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Effects of Temperature Acclimation on the Isozyme Pattern of Liver Lactate Dehydrogenase in the Goldfish, *Carassius auratus* (L.)

With 3 Text-figures

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ABSTRACT Goldfish acclimated to 8°, 18° and 28°C were examined for the LDH isozyme pattern of liver extract. The liver LDH of fish acclimated to 8° or 18° consists of five isozymes while 28°-acclimated fish show only three of them. However, such a reduction in the number of isozymes in 28°-acclimated fish is not stationary because the missing isozymes reappear eventually on prolonged acclimation at 28°C. The process—extinction and reappearance of isozymes—passes faster in well aerated condition than in non-aerated condition. The results obtained suggest that two of the five isozymes of liver LDH which have once been inactivated under the influence of an elevation of the ambient temperature are reactivated on the completion of acclimation to a high temperature.

Effects of temperature acclimation on physiological and biochemical properties of fish were studied extensively by many investigators (Anderson, 1970; Baldwin, 1971; Baslow 1967; Braun *et al.*, 1970; Fry and Hochachka, 1970; Hochachka, 1967; Künnemann *et al.*, 1970; Peterson and Prosser, 1972; Roberts, 1967). We reported earlier that the composition and the thermostability of some tissue proteins of goldfish change in relation to the temperature to which they are acclimated (Ohsawa and Tsukuda, 1964, Tsukuda and Ohsawa, 1971). In these years, we have focused our research in this field on elucidation of the adaptational significance of modifications in the isozyme pattern of lactate dehydrogenase (LDH) which has been found to be somewhat susceptible to changes in the ambient temperature.

Concerning tissue LDH isozymes of poikilotherms, there are some reports on the variations among species (Markert and Faulhaber, 1965; Lush, 1970) and with the developmental stage (Adams and Finnegan, 1965; Ewing and Clegg, 1969; Nakano and Whiteley, 1965). With regard to temperatute acclimation, Hochachka

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(1965 and 1967) found that the five isozymes of goldfish liver LDH which are assumed to be tetramers consisting of two kinds of subunits decrease in number during warm acclimation. On the basis of this finding he has developed vigorously his biochemical investigation on the relation between LDH isozymes and the acclimation temperature (Fry and Hochachka, 1970).

We traced, on the other hand, the change in the isozyme pattern of goldfish liver LDH over prolonged periods of temperature acclimation and obtained results which appear to reflect somewhat different aspects of the problem from those noted by Hochachka.

Materials and Methods

Goldfish, *Carassius auratus* (Linné), ranging in body weight from 13 to 24 g, were kept more than 40 days at an acclimation temperature of 8°, 18° or 28°C, in aquaria 30×60 cm by 30 cm high. Each acclimation aquarium, containing about 48 l of dechlorinated tap water and eight individuals, was controlled thermostatically at a given temperature as reported before (Tsukuda and Ohsawa, 1971) and fish received once a day about 50 mg per individual of the commercial food for goldfish.

The livers isolated from a goldfish acclimated to a given temperature were washed with the barbiturate buffer solution same as was used for electrophoresis and weighed. Then they were ground at ice-temperature with a glass pestle in a small glass motar together with a given volume of the buffer solution (1 ml per g liver). The homogenate was transferred to a plastic tube for centrifugation with an additional same volume of the buffer solution. The homogenate was centrifuged in the cold at 5000 ×g for forty minutes and the supernatant was used for electrophoresis.

For the electrophoretic analyses of liver proteins and LDH isozyme activities were used cellulose acetate membrane and barbiturate buffer of pH 8.6 and ionic strength 0.07. About 5 μl of extract was put on a strip of membrane and a constant current of 0.8 mA per cm of membrane width was applied for sixty minutes. Samples from two different acclimation temperature groups were run parallel.

Immediately after electrophoresis, some membrane strips were stained for proteins with Ponceau red 3 R and the other were treated for LDH activity with the mixture freshly made as follows: Na-lactate (7 g of 50% Na-lactate/100 ml buffer) 5 ml, phenazine methosulfate (0.016 g/100 ml buffer) 5 ml, *p*-iodonitrotetrazolium (0.1 g/100 ml water) 5 ml, and NAD (0.2 g/40 ml water) 2 ml. For LDH analysis membrane strips were immersed in this reaction mixture at 37°C for several minutes in darkness, fixed with 10 per cent acetic acid, washed a few times in water, and dried at room temperature.

Two to six individuals were sampled for a test. No sexual difference was detected.

RESULTS

Experiment I

Three groups of fish acclimated to different temperatures of 8°, 18° and 28° were examined for the protein and the LDH isozyme pattern of liver extract. As for proteins no difference was detectable among the three groups.

