Production of Iodinated Compounds and the Effect on Strobilation in *Aurelia aurita*

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May 2, 1973
(date)

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Abstract

Aurelia aurita scyphistomae were exposed to a radioisotope iodine^{131}, which induced strobilation. Strobilation is an asexual reproductive process unique to certain coelenterates. The scyphistomae were chromatographically analyzed after designated periods of exposure to iodine^{131}. Three iodinated compounds were identified by Rf values in two chromatographic solvent systems. These compounds were monoiodotyrosine, diiodotyrosine and thyroxine.

Iodotyrosines appeared in samples at four and eight hours after exposure to iodine. Thyroxine appeared twelve hours after exposure. Organisms exposed to iodine for just four and eight hours did not strobilate. Those organisms exposed twelve hours or more, did strobilate. This response indicated that thyroxine is required for strobilation.

Two known inhibitors, thiourea and potassium thiocyanate, prohibited strobilation in *Aurelia aurita* scyphistomae. No iodinated compounds were formed when polyps were exposed to iodine and thiourea, and strobilation did not occur. Exposure to potassium thiocyanate and iodine resulted in formation of monoiodotyrosine, diiodotyrosine and thyroxine, but strobilation did not occur. The inhibitory effect of thiourea and potassium thiocyanate on strobilation, and the

initiation of strobilation after the sequential production of monoiodotyrosine, diiodotyrosine and thyroxine, suggests a model for thyroxine action similar to that in vertebrates.

Acknowledgments

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Introduction

Aurelia aurita, the true jellyfish, exhibits the phenomenon of alternation of generations, a reproductive process common to many coelenterates. This life cycle affords a unique model for the study of morphological changes. The organism alternates between a sessile, asexual scyphistoma (polyp) and a free-swimming, sexual medusa. The transitory stage from scyphistoma to medusa is the strobila. Strobilae have been studied, because they represent unusual reproductive structures. Many factors which may influence the initiation of strobilation have been researched. These factors include nutrition (Gilchrist 1937a, Hyman 1940, Berrill 1949a, 1949b), light (Custance 1964, 1967), temperature (Lambert 1936, Kakinuma 1962, Custance 1966, 1967) and iodine concentrations (Spangenberg 1967, 1968).

This investigation is concerned with the relationship between iodine and the initiation of strobilation. A direct relationship between the uptake of iodine and the onset of strobilation has been shown by Spangenberg (1967). This relationship introduces questions concerned with the production of an iodinated compound that may play a role in strobilation initiation. Iodine uptake with respect to strobilation suggests a "hormone-like" effect, that might indicate the presence of a thyroid hormone such as thyroxine. Aurelia aurita has no thyroid gland, but there are certain invertebrates that exhibit the capability of synthesizing

extrathyroidal thyroxine (Berg, Gorbman and Kobayashi, 1959). However, the physiological action of an iodinated hormonal-like compound in marine invertebrates has not been elucidated. Therefore the specific aims of this investigation are as follows:

1) To determine the presence of an iodine metabolism complex in Aurelia aurita scyphistomae and strobilae.
2) To identify iodinated compounds present in scyphistomae and strobilae.
3) To record a sequence of morphological changes in relation to the occurrence of iodinated compounds in A. aurita.
4) To demonstrate that an iodinated compound with a metabolic function is synthesized by A. aurita.
5) To compare the iodine metabolism in A. aurita to that of certain higher forms of animal life.

Review of Literature

A brief account of the natural life cycle of Aurelia aurita is presented, since the morphological changes therein are related to this investigation. Adult medusae produce gametes in the late summer or early fall of the year. The male releases spermatozoa which are taken into the gastrovascular cavity of the female. Eggs are fertilized and released from the mouth to the ciliated grooves of the oral arms. Zygotes develop into ciliated, two layer planula larvae which are free-swimming structures. A planula attaches to a bottom surface and begins to invaginate at the site of the blastopore. This attached organism develops into a scyphistoma, a cone-shaped structure with tentacles surrounding the mouth (Figure 1). The scyphistoma reproduces by asexual budding of more scyphistomae (Figure 2). The scyphistoma undergoes transverse fission at the distal end during late winter. Tentacles are absorbed, and discs are formed while the transverse striations gradually become separated (Figure 3). This process is termed strobilation, and discs formed are called ephyrae. Ephyrae are released from the scyphistoma which then regenerates tentacles, and assumes its previous form. Ephyrae are immature medusae and gradually mature during the warmer periods of the summer. Medusae are sexually mature by late summer. The complete life cycle is illustrated in Figure 4. Hyman (1940) has indicated that second year, and older, medusae become

