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Malate Dehydrogenase Isozymes Of Different Stages Of Chesapeake Bay Jellyfish

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MALATE DEHYDROGENASE ISOZYMES OF DIFFERENT STAGES OF CHESAPEAKE BAY JELLYFISH[1]

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In the Scyphozoa, pronounced morphological changes occur during strobilation which are undoubtedly preceded by changes in gene activity, metabolic pathways, and biosynthetic mechanisms: the long tentacles of the scyphistoma are retracted and a series of transverse constrictions are formed at the oral end; each transverse section develops into an ephyra and subsequently into an adult medusa. This morphological development of *Chrysaora quinquecirrha* has been thoroughly studied by Littleford (1939) and Calder (1972). Influences of environmental and chemical factors on strobilation of both *Aurelia aurita* and *C. quinequecirrha* have been investigated by many workers (Custance, 1964; Spangenberg, 1965, 1967, 1974; Loeb, 1970, 1973; Loeb and Gordon, 1975; Black and Webb, 1973; Olmon and Webb, 1974). Black (1972) reported increased levels of DNA in the strobilae of *A. aurita.*

Isozymes are probably important in regulating tissue function, and such studies may provide information on the molecular basis of cellular differentiation (Markert, 1975; Whitt, 1975). Investigations of the ontogeny of the most extensively studied enzyme, lactate dehydrogenase (EC 1.1.1.27), have been reviewed by Masters and Holmes (1972), and the evaluation of gene structure, function and regulation have been reviewed by Markert, Shaklee and Whitt (1975). The enzyme of interest, malate dehydrogenase (MDH, EC 1.1.1.37), has been studied in a few developing embryos of vertebrates, including the frog (MacBride and Guttman, 1973), fish (Nakano and Whiteley, 1965; Shaklee, Champion, and Whitt, 1974), and chicken (Greenfield and Boell, 1970).

Only a limited number of MDH isozyme studies have been undertaken on the development of vertebrates. These include the nematode, *Ascaris suum* (Zee and Zinkham, 1968), the marine snail, *Ilyanassa obsoleta* (Meizel and Markert, 1967), and the sea urchins, *Arbacia punctulata* (Moore and Villee, 1961, 1962, 1963a, 1963b; Billiar, Brungard, and Villee, 1964; Billiar, Zelewski, and Villee, 1966; Francesconi and Villee, 1968; Villee, 1968) and *Strongylocentrotus purpuratus* (Ozaki and Whiteley, 1970). In this study, the MDH isozyme patterns of different stages of the Chesapeake Bay jellyfish, *A. aurita, C. quinquecirrha,* and *Cyanea capillata,* were investigated as a model system for probing biochemical development of the simplest Animalia (Whittaker, 1969; Margulis, 1971).

Materials and Methods

Medusae of *A. aurita* and *C. quinquecirrha* used in this study were collected from the York River, Virginia near the Virginia Institute of Marine Science in

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the summer of 1972. *C. capillata* medusae were collected during the previous winter season from the same location. Scyphistomae cultures of *C. capillata* and *A. aurita* were started from eggs collected from mature medusae (Calder, 1971). Planulae and planulocysts of *C. capillata* were obtained after placing fertilized eggs taken from medusae into culture. In the case of *C. quinquecirrha,* male and female medusae into culture. In the case of *C. quinquecirrha,* male and female medusae were put in the same container overnight and fertilized eggs were collected from the bottom of the container. Scyphistomae and cysts were obtained by maintaining planulae in filtered York River water (19–21‰ salinity) at 15° C for *C. capillata* and at room temperature (19–21° C) for *C. quinquecirrha* and *A. aurita.* The scyphistomae were fed nauplii of newly hatched *Artemia salina* once a week.

After washing, the organisms were ground with 3% (w/v) sucrose in 0.004 M Tris-0.038 M glycine buffer, pH 8.3 and the homogenate was centrifuged at 2000*g* for ten minutes. The crude extracts of different stages were applied directly to the tops of the gels without the aid of spacer gels. Polyacrylamide gel electrophoresis in a vertical cell (Büchler Instruments, USA), using 7.5% gel made with acrylamide (Eastman) and Tris-HCl-TEMED buffer system, pH 8.9, was performed (Davis, 1964). All gels were polymerized with ammonium persulfate rather than photopolymerization. Electrophoresis was performed at 5 mA per gel in a water-cooled cell (usually 2–3 hours) with bromophenol blue as the electrophoresis dye marker.

