Iodine Induction of Metamorphosis in Aurelia [1]

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ABSTRACT Extensive laboratory investigations revealed two factors of primary importance for the initiation of metamorphosis (strobilation) in *Aurelia aurita*. It was found that sufficient quantities of iodide ions (in the form of either potassium iodide or elemental iodine) must be present for scyphistomae preconditioned at a low temperature to strobilate. The iodide ion requirement represents a very low dosage ($1:10^8$ dilution). Thyroxin, also in very minute quantities, likewise induces strobilation in temperature-preconditioned *Aurelia*. This response to thyroxin represents the first clear-cut demonstration of a morphogenetic response of a coelenterate to a hormone which is biologically significant in vertebrates.

A recommended temperature for preconditioning this Texas strain of *Aurelia* to strobilate is 19°C for at least one month prior to testing. Organisms maintained at 27°C can be induced to strobilate for several months, but they ultimately lose their ability to respond to induction stimuli. A higher test temperature of 27°C does, however, hasten the strobilation of scyphistomae in iodine-containing media.

Other environmental factors i.e., nutrition, and pH and hypotonicity of the medium, were found to be of little importance (within limits) to the mechanisms involved in the induction of strobilation (SI) in these organisms.

Several different environmental factors have been implicated in the initiation of metamorphosis (strobilation) in marine jellyfishes. Earlier workers believed that low temperatures play a role in the initiation of strobilation (SI) because they noted the occurrence of strobilae usually in winter or early spring. Agassiz (1862) noted the presence of strobilating *Aurelia* at Boston Harbour in March or early April, while Chuin ('28) reported that strobilation in scyphistomae was known to occur in November and December. Hargitt and Hargitt ('10) found strobilating *Cyanea arctica* at Wood's Hole in April and early May and strobilating *Aurelia* at this location from March to May. Lambert ('35) and Delap ('05, '06) observed that strobilation occurred seasonally in organisms maintained in their laboratories. Delap maintained scyphistomae of *Cyanea capillata* for two years and observed strobilation in them each winter when the minimum daily temperature fell below 45°F. Lambert kept both *Aurelia* and *Chrysaora* polyps in aquaria for many years and found that strobilation occurred regularly during midwinter when temperatures were minimum. Kakinuma ('62) found that *Aurelia aurita* strobilated readily in the spring and autumn when maintained at a laboratory room temperature which varied seasonally. Most recently, Sugiura ('65) reported that *Mastigias papua* had critical temperature requirements for SI.

Other investigators have ascribed a role in SI to nutrition and light. Lambert ('36) reported that the necessary conditions for strobilation included a "heavy diet of suitable food," and pointed out that some "hydra tubas" appeared to require molluscan food to strobilate, while others did not strobilate until they had been fed on small Nereids. Hyman ('40) stated that lack of food inhibits strobilation. Recently, Custance ('64), while studying *Aurelia*, reported that strong light inhibits SI. Kakinuma ('62), on the contrary, found that light played a positive role in SI in his *Aurelia aurita*.

Previous investigators, however, have not reported any chemical requirements for SI in jellyfish.

This paper reports the results of a five-year investigation of the effects of environmental factors on SI in *Aurelia*. The effects of temperature preconditioning, iodide ions (in the form of either potassium iodide or elemental iodine), thyroxin, nutrition, and hypotonicity and pH of the culture medium

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on SI were studied. A successful, easily reproducible method for SI in *Aurelia* was developed and, through this achievement, new insights concerning the mechanisms involved in SI were acquired. The necessity for adequate temperature preconditioning and for the presence of a minute amount of iodide ion for SI was demonstrated. A definite hormone-type influence on SI was established by the successful induction of strobilation by minute quantities of thyroxin. The possible relationship between iodide ion requirements for SI in *Aurelia* and the iodine-thyroxin accelerated metamorphosis in higher animals is discussed.