Figure 1 - Aurelia aurita scyphistoma

Figure 2 - Aurelia aurita scyphistoma, exhibiting early budding

Figure 3 - Aurelia aurita strobila

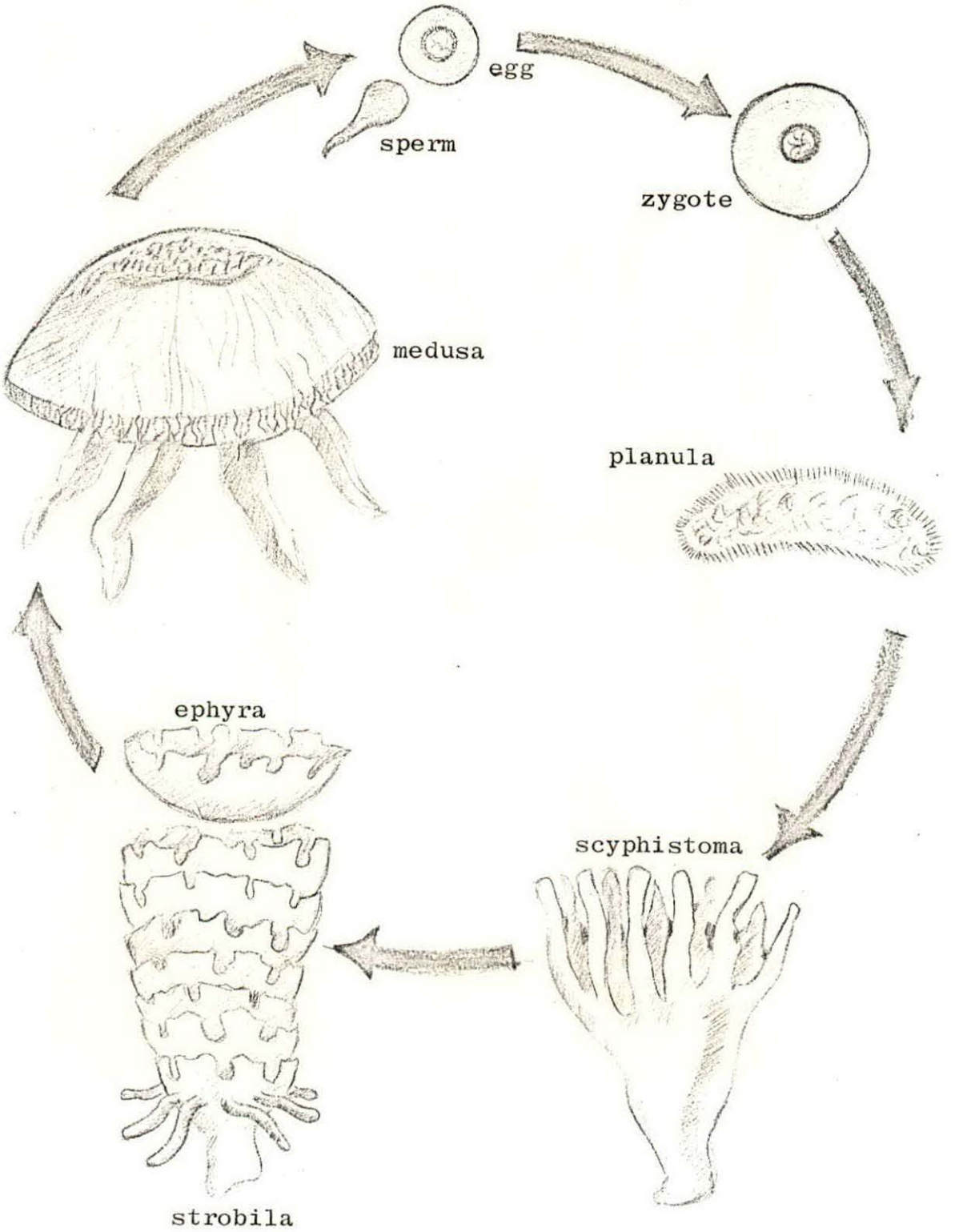


Figure 4 - Life Cycle of Aurelia aurita

sexually active in the late summer and produce gametes concurrently with the sexually reproductive youngest medusae. The life span of scyphistomae is unknown, but laboratory cultures have been maintained for at least three years.