In order to determine whether the observed MDH isozyme differences were associated with the mitochondria or cytosol, mitochondria were prepared from medusae of *A. aurita* and *C. quinquecirrha.* The tentacles and oral arms of fresh medusae were mixed with 0.6 M sucrose-Tris-glycine buffer, pH 8.3 (1 : 1, w : v). After the homogenate was centrifuged at 2000*g* for five minutes, the supernatant was removed and the precipitate discarded. The 2000*g* supernatant was centrifuged in a Sorvall centrifuge at 7000*g* for thirty minutes, the supernatant discarded, and the precipitate (mitochondrial fraction) resuspended in fresh sucrose buffer and centrifuged again. After this washing process was repeated four times, the mitochondrial fraction was homogenized with 3% sucrose-Tris-glycine buffer, and the homogenate applied directly to the top of a 7.5% gel without the aid of a spacer gel. Electrophoresis was carried out as usual (Zubkoff and Lin, 1975).

Results

Aurelia aurita

MDH isozyme patterns of *A. aurita* are shown in Figure 1. The scyphistomae possess four bands as described previously (Lin and Zubkoff, 1973). There is little difference in MDH isozyme patterns among planulae, scyhistomae, strobilae, and medusae. In these four developmental stages, the major isozyme is the 0.43 mitochondrial form. In *A. aurita,* the fast migrating bands (0.39 and 0.43) are the mitochondrial form and the slow moving bands (0.33 and 0.29) are the isozymes of the cytosol (Figure 2). Although this MDH isozyme pattern differs from that of most organisms in which the mitochondrial MDH moves more slowly toward the anode than the cytosol MDH, it is similar to that for tuna (Kitto and

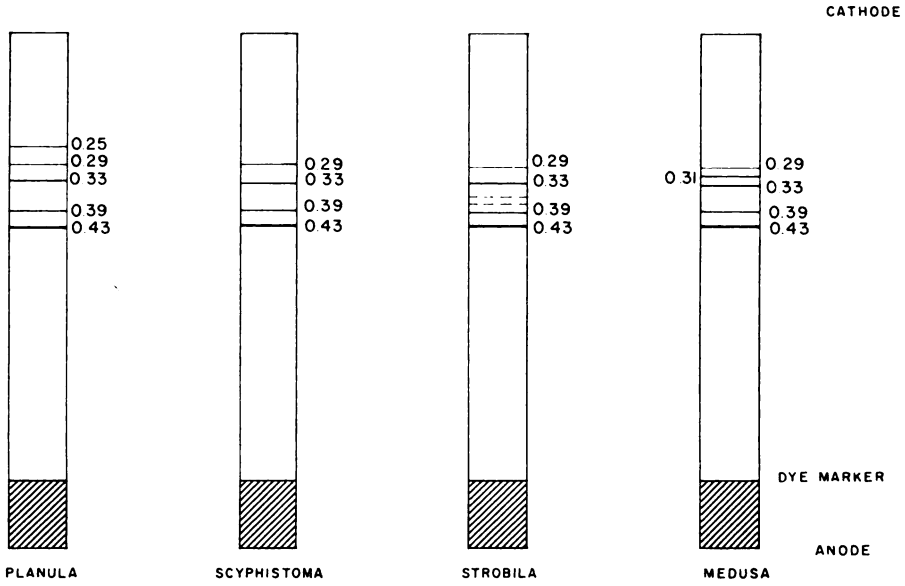

FIGURE 1. MDH isozyme patterns of different stages of *Aurelia aurita.*

Lewis, 1967), the marine snail, *Ilyanassa obsoleta* (Meizel and Markert, 1967) and the sea urchin, *Strongylocentrotus purpuratus* (Ozaki and Whiteley, 1970). Although no differences of the mitochondrial MDH (0.39 and 0.43) were observed among planulae, scyphistomae, strobilae, and medusae, the planulae do possess an additional slow moving band (0.25) which is lacking in the other polymorphs. The only difference among the strobilae is the presence of two trace bands which are not detected in any other stage. Of the thirty *Aurelia* medusae studied, two kinds of MDH isozyme patterns were observed: the isozyme pattern of the medusa in which an additional 0.31 band is present (Figure 1) represents twenty of the *A. aurita* medusa population studied, whereas the other ten medusae have the same isozyme pattern as the scyphistomae. Although ephyrae were not analyzed due to a lack of material, their MDH isozyme patterns would undoubtedly be the same as that of the scyphistomae and medusae.