METHODS

Scyphistomae of *Aurelia aurita* obtained from a single medusa from the Texas Gulf Coast were used in these studies (Spangenberg, '64). These organisms have been maintained in this laboratory for over four years in an artificial sea water modified by Spangenberg ('65) (ASW_S). They were fed twice weekly with newly hatched *Artemia*, and transferred after each feeding to clean culture solutions and dishes.

Testing procedure. Testing of groups of organisms was accomplished by transferring groups of 45 organisms from their culture solution (ASW_S) into 50-ml parafilm-covered beakers containing the test solution. Testing of individual organisms was done in 15 ml of test solution in 24 × 82 mm Titeseal vials. All organisms were tested at 27°C, usually for 14 days, and examined under the dissecting microscope periodically to determine accurately the number of strobilating organisms. They were not fed during the test period.

The cultures of polyps from which the test organisms were derived were examined carefully in order to detect possible spontaneous strobilation in the cultures.

EXPERIMENTAL RESULTS

Temperature preconditioning

The need for low temperature preconditioning for SI in *Aurelia* was discovered while testing organisms in natural sea water (NSW). It was found that, when organisms maintained in artificial sea water (ASW_S) at 27°C were transferred to NSW at the same temperature, they frequently strobilated (table 1). After eight months, however, these organisms failed to strobilate when changed to a new saline environment. Since it was believed that long exposure to the higher temperature of 27°C may have altered the organisms' ability to strobilate, all of the cultures were transferred to a lower temperature, 19°C.

After a month's preconditioning at 19°C, groups of organisms from different cultures were tested at 27°C periodically over a period of six months. A large percentage of these organisms strobilated regularly even though new "batches" of sea water were used during these studies (table 2). Low temperature - conditioned organisms were used in all of the following experiments.

Iodide ion induction

The importance of iodide ions for SI was demonstrated through the comparison of the influence of various artificial sea waters on SI:

Modified Von Flack's artificial seawater (ASW_F) (Needham, '37) was tested in the

TABLE 1

Reduced strobilation initiation in scyphistomae maintained for more than a year at 27°C

	No. of organisms	% strobilae
Organisms kept in ASW_S for less than a year at 27°C tested in NSW at 27°C	297	47.4
Organisms kept in ASW_S for more than a year at 27°C tested in NSW at 27°C	415	1.2

TABLE 2

Initiation of strobilation in Aurelia transferred from ASW_S at 19°C to NSW at 27°C

Length of time at 19°C	No. of organisms	% strobilae
days		
34	375	68.2
60	180	48.8
79	225	65.7
123	225	80.4
148	450	50.0
169	450	64.4
185	270	83.0

search for a chemically defined solution that induced strobilation as effectively as NSW (table 3). Scyphistomae were tested in this solution, which did not contain iodide ions. Organisms in this formula did not begin strobilating even after 41 days, although 100% of those in NSW rapidly strobilated.

Instant ocean (ASW_{IO}) was likewise tested for SI ability in preconditioned organisms (table 4). It was discovered that the SI response was usually greater than in organisms tested in NSW. Eighty percent of a total of 1,360 organisms strobilated within a 14-day period at 27°C. The change of organisms from ASW_S to ASW_{IO} was found to be, therefore, of primary importance in SI. To determine whether one or more components in either of the artificial sea waters (ASW_S and ASW_{IO}) was responsible for SI response, each of the major and minor ingredients of ASW_{IO} were tested.

The major ingredients of ASW_{IO} were added collectively to distilled water according to the formula published for ASW_{IO} (Segedi and Kelley, '64). These are: sodium chloride, magnesium sulfate, magnesium chloride, calcium chloride, potassium chloride, sodium bicarbonate, potassium bromide, strontium chloride, manganese sulfate and sodium acid phosphate. Three hundred and sixty preconditioned organisms were transferred from ASW_S in five groups into the major ingredients of ASW_{IO} (partial ASW_{IO}) and tested at 27°C for periods up to 25 days. Control organisms were tested in complete ASW_{IO}. No strobilae developed in this partial ASW_{IO} although high percentages of organisms strobilated in the complete ASW_{IO}.