Originally, the scyphistoma and medusa stages of Aurelia and related genera were taxonomically divided. Reid (1848) first described the relationship of scyphistoma to medusa, and the role of the strobila in the alternation of generations exhibited by Aurelia. Later, Mayer (1910) and Hyman (1940) clearly described the transitory role of the strobila, and the morphological changes that occur in strobilation. The complete life cycle is well understood, but the factors which influence and control the cycle are of great interest to researchers. A key point of interest is the action of a mechanism which controls the change of scyphistomae to strobilae in order to complete the alternation of generations rather than the reproduction of more scyphistomae.

Percival (1923), Gilchrist (1937a) and Hyman (1940) were the first to describe factors that may influence the initiation of strobilation. These researchers concluded that variations in nutritional levels were significant in strobilation initiation. Berrill (1949a) did extensive work on nutrition and strobilation, with emphasis on morphological variation. He noted that the following two types of strobilation occurred: polydisk formation, in which many ephyrae are produced simultaneously and monodisk

formation in which only one ephyra is produced. Apparently, both nutrition availability and the size of the scyphistoma determine which of the two types occur. Later, Berrill (1949b) stated that other factors may also be influential in determining the type of strobilation; such as the shape and size of the tentacular end of the scyphistoma. Spangenberg (1967) found that nearly 100% of Aurelia aurita, chemically conditioned, would respond by strobilating after two months of food deprivation. Starvation was determined by noting a reduction of test organisms. Strobilation response during starvation indicated that nutritional levels may be considered secondary in importance to strobilation initiation.

Factors other than nutrition have been considered as controlling factors in strobilation. Custance (1964) experimented with light as an inhibitor of strobilation in laboratory cultures. The fact that strobilation occurs during the shorter daylight period of winter led to the research of Custance. His results indicated there was no apparent relationship between light and strobilation. Later studies (Spangenberg 1965b, Custance 1967, Matsueda 1969) substantiated this conclusion.

The effect of temperature on strobilation in A. aurita has been more widely studied because of the temperature fluctuations which occur during the seasonal changes of the life cycle. Researchers, have maintained scyphistomae under controlled conditions (Lambert 1935, Gilchrist 1937b), and

found that strobilation occurs primarily at lower temperature levels which correspond to those occuring during winter. Lambert (1935) stated that a low minimal temperature is required for strobilation. Other researchers (Jachowski 1963, Kakinuma 1962, Custance 1966) have studied temperature levels of laboratory maintained Aurelia, and how they relate to the life cycle. Kakinuma (1962) found that 15°C was the optimum level for strobilation, with 30°C being almost inhibitory. Custance (1966) reported a statistical study that indicated the optimum temperature for strobilation is between 5°-10°C. Spangenberg (1967) was able to initiate strobilation, on chemically conditioned scyphistomae, over a wide temperature span. However, organisms maintained at 27°C ceased to respond after eight months, but could be induced to strobilate after pre-conditioning at the optimum temperature of 19°C. She stated that in nature the pre-conditioning temperature could be of greater importance than the existing temperature at the time of strobilation. This more closely coincides with the natural occurrence of strobilation, since marine temperatures change slowly from a maximum summer level to a minimum level in late winter.

Spangenberg (1967, 1968) was the first researcher to study the effect of chemicals on strobilation. She determined that organisms deprived of an iodide content in sea water media strobilated, when iodine (I_2) at 10^{-6} molarity (M) was added. In a series of tests, 100% of those scyphistomae pre-conditioned at 19°C strobilated within five days

after iodine administration. In another study Spangenberg (1971), discovered that other chemicals also showed a positive effect on strobilation. All chemicals that had an effect contained iodide atoms on a larger, organic molecule. One such organic molecule, tyrosine, has been identified in the extracellular fluids of Aurelia aurita (Lane et al. 1965). This response to such iodinated organic molecules indicates the presence of a biological process utilizing iodine, which closely resembles a hormonal system involving thyroxine or related substances.

To adequately understand iodine metabolism at this organizational level, and its evolutionary relationship, one must consider an overview of iodine function within the animal kingdom, especially among the invertebrates. In an extensive work, Gorbman, Clements and O'brien (1954), determined that all major phyla of invertebrates, except Protozoa and Echinodermata, showed evidences of iodine uptake. In some organisms, they determined that certain iodinated compounds were synthesized.