Chrysaora quinquecirrha

The MDH isozyme pattern of developing *C. quinquecirrha* is shown in Figure 3. As previously described (Lin and Zubkoff, 1973), the scyphistomae have 7 bands: the major bands with strongest intensity of staining are the 0.53–0.54 doublet, and the 0.40 band. Although changes have occurred through the development of *C. quinquecirrha,* the only difference between scyphistomae and strobilae is that the strobilae have a slow-moving band (0.19). Whether this 0.19 band is due to dissociation of the 0.21 band, or is a band unique to the strobilae, is unknown at this time. During the ephyra stage, the intermediate migrating bands (0.31, 0.37, and 0.40) show a decrease in their activities. The 0.40 band which is intense in

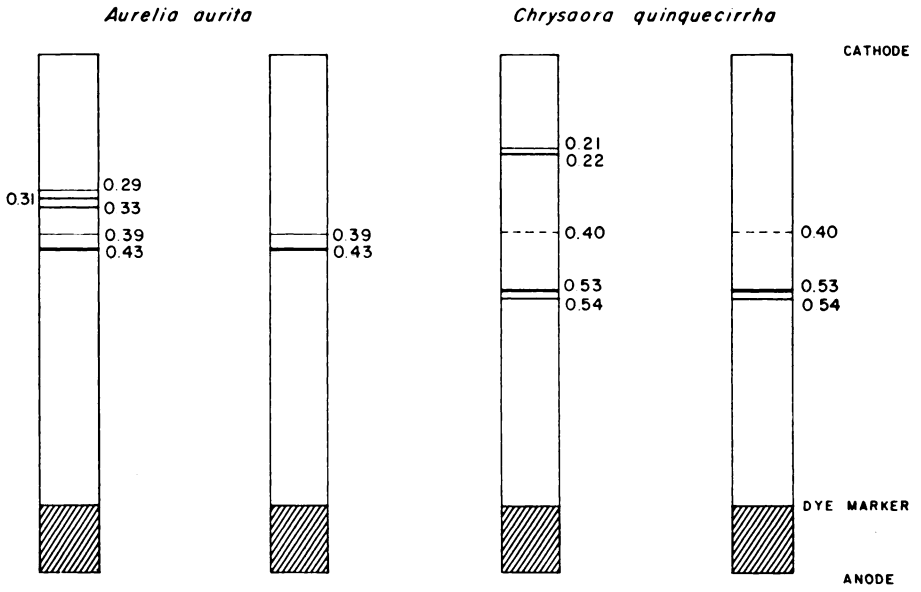

FIGURE 2. Mitochondrial MDH isozyme patterns of *Aurelia aurita* and *Chrysaora quinquecirrha* medusae. Left: whole tissue homogenate; Right: mitochondrial fraction.

the scyphistomae can be observed only as a trace band in the ephyrae; the 0.33 band of the scyphistomae is not observed in the ephyrae. In the medusae, there is a further reduction in the activities of the intermediate migrating bands clearly observed in the scyphistomae and strobilae: only the 0.40 band can be observed, whereas the other three bands (0.31, 0.33, and 0.37) are absent. In these four stages, scyphistomae, strobilae, ephyrae, and medusae, the major bands are at 0.53 and 0.54. In medusae, 0.40, 0.53, and 0.54 bands are the mitochondrial MDH and 0.21 and 0.22 bands are MDH of the cytosol (Figure 2). In addition to the loss of the intermediate migrating bands (in progressing from scyphistomae to the medusae), there is also an increase in the activities of the cytosol MDH (0.21 and 0.22 bands) in the medusae. Thus, there is a shift from many MDH isozymes in the scyphistomae to fewer isozymes in the medusae. In podocysts, the slow migrating doublet (0.21–0.22) associated with the cytosol predominates; the other bands associated with the scyphistomae and medusae are not detected.