The minor ingredients, those present in less than 1 mg% in ASW_{IO}, were also tested for SI. They were divided into three separate groups which were added to ASW_S. Forty-five organisms from three different cultures were tested in each of these groups of salts at 27°C (table 5). The SI factor was found to be present in group III, containing zinc sulfate, potassium iodide (KI), cobalt sulfate and copper sulfate. When the components of group III were added separately to ASW_S while testing groups of 45 organisms, it became quickly evident that the SI factor was KI (table 6).

Potassium iodide versus iodine studies. Tests were performed to determine whether

TABLE 3

Effect of ASW_F on initiation of strobilation in Aurelia

Test solution	Experiment duration	No. of organisms	% strobilae
	days		
NSW	6	75	100.0
ASW_F	41	75	0.0

TABLE 4

Initiation of strobilation in Aurelia transferred from ASW_S at 19°C to ASW_{IO} at 27°C

No. of organisms	Preconditioning time at 19°C	Experiment duration	% strobilae
	days	*days*	
135	239	14	32.5
225	274	14	61.7
225	296	8	100.0
225	308	14	84.7
225	375	5	100.0
225	379	6	100.0

TABLE 5

Groups of 135 Scyphistomae tested for strobilation initiation in minor components of ASW_{IO}

	% strobilae	Experiment duration
		days
LiCl Na_2MoO_4 NaS_2O_3	0	12
$Ca(C_6H_{11}O_2)_2$ $Al_2(SO_4)_3$ RbCl	0	12
$ZnSO_4$ KI $CoSO_4$ $CuSO_4$	100	7

TABLE 6

Initiation of strobilation by KI at 27°C in groups of 135 scyphistomae transferred from ASW_S at 19°C

	% strobilae	Experiment duration
		days
$ZnSO_4$	0.0	11
KI	100.0	9
$CoSO_4$	0.0	11
$CuSO_4$	0.0	11
ASW_{IO}	67.5	11
ASW_S	0.0	11

TABLE 7

Strobilation initiation in groups of 45 Aurelia exposed to I_2 and KI

		% strobilae				Time of maximum strobilation
		Experiment I	Experiment II	Experiment III	Overall average	
						days
I_2	$1:10^7$	100	100	100	100	11
	$1:10^8$	73	60	62	65	14
KI	$1:10^7$	87	99	100	95	14
	$1:10^8$	49	84	66	66	14
ASW_S		0	0	0	0	14

elemental iodine (I_2) was as effective in SI as was (KI), since natural sea water contains iodide ions (I^-) in its elemental form (I_2). One hundred and thirty-five polyps in three separate experiments were exposed to dilutions of $1:10^7$ (100 μgm/l) and $1:10^8$ of KI and I_2. The results in table 7 show that both forms of I^- are equally effective in SI in *Aurelia*.

Iodine concentration tests. The quantitative range of activity of I_2 in SI was determined by testing groups of 45 organisms is ASW_S containing I_2 in dilutions of $1:10^8$. It can be seen in table 8 that some organisms were induced to strobilate by dilutions of I_2 as low as $1:10^9$. One hundred per cent of the organisms strobilated in response to dilutions of $1:10^8$ and $1:10^7$. Those in the higher concentration usually strobilated a few days earlier than those in the $1:10^8$ concentration.

Thyroxin. It became of interest to determine whether thyroxin could induce strobilation since I_2 is a major component of thyroxin ($C_{15}H_{11}I_4NO_4$). Seven groups of 45 organisms from different cultures were tested in concentrations of $1:10^7$ thyroxin. Table 9 reveals that 31 to 87% of organisms could be induced to strobilate over a 14-day period. The overall average of 61% SI is lower than that of organisms induced to strobilate by I_2.

Test temperature

To determine the importance of a test temperature higher than the maintenance temperature, comparisons were made of preconditioned organisms tested in ASW_{IO} at 27°C and 19°C (table 10). Although the percentage of strobilae obtained within a two-week period was somewhat lower in organisms tested at 19°C than in those tested at 27°C, considerable strobilation did occur. From this data, it was concluded that temperature increase is not necessary for SI although, in many scyphistomae, a higher temperature does increase the rate of SI.