Diiodotyrosine (iodogorgoic acid) was first isolated in the coelenterate corals (Roche, 1952). Monoiodotyrosine is the iodoprotein form most commonly found in invertebrates, but high levels of thyroxine have been found in certain Molluscan forms (Gorbman, et al. 1954). Many invertebrates contain iodoprotein associated with the ectoderm or external skeleton. In Annelids, the greatest amount is found in the cuticle. The foot and shell of Mollusks contain the greatest

amounts of iodoprotein. However, there is some indication of higher concentrations near the oral opening, especially in the areas of the pharyngeal teeth and radula.

In protochordates, iodoprotein-forming tissue is located in the mid-ventral pharynx. During metamorphosis of the lamprey, iodoprotein tissue of the larval form is almost completely separated from the pharynx and releases iodoprotein into the pharynx through a duct. The adult lamprey has a distinct, follicular thyroid gland.

This sequence of development led Gorbman and Bern (1962), to propose a theory concerning the evolution of thyroid function. First, primitive invertebrate types synthesized iodoproteins mainly as scleroproteins which were evenly distributed through the body surface. At some point, thyroxine, or an iodoprotein, began to be utilized in metabolic processes. Survival value increased the occurrence of internal iodoprotein-forming tissue located in the pharynx, thereby establishing a characteristic for the second invertebrate type, which is more advanced. Third, the concentration of iodoprotein-forming tissue in the pharynx is represented by the amphioxus, a protochordate. Fourth, the lamprey represents the transitory stage of iodoprotein-forming tissue in which pharyngeal tissue evolves to a functional thyroid gland by metamorphosis. The thyroid gland in higher vertebrates differs little from this early lamprey form.

Based upon these background observations this investigation is involved with the pursuit of the following specific goals:

1) To determine the presence of an iodine metabolism complex in Aurelia aurita scyphistomae and strobilae.
2) To identify iodinated compounds present in scyphistomae and strobilae.
3) To record a sequence of morphological changes in relation to the occurrence of iodinated compounds in A. aurita.
4) To demonstrate that an iodinated compound with a metabolic function is synthesized by A. aurita.
5) To compare the iodine metabolism in A. aurita to that of certain higher forms of animal life.

Materials and Methods

Maintenance of the Organism

The scyphistomae used in this study were collected from the Gulf of Mexico, near Galveston, Texas. They were maintained under laboratory conditions for five years. An artificial sea water (ASW) medium was used to culture the organisms. The necessary salts were added to twice distilled water according to Lyman and Fleming (1940) and modified by Spangenberg (1965a). Salts and amounts added are listed in Table 1.

Table 1 - Artificial Sea Water Medium*

Salts	grams/liter of H_2O
Sodium Chloride	23.47
Magnesium Chloride	4.98
Sodium Bisulfate	3.91
Calcium Chloride	1.10
Potassium Chloride	0.665
Sodium Bicarbonate	0.192
Potassium Bromide	0.096
Boric Acid	0.026

*Lyman and Fleming (1940), modified by Spangenberg (1965b)

The major nutrient supply was freshly hatched *Artemia*. The scyphistomae were fed twice weekly and transferred by pipette to fresh media 24 hours after feeding. The medium for the organisms, and for the *Artemia* hatch, was filtered through an ion exchange resin to eliminate iodides. This procedure contributes to the iodine conditioning that has been achieved

with these Aurelia. The resin used was Dowex 1-X-2, 400 mesh. The reservoirs for culturing were dishes of 5 inch and 10 inch diameter each containing 250 - 500 polyps. The culture dishes were stored in a Psycrotherm Controlled Environment Incubator, manufactured by the New Brunswick Scientific Company. Temperature was kept constant at 19°C. The incubator is equipped with one glass window on the door which produces almost normal diurnal light conditions. Organisms, pre-conditioned at 19°C and free from iodides, readily responded to iodine exposure by strobilating. This response expedites the study of iodine metabolism, because of the certainty of iodine uptake.