There was a consistent difference in the number of LDH isozymes between 8°- and 28°-acclimated fish. The liver LDH of 8°-acclimated fish always showed five isozymes while that of 28°-acclimated fish had four or three isozymes (Fig. 1). This reduction in the number of isozymes involved disappearance of LDH 3 and further extinction of a component charged more negatively (Fig. 2). The latter is assumed to be LDH 2 but cannot be identified.

The result obtained with goldfish by Hochachka (1965) coincides in general with that mentioned above, but differs from it in that LDH's 1 and 2 disappeared following transfer from 4–5° to 20–22°C. It is not certain whether the difference may be ascribed to that in the acclimation temperature or may depend on other factors.

When the acclimation to a high temperature was prolonged, the missing LDH isozymes tended to appear. Fish kept at 28°C for about one year were found to have five isozymes. Moreover, fish which were purchased in summer possessed five isozymes despite of having been kept in a high water temperature of 32°C.

These facts suggested that the liver LDH isozymes might decrease in number under the influence of rising temperature but be restored to the initial number when acclimation to the high temperature was completed or that the number of LDH isozymes might be related not only to the temperature but also to other factors such as

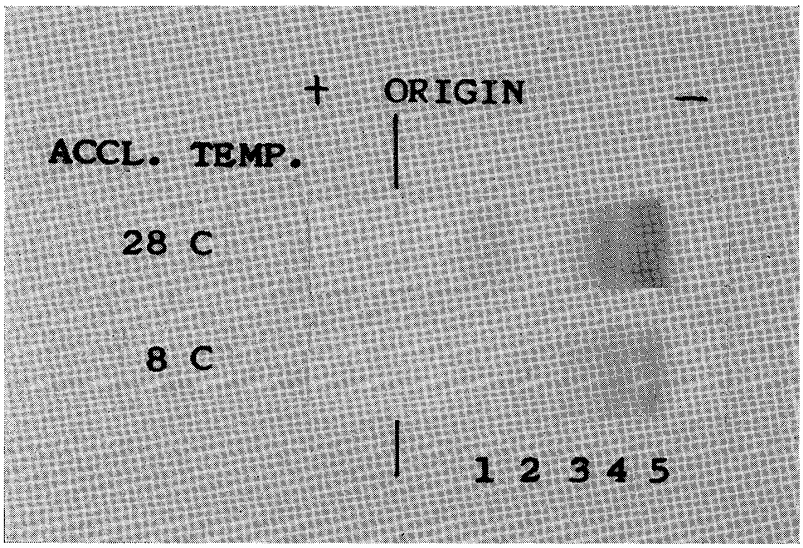

Fig. 1. Electrophoretic patterns of liver LDH of goldfish acclimated to 28° (upper) and 8°C (lower). Isozymes are numbered at the bottom from anode to cathode.

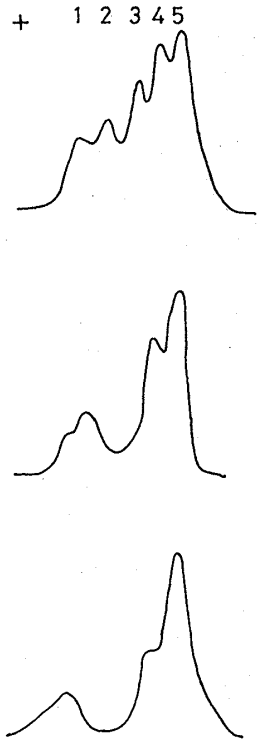

Fig. 2. Typical zymograms of goldfish liver LDH, consisting five (upper), four (middle), and three (lower) isozymes.

nutritive condition, oxygen tension (Altman and Roben, 1969), and photoperiod (Massaro and Booke, 1971). According to Hochachka (1965) LDH's 1 and 2 disappear at 20–22°C when the water is aerated but only LDH 1 does without aeration.

The following experiment was performed to trace the time course of the change in the LDH isozyme pattern during acclimation to high temperatures. Influence of oxygen concentration on the change was also observed.

Experiment II

Goldfish purchased in July and August were maintained at 18°C and those in November at 15°C for about a month before warm acclimation. In the summer series fish in the first acclimation at 18°C were divided at random into three groups for the second acclimation. One group was left at 18°C and the other two groups were transferred to 28°C, one of them being aerated continuously. In the November series fish in the first acclimation at 15°C were divided into four groups for the second acclimation. Two groups were transferred to 28°C and the other two were left at 15°C. One group of either second acclimation was aerated. Average oxygen saturation determined by the Winkler method was 96% in aerated and 27% in non-aerated aquaria. After transfer to the second acclimation temperature of 28°C the number of liver LDH isozymes was determined at intervals by electrophoresis in comparison with that of the control fish which were continually maintained in the cold.