Effects of other environmental factors on strobilation initiation

The influence of various hydrogen ion concentrations in ASW_{IO} on SI was tested. Groups of 90 organisms preconditioned at 19°C in ASW_S were exposed to ASW_{IO} buffered at various pH values at 27°C. Organisms were transferred to newly buffered solutions every two days. Each day

TABLE 8

Strobilation induction in two groups of 45 scyphistomae tested in various dilutions of I_2

Iodine dilutions	% strobilae	Experiment duration
		days
$1:10^7$	100	10
$1:10^8$	100	12
$1:10^9$	25	14
$1:10^{10}$	0	14
ASW_S	0	14

TABLE 9

Induction of metamorphosis in groups of 45 Aurelia by $1:10^7$ dilution of thyroxin over a period of 14 days

Culture no.	% strobilae in thyroxin	% strobilae in ASW_S
15	77	0
20	53	0
24	31	0
10	71	0
19	68	0
10	43	0
7	87	0

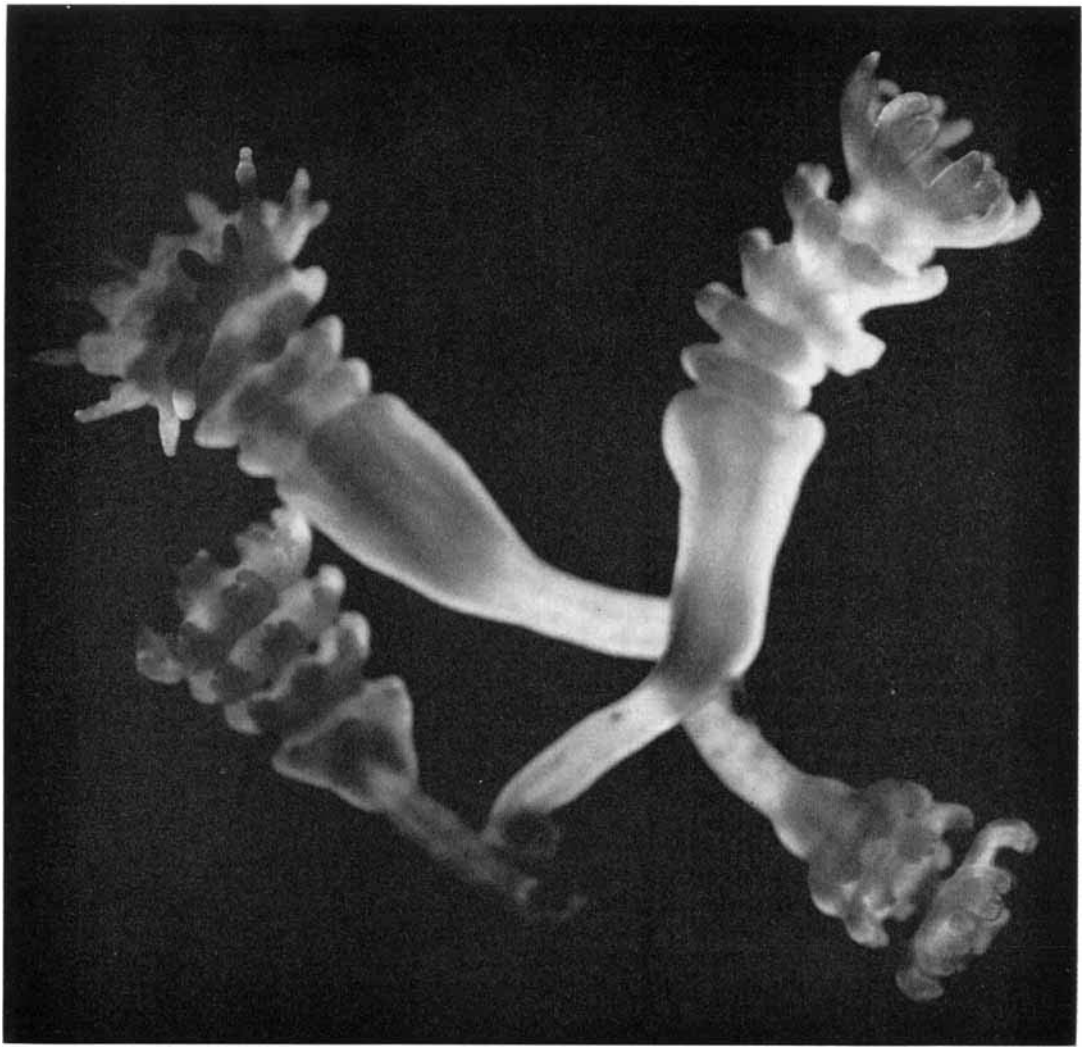

Fig. 1 Iodine-induced strobilation in *Aurelia*. (Photographed by Dr. Clarence Flaten.)

TABLE 10

Induction of metamorphosis in groups of 45 Scyphistomae transferred from ASW_S at 19°C to ASW_{10} at 19°C

Experiment 1		Experiment 2		Experiment 3	
Maximum % strobilae	Experiment duration	Maximum % strobilae	Experiment duration	Maximum % strobilae	Experiment duration
	days		*days*		*days*
93.3	14	44.4	12	100.0	13
100.0	10	100.0	12	73.3	13
44.4	14	26.6	12	20.0	13
100.0	14	17.7	12	100.0	13
100.0	14	13.3	12	66.6	13

the pH values of each soltuion were determined by a pH meter. The pH values did not change during the two-day period between changes. From table 11 it can be seen that SI is not pH-specific, in that organisms strobilated in solutions ranging from pH 6.5 to 10.0. This represents a remarkable capacity for organisms whose natural habitat is a well buffered sea water.

The effect of hypotonicity on SI was tested using dilute concentrations of ASW_{10} at 27°C. Groups of 45 organisms were exposed to solutions of ASW_{10} ranging from 4.02% to 1.15% total salt (table 12). It was found that 100% of the organisms strobilated in solutions less than one-third the concentration of natural sea water. It is perhaps noteworthy, however, that the KI concentration was within the range of SI activity even at this low (1.15%) total salt concentration.