Strobilation Testing

The scyphistomae were exposed to iodine and iodine related compounds to achieve optimum time levels for strobilation, and to determine relationships between the various compounds. The compounds used were monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T_3), thyroxine (T_4), thyroglobulin (Thy), tyrosine (Tyr) and iodophenylalanine (IPA). All organic compounds used in this investigation were obtained from Sigma Chemical Company. For each chemical tested, 25 ten milliliter (ml.) samples were prepared in the deionized artificial sea water at concentrations of 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} molar. Polyps were exposed to each 10 ml. sample in 15 ml. vials, one polyp per vial. The vials were sealed and stored at 19°C. A check for strobilation, of the 25 units for each sample, was made every day for two

weeks. Strobilation was determined by visually observing morphological changes related to the formation of transverse striations.

Chromatographic Analysis

Due to the small size and weight of an A. aurita scyphistoma, several organisms had to be prepared for analysis. A minimum weight of 30 milligrams (mg.) of polyps was required for analysis. To obtain the required weight, 50 to 60 polyps were sacrificed.

Chromatographic analyses were performed on the organisms to identify iodoamino acids. These analyses were made by the employment of a radioisotope tracer, iodine^{131} (I^{131}), which has a half-life of 8.05 days. The tracer was obtained from Environmental Nuclear Engineering Company, Dallas, Texas, in the form of NaI.

Thirty mg. of polyps were used for one sample. The polyps were introduced into 50 ml. of ASW containing 1 microcurie of tracer per ml. of media. A. aurita absorbs the tracer iodides with a rate equal to the absorption of non-tracer iodides. Each 30 mg. sample was exposed to the tracer for a distinct time period. Sample periods were for 4, 8, 12, 16, 20, 24, 28, 32, 40, 48, 72, 96 and 120 hours duration. These periods were selected after previous experiments indicated the presence of iodine containing compounds within these hours. Extra polyps were included in each sample for purposed of observing morphological changes. Morphological changes were recorded and correlated

with the chromatographic analyses.

The polyps were removed from the isotope medium at the end of the specified time period, and rinsed in a series of fresh ASW to remove any external isotope. Almost all ASW was removed from the polyps, and 3 ml. of Tris buffer (pH 8.0) were added. The organisms were homogenized in a Potter-Elvehjem homogenizer, and the mixture was centrifuged. The precipitate was rinsed once with trichloroacetic acid (TCA), then centrifuged again according to Heninger and Albright (1966). The precipitate was added to 2 ml. of Tris buffer (pH 8.0) that contained 20 mg. of Protease, Grade IV. This mixture was incubated at 37°C for four hours to hydrolyze the protein into amino acids. Two hundred microliters of hydrolyzed precipitated mixture were spotted on Whatman number 1 paper chromatography strips, with dimensions of one inch wide and 65 centimeters (cm.) long. Duplicate strips for each test sample were analyzed using descending chromatography in two solvent systems. Spots of known iodocompounds were analyzed simultaneously for comparison purposes. The known compounds were: iodine, monoiodotyrosine, diiodotyrosine, triiodothyronine and thyroxine.

Isotope Tracer Determinations

The chromatography strips were assayed to determine tracer peaks of the isotope I^{131}. The I^{131} could have been part of other molecules, but disintegrations would still be detected. A scanning device was used to detect locations of

radioactivity on the chromatography strip. These tracer peaks were then correlated to the colorimetric analysis of the strip.

A Geiger-Mueller (G-M) tube was used to scan the strip and read the beta decay from I^{131}. Scanning was made possible by keeping the G-M tube stationary and using a motor drive to pull the strip under the G-M tube. The G-M tube fed the impulses into a ratemeter, from which counts-per-minute readings could be determined. However such a reading was only valid for a particular location under the G-M tube. No accumulative data was made available by the ratemeter. The impulses from the ratemeter were amplified to a chart recorder, and the radioactivity was graphically recorded as relative radioactivity versus chromatogram length. The peaks on the charts did not specifically show counts per minute, however recordings from the ratemeter were utilized to relate activity at different locations on the chromatogram. Rf values of the radioactive peaks were determined and compared to the Rf values of expected compounds. In determining the distance from the origin, the highest point of the peak was used as being definitive for that substance.

Each of the chromatographic analyses were performed in two different solvent systems. This was done to insure that the data obtained could be reproduced in different solvent systems.

Colorimetric Determination

The levels of iodoamino acids from the Aurelia are usually too low to provide reliable colorimetric determinations by the use of standard reagents, such as ninhydrin. Most of the determinations on unknowns were, therefore, made by isotope detection. Strips with known amounts of iodoamino acids and iodine were run simultaneously with the sample tissue. The strips were identified by one of three methods: (1) iodoamino acids were detected with Pauley's reagent, in which thyronines appear purple and tyrosines appear bright orange; (2) iodoamino acids also appear as darker areas under ultraviolet light; and (3) iodine is not sensitive to either of these methods so a reagent specific for iodine was used (ceric sulfate-arsenious acid reagent).