In all of the 18°- and 15°-acclimated fish tested the five isozymes were found consistently. Distribution of the number of isozymes among fish sampled during the second acclimation at 28°C is summarized in Table 1. In the summer series in which fish were transferred from 18° to 28°C, the group without aeration retained the original number of five throughout 49 days of the second acclimation, while in the aerated group the number of isozymes decreased in about 21 days and became four or three in about 40 days after transfer. Then it returned to the original state, i.e., five. In the November series in which fish were transferred from 15° to 28°C, the aerated group showed reduction in the number of isozymes in about 60 days and then recovered the original number, while in the group without aeration the change took place considerably later.

Table 1
Distribution of the number of liver LDH isozymes among goldfish sampled during acclimation at 28°C after transfer from 18°C in July to August and from 15°C in November.

Days after transfer to the second acclimation temperature of 28°C	No. of isozymes	First acclimation at 18°C in July–Aug.		First acclimation at 15°C in November	
		Not aerated	Aerated	Not aerated	Aerated
		3 4 5	3 4 5	3 4 5	3 4 5
7– 14		0 0 6	0 0 6		
20– 28		0 0 6	0 1 5		0 0 3
35– 42		0 0 5	3 2 0		0 0 3
47– 49		0 0 4	0 0 4	0 0 3	
55– 57					0 2 0
63– 64					2 0 0
74– 84				0 1 1	0 1 2
88– 93				2 2 0	0 0 6
96– 98				2 0 0	
100–111				0 0 4	

The results described above indicate that the reduction in the number of liver LDH isozymes in fish transferred to a higher temperature is temporary in nature and that the isozymes which have disappeared in the course of acclimation to the high temperature reappears eventually with the completion of acclimational process. Higher oxygen tension seems not to be an indispensable factor for the isozymes to disappear. It certainly has an accelerating effect on the process concerned. Thus the group without aeration in the summer series would have shown a reduction in the number of isozymes, if the experiment had been continued for additional several weeks.

Figure 3 shows the change in the ratio of the wet weight of liver to that of body during the acclimation. In goldfish wich had been purchased in July and August and kept at room temperature ranging 28–30°C the mean ratio was 1.8 for males and 1.6 for females. When fish were transferred to 18°C the ratio increased for about 30

days and then decreased until it reached relatively stationary levels in 70 days after transfer. On elevating the water temperature from 18° to 28°C when the ratio was in the increasing phase, it began immediately to decrease and reached stationary levels