TABLE 11

Studies of strobilation initiation of 90 scyphistomae tested at each pH value in ASW_{10} for eight days

pH value	% strobilae	pH value	% strobilae
6.0	0.0 (80% degenerated)	8.5	100.0
6.5	100.0	9.0	100.0
7.0	100.0	9.5	87.7
7.5	100.0	10.0	100.0
8.0	100.0		

TABLE 12

Effect of hypotonicity of ASW_{10} on strobilation initiation in Aurelia tested at 27°C within a 14-day period

Total salt concentration	% strobilae	No. of organisms
%		
4.02	100.0	90
2.65	100.0	90
2.01	100.0	90
1.60	100.0	90
1.34	100.0	90
1.15	100.0	90

The effect of starvation on strobilation initiation was tested when two large cultures at 19°C were deprived of nutrition for two months. During this time, groups of 45 organisms from each culture were exposed weekly to ASW_{10} at 27°C. Table 13 demonstrates that the organisms may be induced to strobilate even after sixty-two days deprivation of food, although 100% strobilation was not obtained after twenty-seven days. The organisms became very reduced in size after 30 days without food and usually only produced one ephyra per strobila after extreme starvation.

DISCUSSION

The remarkable requirement for I^- for SI in *Aurelia* was discovered through exploration of the effects of environmental factors. Many scyphistomae, when transferred from ASW_S to ASW_{10} or NSW rapidly began strobilating, while organisms tested in artificial sea waters lacking I^- (Spangenberg's (ASW_S) and Von Flack's (ASW_F) did not strobilate. Testing of individual ingredients of ASW_{10} demonstrated that KI was the only factor in this solution capable of inducing strobilation in *Aurelia*. Laboratory tests further demonstrated that I_2 was equally as effective in SI as KI, and it is therefore probable that the I_2 in NSW plays the major role in SI in organisms tested in this solution.