All of these methods were used on known substances and those Rf values obtained were compared to the Rf values characteristic of the radioactive peaks. Therefore, two parameters of measurement were used, but comparisons could be made from the Rf values alone. The Rf value is the ratio between the sample distance from the origin and the distance of the solvent front from the origin. The value will always be less than one.

Inhibitors of Strobilation

An effort to possibly relate and compare, the iodine metabolism of the Aurelia aurita with higher animal forms was made by testing chemical inhibitors that show a thyroid inhibitory action. Certain chemicals inhibit the ability

of the thyroid gland at two different levels in mammalian systems (Gorbman, 1963). One group of chemicals, including thioureas, thiouracils and sulfonamides, inhibit the uptake of iodine and thus prevent the formation of iodotyrosines. A second group of chemicals include thiocyanates, which apparently inhibit the action of iodinated compounds rather than the synthesis of a hormone. The two chemicals used in this study, representing the two inhibitory levels, were thiourea and potassium thiocyanate.

Low levels (1 mg.%) of the inhibitory chemicals were added to 50 ml. of ASW that contained 50 microcuries of I^{131}. Sufficient scyphistomae to provide 30 mg. of tissue, per time interval sample, were introduced to the test medium. The organisms were removed at each time interval of 4, 8, 12, 16, 20, 24, 48, 72, 96 and 120 hours. The organisms were prepared, chromatographed and scanned by the procedure described above. Strips obtained by this process were compared to those strips for samples with I^{131} alone, added to the medium.

Results

On the basis of results from preliminary tests it was determined that strobilation by induced methods occurred within 10-14 days, therefore, a test limit of 14 days was established. Table 2 shows the average time, in days, in which 50 per cent of the organisms in each sample strobilated after incubation with the various iodinated compounds. These are average values obtained from three different series of tests. For purposes of statistical analysis, the maximum of 14 days was given to those samples that did not initiate strobilation.

Table 2 - Time, in Days, in which 50 per cent of the Sample Population Strobilated at Various Molar Concentrations of Eight Compounds

Chemical	Molar Concentrations 10^{-6}	10^{-7}	10^{-8}	10^{-9}
Iodine	4.8	5.8	7.6	10.2
Thyroxine	6.2	11.2	14.0	14.0
Triiodothyronine	8.4	12.8	14.0	14.0
Diiodotyrosine	10.2	12.2	14.0	14.0
Monoiodotyrosine	10.6	13.4	14.0	14.0
Thyroglobulin	12.2	14.0	14.0	14.0
Tyrosine	14.0	14.0	14.0	14.0
Iodophenylalanine	14.0	14.0	14.0	14.0

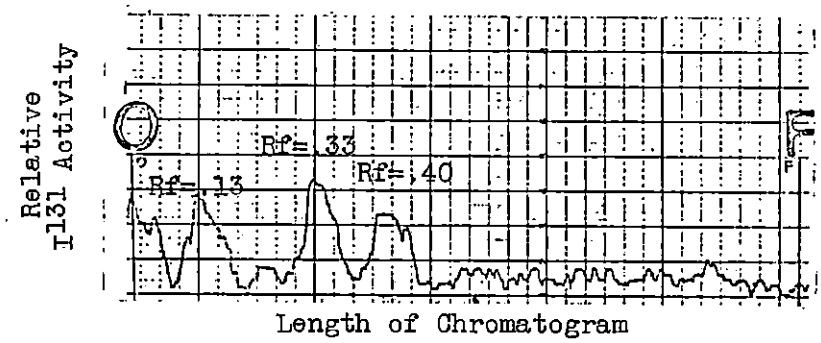
Statistical analysis of eight iodinated compounds at four various molarities from Table 2 was performed in order to determine which compounds at which molarity were more effective (with respect to time), in initiating strobilation. Sum of squares for each of four variables was obtained and analyzed according to degrees of freedom (DF) and F distribution. Results of the analysis are shown in Table 3.