At times, both red-pigmented and white-pigmented *Chrysaora* medusae occur. Although we suspect that this pigmentation is due to either a genetic difference and/or the influence of environmental factors such as food and light, a single MDH isozyme pattern is observed in both the white and red *C. quinquecirrha* medusae populations.

Cyanea capillata

Lin and Zubkoff (1973) observed that the scyphistomae of *C. capillata* have a single fast moving band (0.50) of great staining intensity and a slow moving

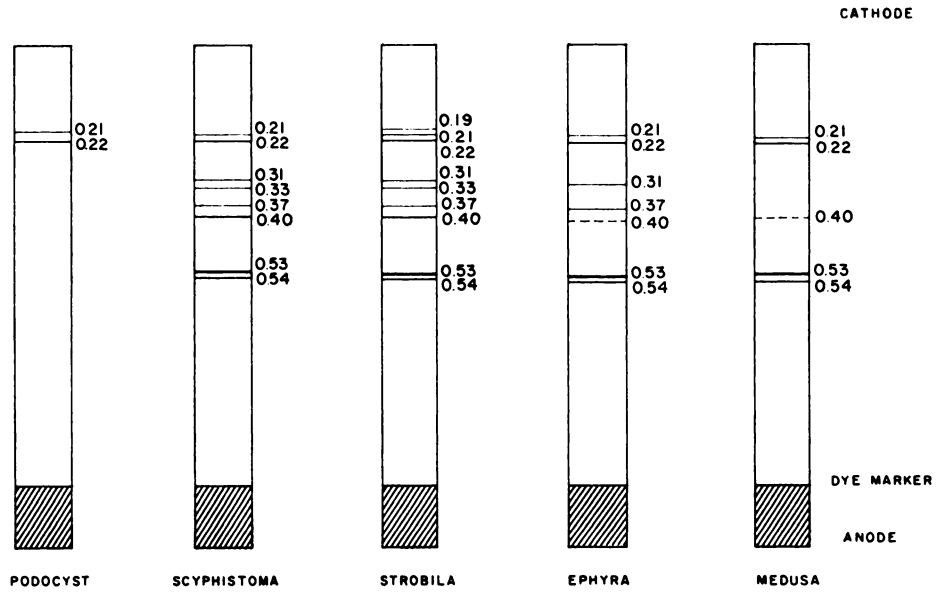

FIGURE 3. MDH isozyme patterns of different stages of *Chrysaora quinquecirrha.*

triplet (0.25). The medusae of *C. capillata* have the same MDH isozyme pattern as the scyphistomae, but there is an increased activity associated with the slow moving bands (inferred to be cytosol in anology with that of *A. aurita* and *C. quinque-*