The fact that strobilation can be induced with minute quantities of thyroxin substantiates the theory that the I^- effect is hormonal in nature. It is not known whether the thyroxin provides the necessary I^- for SI or whether the organisms are using the I_2 or I^- to synthesize thyroxin or a thyroxin-like hormone. The fact that the thyroxine can be taken in by the organisms and utilized in some manner necessory for SI is significant. It is therefore sufficient to point out that a morphological response to a hormone has occurred in these lower invertebrate animals, as shown by SI. This response represents extensive mitotic activity and cellular differentiation, as the polyp's tissues give rise to new organisms (ephyrae) in a well regulated growth pattern.

Goldsmith ('49) reviewed the literature concerning the effects of thyroxin on invertebrate organisms. Although claims have been made that thyroid derivatives induced morphological responses in some invertebrates, results were sometimes inconclusive and often not reproducible. Goldsmith concludes that thyroid gland

substance appears to influence metabolism in "isolated tissues of invertebrates."

Gorbman ('63) points out that while iodinated proteins, including monotyrosine, diiodotyrosine, iodinated histidines and thyroxin have been found in numerous invertebrates, "no clear evidence has yet been presented to show that treatment of an invertebrate with thyroid hormone results in any kind of response."

It is known therefore that iodinated proteins occur in numerous invertebrates but their role in these organisms is uncertain. In *Aurelia,* proper temperature preconditioning is of critical importance for the SI by I^-. Perhaps the existence of other thyroxin or I^--mediated effects in invertebrates is hidden beneath obscure preconditioning requisites which must be determined before these effects can be easily demonstrated.

Preconditioning

Cultures of Texas *Aurelia* should be maintained at a low temperature at least one month in order to be induced to strobilate over long periods of time. Organisms maintained at a higher temperature, 27°C, can be induced to strobilate over a period of eight months but eventually they cease to respond to stimuli which initiate strobilation. The range of low temperature-conditioning in these organisms is not yet known, but Texas *Aurelia* maintained at 10°C do not survive.

Low temperature has long been recognized as a potential requisite for SI. Lambert ('35) stated that a critical low temperature is necessary for SI and he and Delap ('05, '06) pointed out that organisms maintained in their laboratories strobilated each winter (laboratory temperatures were not cited). Kakinuma ('62) also found seasonal SI in his laboratory-reared *Aurelia* which strobilated readily in the spring and autumn. Since his laboratory room temperature may have ranged from 5 to 28° it is possible that low maintenance temperatures could have played a role in this seasonal response.

The "critical low temperature" necessary for SI has been determined only for the jellyfish reported herein and the *Mastigias papua* of Sugiura ('65). Sugiura found that *Mastigias papua* required a critical low temperature range of 20–22°C. After prolonged exposure to 27°C, organisms frequently failed to strobilate and had to be cooled to 20°C for at least a month before they would strobilate.

It is quite probable that various strains, even of the same species of jellyfish, may require a different low temperature-preconditioning, possibly depending upon the winter temperatures of the waters of their natural habitats. *Aurelia* from England (Custance, '64) apparently require a lower preconditioning temperature than Gulf Coast *Aurelia,* and indeed, they tolerate much lower temperatures than Texas *Aurelia.*

It is interesting to note that low temperatures previously have been correlated with increased thyroid activity in frogs and salamanders (Wolf, '34; Morgan and Moyer, '36; and Morgan and Fales, '42).

Increased temperature following preconditioning. Although a temperature above 19°C is not necessary for SI, SI is expedited with increased temperatures. Scyphistomae kept at 19°C and tested at 27°C in I_2 often strobilate in large numbers within a five-day period, whereas only a few of those not transferred to the higher temperature strobilate in this time. Kakinuma ('62) reported that the most favorable temperature for SI in his *Aurelia* was 15°C, although he tested them at temperatures up to 30°C. Higher temperatures may also facilitate strobilation in nature as organisms preconditioned by winter temperatures could respond more readily to induction stimuli in the spring.