Table 3 - Summation of F Distribution of Four Variables on Strobilation Initiation

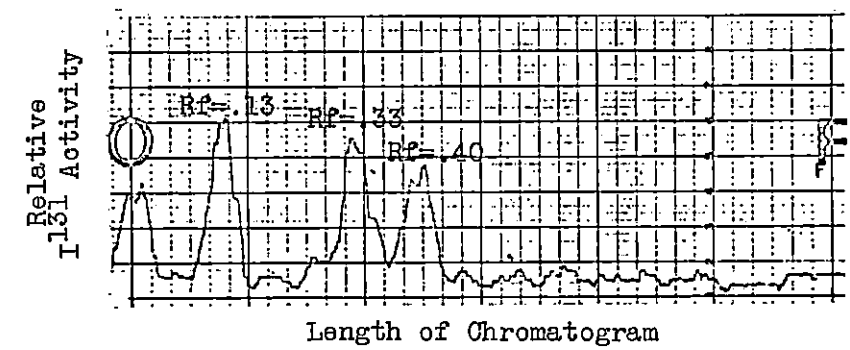
Variation	Sum of Squares	DF	Means Squared	F Distribution
Chemicals	320.2	7	45.74	$91.48 : F_{7,169} = 2.76$
Concentration	292.6	3	97.53	$195.06 : F_{3,160} = 3.91$
Interaction	149.9	21	7.14	$14.28 : F_{21,160} = 2.00$
Error	80.9	160	.50	

The level of significance was the .01 level, and the large F values indicate that chemicals, concentration and interaction of these two were significant in their effects. A one-way analysis of variance was done at the 10^{-6} molar level. This analysis indicated that the effects of iodine and thyroxine on strobilation were significantly different (at the.01 level) from the other six chemicals. Therefore, statistical analysis indicates that iodine is the most effective compound on strobilation initiation with respect to time.

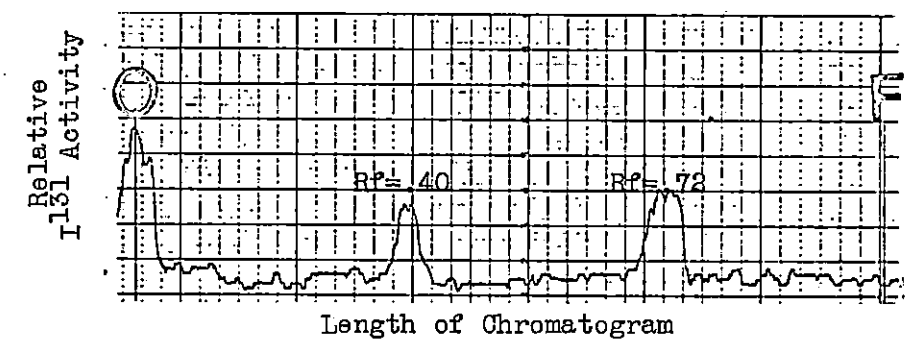
Chromatographic analysis of organisms exposed to the I^{131} tracer is described according to time levels used. Compounds are identified by Rf values calculated from tracer peaks presented in Figures 5, 6 and 7.



