. The adult distribution of liver LDH activity, where group (d) is pronounced and group (b) is smeared, is achieved in the salmon at the smolt stage and in the trout at the prespawning stage.

Thus, the developmental LDH isozyme pattern of early organogenesis in the 2 salmonids studied seems to parallel observations in *Xenopus laevis*[5,6]: Once the yolk disappears – either as intracellular platelets, as in *Xenopus*, or concentrated extracellularly in a yolk sac, as in salmonids – the adult number of bands in all organs tested (liver excepted) is established. The presence of yolk may have some influence on gene regulation or directly on enzyme expression. Also, when the yolk is absorbed or immediately afterwards, eye, heart muscle and brain show adult distribution of activity. However, in skeletal muscle and in the digestive tract (intestine in *Xenopus* and liver in salmonids), the adult patterns emerge gradually and are fully established at a very much later stage.

Zusammenfassung. Die ontogenetischen Änderungen des Isoenzym-Musters der Laktatdehydrogenase (LDH) von *Salmo salar* und *S. trutta* wurde mittels Stärkegel-Elektrophorese verfolgt. Während der frühen Entwicklungsstadien (Furchung und Gastrulation) sind allein die «Herz-Banden» vorhanden. In allen geprüften Organen wird die adulte Bandenzahl unmittelbar nach der Dottersack-Resorption erreicht. Im selben Statium weisen Herzmuskel, Auge und Gehirn bereits das adulte Verteilungsmuster auf, während dieses im Skelettmuskel und der Leber erst nach 2–3 Jahren, vor Erreichung der Geschlechtsreife, auftritt.

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Metabolic Changes Induced by Galactose

Dietary carbohydrates have been reported to decrease blood cholesterol in man[1,2], but lipaemia rises with glucose, sucrose and fructose[3–10]. We have studied glucolipidic metabolism after an oral dose of galactose.

Material and methods. Blood samples were taken from 9 voluntary healthy subjects after a fasting period of 8 h, and immediately after-wards 100 g of galactose dissolved in water were administered orally. Further blood samples