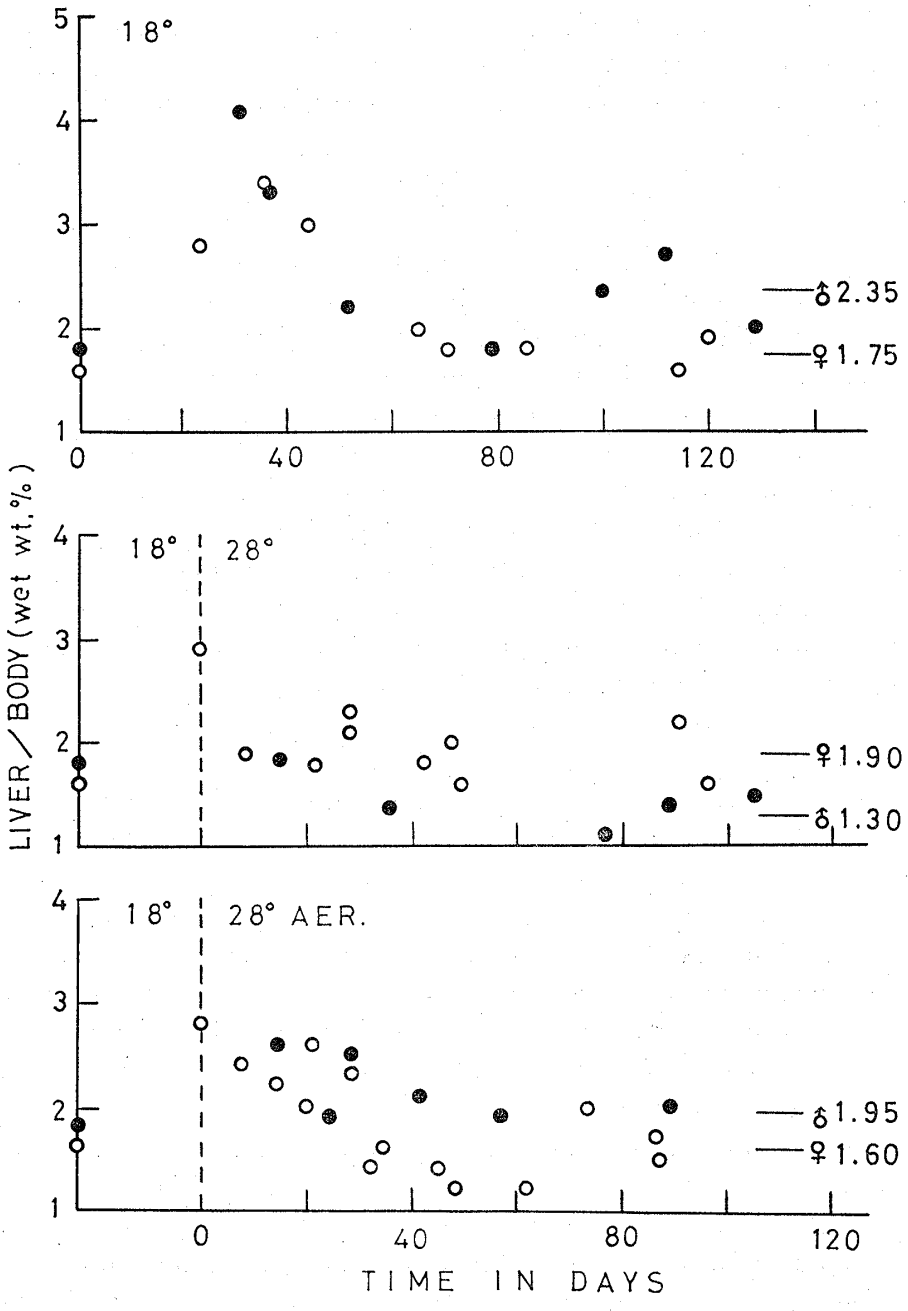

Fig. 3. Relative wet weight of liver of goldfish sampled during acclimation at 18° (upper) after transfer from room temperature ranging within 28–30°, and at 28° without aeration (middle) and with aeration (lower) after transfer from 18°C. Horizontal segments with figures on the right side are stationary levels attained. Solid circles denote males and empty circles females.

in about 40 days.

The liver weight is known to increase in a cold environment (Barnett, 1965; Heroux, 1961; Tsugawa, 1971). The results obtained by the present experiment, however, indicate that the process is not so simple as generally accepted. The liver weight increases initially under the stimulation of a depression of temperature but afterwards it decreases to a stationary level ranging within 1.5–2.0, which is considered as the normal level independent of ambient temperature.

DISCUSSION

A number of physiological and biochemical properties of poikilotherms have been reported to be modified in the course of acclimation to altered ambient temperatures. Most of them are rate processes which increase or decrease progressively till stationary levels at acclimation temperatures are attained. They are usually regarded as adaptive in substance, including, for example, tolerance to temperature extremes, activities and organismal metabolic rate. Other properties such as have been dealt with in the present study are different in nature from them informing apparently of no adaptational significances. Modifications in properties of the latter category must nevertheless be basic for changes in those of the former category, and are not always progressive but may reverse their direction in the process of acclimation. Thus the disappearance of some liver LDH isozymes after transfer to a high temperature as well as the increase in liver weight following a depression of temperature are considered to be reflecting initial preparatory phases of acclimation.

According to Fry and Hochachka (1970) cold adaptation involves not only acceleration of enzyme synthesis and consequently increase of activity in general, but increase of a specific type enzymes. Namely, there are two types of enzymes, i. e., cold and warm enzymes. Cold type enzymes have smaller Km values in cold than warm type ones and are favorable to exhibit sufficient activity in cold at physiological levels of substrates. Thus the induction of specific new isozymes which are adaptive in functioning in a cold environment means the establishment of a new state in metabolism. Hochachka (1967) shows that the disappearance of one or two isozymes at a high temperature is due to a direct effect of the high temperature on the enzyme protein configuration.

According to our present results, it is certain when goldfish are transferred from cold to warm one or two of the five isozymes of liver LDH disappear. This represents, however, the initial intermediary phase of acclimation which is presumably induced by stimulation of a rise in the environmental temperature, and the isozymes missing or inactivated temporarily reappear toward the completion of acclimation, contradicting with the interpretation of Hochachka. Though no exact information is available about the time course of acclimation to high temperatures in goldfish, it is surmised from the data on the guppy, *Lebistes reticulatus*, reported earlier (Tsukuda, 1960) that the "cold enzymes" are reactivated after the completion of acclimation.

Biochemical mechanisms underlying the inactivation and reactivation of "cold enzymes" as well as the biological significance of the phenomenon certainly represent essential problems in the research of physiological temperature adaptation.

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