Terni ('19) and Eggert ('36) found that higher temperatures increased the rate of thyroxin-induced metamorphosis in the amphibia. However, as in the case of jellyfish, other authors (Belkin, '33; Tchepovetsky, '34; and Fosi, '35) point out that a temperature increase alone will not suffice to induce metamorphosis in amphibia.

Light. Custance ('64) found that light was an inhibitory factor in SI in his strain of *Aurelia.* He postulated that the seasonal periodicity of strobilation in nature may relate directly to the amount of daily illumination available at certain times of the year. Kakinuma ('62) obtained higher percentages of strobilae in *Aurelia* illuminated by a ten watt fluorescent bulb than

TABLE 13

Effect of starvation on metamorphosis induction in groups of 90 organisms transferred from ASW_S at 19°C to ASW_{10} at 27°C

Starvation time	% strobilae	Maximum strobilation time
days		*days*
0	100.0	6
6	100.0	5
13	100.0	5
20	100.0	7
27	78.8	14
34	100.0	5
41	28.8	14
48	8.9	14
55	2.2	14
62	2.2	14
76	0.0	14

in organisms tested in darkness. Darkness has also been found to affect thyroid function by Cehovic ('57), who found that continued darkness increases thyroid function as measured by increased I^{131} uptake.

Nutrition. Proper nutritional conditioning for SI in jellyfish has been emphasized in the past. Lambert ('35) refers to the need for a heavy diet of suitable food, and Hyman ('40) points out that lack of food inhibits strobilation. Nutritional studies in this laboratory indicate that scyphistomae of *Aurelia* have a remarkable ability to strobilate even after two month's deprivation of food, although the small starved polyps usually produce only one ephyra. Large percentages of organisms strobilate after one month's deprivation of food, when organisms are induced to strobilate in ASW_{10} containing I^-. Although the true nutritional state of the organisms is not known at this time, it is deduced that they are in a state of starvation because of their marked reduction in size. A heavy diet of food may not be as necessary as previously believed for SI.

pH and hyoptonicity. Environmental factors which do not appreciably affect SI in *Aurelia* are pH and hypotonicity (within limits), of the surrounding medium. It was found that *Aurelia* can be induced to strobilate in ASW_{10} with pH values ranging from 6.5 to 10.0. Bradway ('36) also found that metamorphosis could occur in *Clavelina* in ASW over a wide range of pH values.

Hypotonicity of the surrounding medium did not alter SI in *Aurelia* in the concentrations studied. Dilution of salts to less than one-third the total concentration of NSW and of ASW_{10} did not prevent SI. It is interesting to note that, even at this dilute concentration, sufficient I^- is present in the ASW_{10} to be physiologically active. Of further importance, however, is the fact that the dilution of other major salts did not appreciably affect development in these organisms.

CONCLUSIONS

This study of the effects of environmental factors on SI in Texas *Aurelia* has led to the following conclusions:

1. The two significant factors involved in SI in *Aurelia* are low temperature preconditioning of the scyphistomae and the presence of sufficient quantities of I^- in the environment.
2. I_2, in quantities as low as $1:10^7$ dilution, is as effective in initiating strobilation as KI, and may, therefore, be active in SI in nature.
3. Thyroxin, also in minute quantities, induces strobilation in *Aurelia*. For the first time, a hormone of known biological significance in higher animals has been shown to induce a definite morphogenetic response in a coelenterate.
4. In order for I_2, KI, or thyroxin to induce strobilation, organisms must be temperature-preconditioned to respond to them.
5. Preconditioning for SI requires the exposure of scyphistomae to a temperature of 19° for a least a month prior to testing. Preconditioned organisms have continually been induced to strobilate for a year and a half.
6. Organisms maintained at 27°C can be induced to strobilate for several months, but they eventually lose their ability to respond to SI stimuli.
7. Transfer of organisms from 19°C into KI-containing test solution at 27°C expedites SI, but a temperature increase is not necessary for SI.
8. Environmental factors found to be of little importance (within limits) to the mechanisms involved in SI are nutrition, and the pH and hypotonicity of the surrounding medium.

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