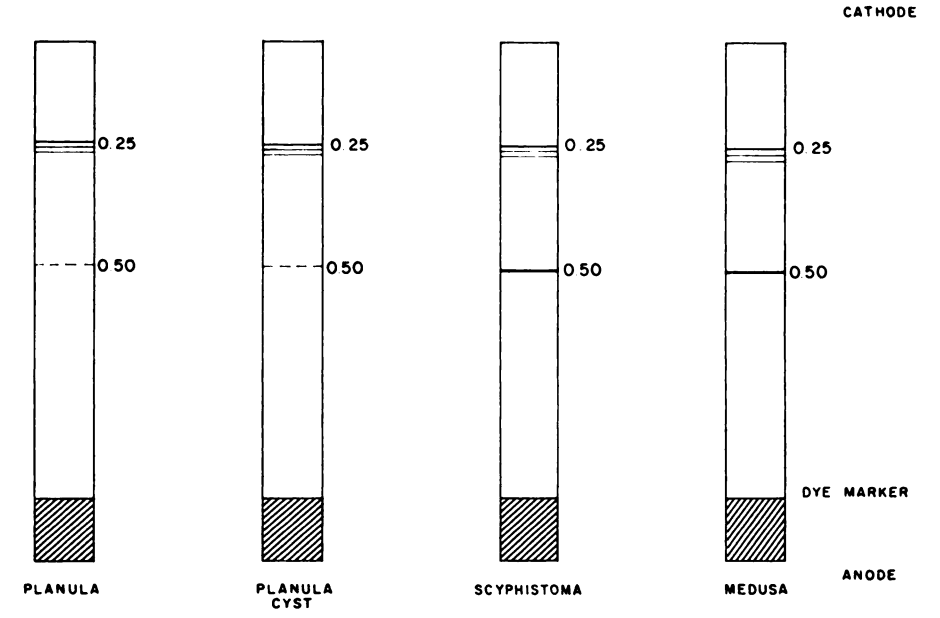

FIGURE 4. MDH isozymes patterns of different stages of *Cyanea capillata.*

TABLE I

Summary of MDH isozyme patterns of different stages of Chesapeake Bay jellyfishes. Parenthesis indicate the number of homogenates prepared; brackets, the number of organisms in each homogenate; face brackets, μg of protein in homogenate. The a indicates polymorph not observed; b, not available in sufficient quantity; c, trace bands appear in some preparations; d, inferred (see Discussion).

	Planula	Cyst	Scyphistoma	Strobila	Ephyra	Medusa
Aurelia aurita	(7)	a	(30)	(4)	b	(30)
	[20–40]		[7–9]	[6–9]		[<1]
	{40–60}		{20–30}	{20–30}		{100–200}
0.25	+					
0.29	+		+	+		+
0.31						+++
0.33	+++		+++	+++		+
0.36				c		
0.38				c		
0.39	++		++	++		++
0.43	++++		++++	++++		++++
Chrysaora quinquecirrha	b	(18)	(41)	(16)	(11)	(30)
		[40–60]	[7–9]	[6–9]	[20–40]	[<1]
		{120–340}	{20–30}	{20–30}	{10–25}	{100–200}
0.19	++d	++	+	+		
0.21	++d	++	+	++	+	++
0.22	++d	++	++	++	++	++
0.31			++	++	+	
0.33			++	++		
0.37			+	+	+	
0.40			+++	+++	+	c
0.53	+d		++++	++++	+++	++++
0.54	+d		+++	+++	++	+++
Cyanea capillata	(14)	(7)	(23)	b	b	(12)
	[30–60]	[40–60]	[15–20]			[<1]
	{40–60}	{120–200}	{20–30}			{100–200}
0.25a	++++	++++	+			++
0.25b	+++	++	+			++
0.25c	++	++	+			++
0.50	c	c	+++			++++

cirrha). Although the planulae and planulocysts of *C. capillata* have the same isozyme patterns as the scyphistomae, there is also a shift in the intensity of the fast and slow migrating bands: the slow migrating bands are the dominant form and only a trace amount of the fast moving band exists in the planulae and planulocysts. This transition of MDH isozyme dominance from planulocysts to scyphistomae is clearly demonstrated in the newly emerged scyphistomae: the 0.50 band, the band that is dominant in aged scyphistomae, is as intense as the 0.25 isozyme triplet. Within the medusae population analyzed, only one isozyme pattern was observed.

In contrast to MDH isozyme patterns which are summarized in Table I, the glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) of *A. aurita* has only one band (0.20) (Zubkoff and Lin, 1975). The G6PD of *C. quinquecirrha* and *C. capillata* also has a single band (0.20) and no change in the G6PD isozyme patterns is observed during the developmental stages of these three species.

Discussion

Changes in isozyme patterns indicate gene expression and may reflect genetic or epigenetic events taking place within the organism (Whitt, 1975). In this study, MDH isozyme changes during the process of strobilation are reported. In the scyphistomae of *A. aurita,* two intermediate migrating bands are found. In the scyphistomae of *C. quinquecirrha,* an additional trace band (0.19) is observed. Although the interpretation that the appearance of the new MDH isozyme is probably due to derepression of the gene is favored, it is possible that either the slowing down of the rate of degradation of that isozyme, the presence of inhibitors, or an epigenetic event may change the isozyme patterns during development. Dissociation or association of an isozyme subunit caused by the binding of metal ions or other compounds which change the electrophoretic mobility of that isozyme is not ruled out.

The MDH isozyme patterns of developing *C. capillata* reveal changes. In the four developmental stages studied, all have two groups of bands (0.25, 0.50). However, there is a shift from the dominant slow moving triplet of MDH of the planulae and planulocysts to the multiple forms of MDH of the scyphistomae and medusae. Whether this transition is due to the change in isozyme activities or the amount of isozyme, is still unknown. It is apparent that all the genes which are responsible for the synthesis of these isozymes are activated in these developmental stages.

The MDH isozyme pattern changes in different stages of *C. quinquecirrha* reveal that the mitochondrial MDH dominate in the scyphistomae and medusae. But the presence or absence and the relative activities of certain isozymes contribute to its complexity. Only minor differences between scyphistomae and strobilae are observed with pronounced MDH isozyme patterns first appearing in the ephyrae. The disappearance of the 0.33 band and the decreasing activities of other intermediate migrating bands are the major evidence of the change of the MDH isozyme pattern. After the ephyrae mature to medusae, the difference is more pronounced: there is only one intermediate migrating band (0.40) in the medusa, the rest of these intermediate migrating bands (0.31, 0.33, and 0.37) have disappeared either by degradation, inhibition, or lack of synthesis. On the other hand, there is an increase in the MDH of the cytosol in the medusae. Unfortunately, because of a lack of planulae of *C. quinquecirrha,* MDH isozyme analysis could not be undertaken. The planula stage which serves as a transition stage between eggs and scyphistomae is very short. However, from the data obtained from *C. quinquecirrha,* and the evidence from *C. capillata,* one can infer that, in planulae, the dominant bands would be the 0.21–0.22 doublet of the cytosol and that the activities of the other isozymes are increasing.

The increased mitochondrial MDH in the scyphistomae and medusae of *C. quinquecirrha* is certainly associated with energy production. When the animal is dormant, as in the cyst, it requires very little energy for maintaining its metabolic activities. When the planulocyst transforms into a scyphistoma, a greater energy requirement exists for maintaining its normal activities, and probably an even greater energy requirement is necessary in the strobilae and medusae. Thus, the genes for mitochondrial MDH are activated and responsible for the production of more energy and biosynthetic precursors.

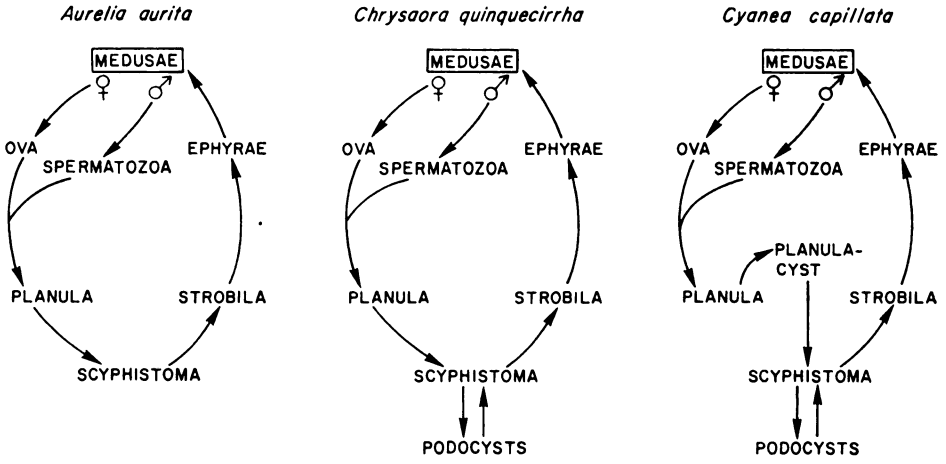

FIGURE 5. Life cycles of Chesapeake Bay jellyfish.

In *A. aurita,* the change of the MDH isozyme pattern is restricted to the cytosol MDH. Because the genes which are responsible for the synthesis of mitochondrial MDH are always in the same activated states, the explanation that certain genes for energy production are activated is not applicable. It should be noted that differences in the life cycles of these three Chesapeake Bay jellyfish exist (Figure 5). *C. capillata* planulae develop into planulocysts, which undergo further development into scyphistomae. The *C. quinquecirrha* planulae develop directly into scyphistomae, without formation of the planulocysts, and the scyphistomae normally form numerous podocysts. Furthermore, the scyphistomae may undergo transformation to form podocysts under adverse conditions, and may revert back to scyphistomae when the adverse conditions are removed. In contrast, cyst formation in the life cycle of *A. aurita* from Chesapeake Bay has not been observed. This may account for differences in the MDH isozyme patterns changes in *A. aurita* when compared to the other two scyphozoans.

The electrophoretic pattern of glucose-6-phosphate dehydrogenase is unlike that of other enzymes of scyphozoan jellyfish (Lin and Zubkoff, 1973; Zubkoff and Lin, 1975). MDH, superoxide dismutase (SOD, EC 1.15.1.1 = terazolium oxidase), and esterase of Chesapeake Bay jellyfish all have isozyme patterns whereas G6PD appears as a single band in the polyacrylamide gel enzyme assay. Although Blanquet (1972) indicated that scyphistomae of *C. quinquecirrha* have an increased G6PD activity during cold acclimation, there is no electrophoretic change in the G6PD pattern during the development of these three species. Blanquet (1972) also suggested that the increased G6PD activity makes more NADPH available for synthesis of fatty acids to be stored in the cysts. Since MDH of jellyfish can use either NADP or NAD as a co-factor (Lin and Zuboff, 1973), it may also serve as a potential NADPH supplier for this purpose.

The Chesapeake Bay jellyfish medusae appear at different seasons: *A. aurita* and *C. quinquecirrha* appear in the summer and *C. capillata* in the winter. The MDH isozyme pattern changes during development are very different between

these two summer jellyfish. In *A. aurita,* the changes are small and confined to the cytosol MDH. In *C. quinquecirrha,* changes of the intermediate migrating MDH isozymes (0.31, 0.33, 0.37, 0.40) occur. In *C. capillata,* the presumed MDH isozymes (0.25) of the cytosol are more prominent in the planulae and planulocysts whereas the presumed mitochondrial MDH (0.50) is most prominent in the scyphistomae and medusae.

Different MDH and SOD isozyme patterns are associated with scyphistomae from distant geographical locations which have significantly different seasonal patterns (Zubkoff and Lin, 1975). However, it is doubtful that temperature directly influences the change of these MDH isozyme patterns during development. In order to study the short term temperature effect on the MDH isozyme patterns of *C. quinquecirrha* and *A. aurita,* the scyphistomae of these two species cultured under different temperatures were compared. The results showed that scyphistomae maintained at both a lower temperature (12–15° C) and at room temperature (18–21° C) for one year have the same isozyme patterns. However, the substrate affinity and the free energy of activation of MDH in cold and warm cultured scyphistomae are different (Lin and Zubkoff, in preparation).

Numerous questions remain unresolved with respect to the development of jellyfish. In *C. quinquecirrha,* can scyphistomae derived from planulae be distinguished from scyphistomae derived from podocysts? Can scyphistomae which have undergone strobilation and reverted back to scyphistomae be differentiated from scyphistomae which have never strobilated? Since there is no structural difference between these scyphistomae, physiological, biochemical and genetic approaches must be employed.

Parts of this study were carried out under Public Law 89–720, The Jellyfish Act, in cooperation with the National Marine Fisheries Service of the U. S. Department of Commerce and the Commonwealth of Virginia. We thank Dr. R. E. Black for reviewing the manuscript and Mr. Michael A. Gorey for maintaining the cultures.

Summary

1. In *Aurelia aurita,* there is no change in the mitochondrial MDH isozyme pattern in different stages, although a relatively small change in the cytoplasmic MDH isozyme pattern can occur.

2. In *Chrysaora quinquecirrha,* three MDH isozyme patterns are observed: a dominant cytosol pattern in the cysts, a complex pattern in the scyphistomae and strobilae, and a simpler cytosol and mitochondrial pattern in the ephyrae and medusae.

3. In *Cyanea capillata,* two MDH isozyme patterns occur in the different stages: the planula and planulocyst pattern and the scyphistoma and medusa pattern.

4. Temperature induced differences were not observed in MDH isozyme patterns of the scyphistomae of *A. aurita* and *C. quinquecirrha* when cultured under different temperatures.

5. No differences in the MDH isozyme patterns of the red and white medusae of *C. quinquecirrha* were detected.

6. Changes in G6PD enzyme electrophoretic mobility during the development of *A. aurita, C. capillata* and *C. quinquecirrha* were not observed.

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