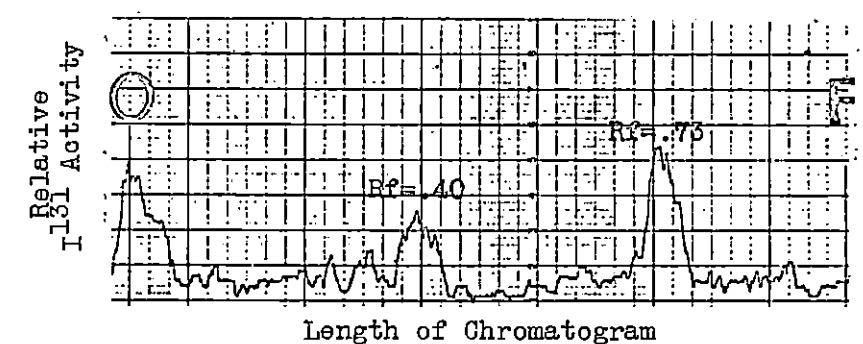
A: Four hours of I^{131} exposure



B: Eight hours of I^{131} exposure

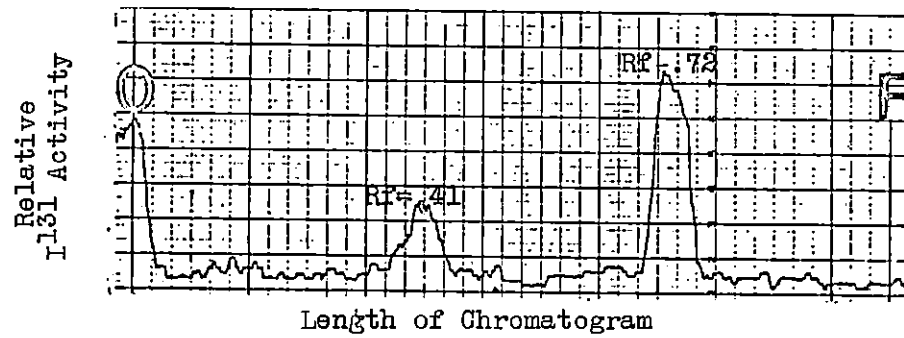


C: Twelve hours of I^{131} exposure

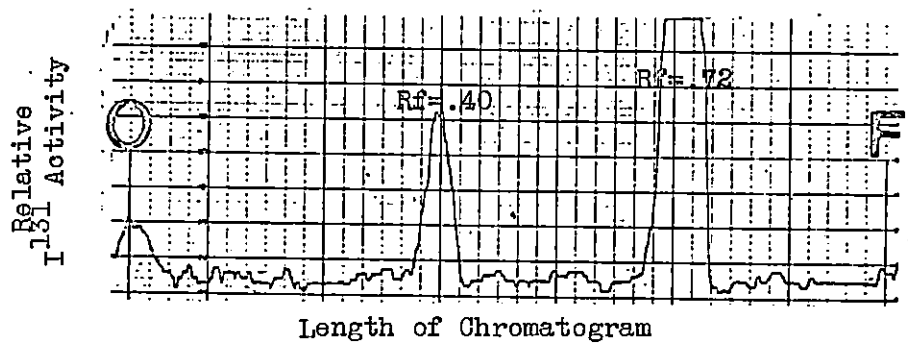


D: Sixteen hours of I^{131} exposure

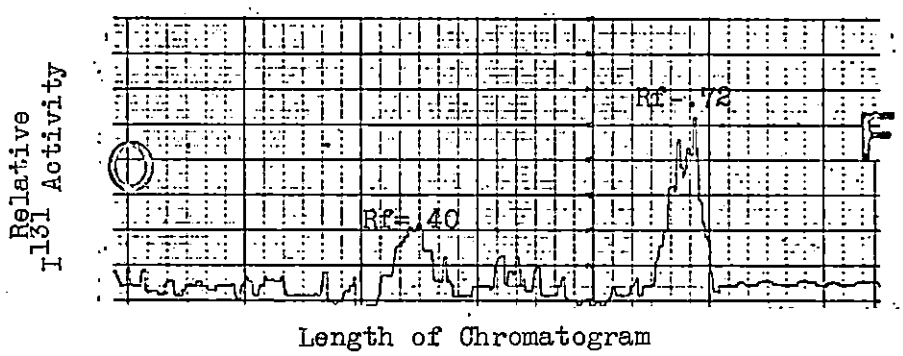
Figure 5 - Radiochromatographic strips indicating tracer peaks present in 4, 8, 12 and 16 hours exposure to I^{131} of A. aurita polyps. (O - origin, F - solvent front)



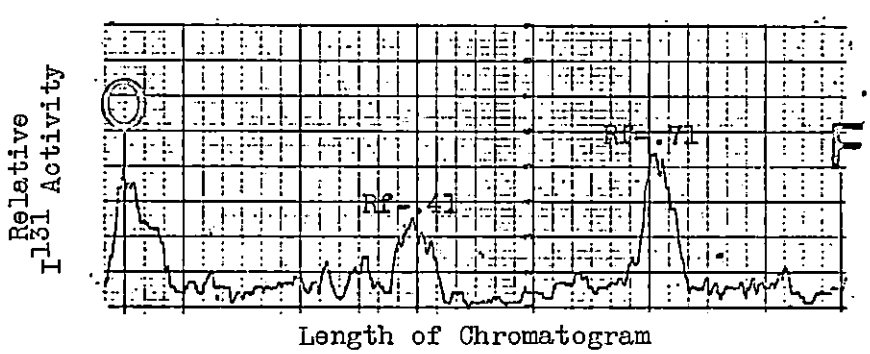
A: 20 hours of I^{131} exposure



B: 24 hours of I^{131} exposure

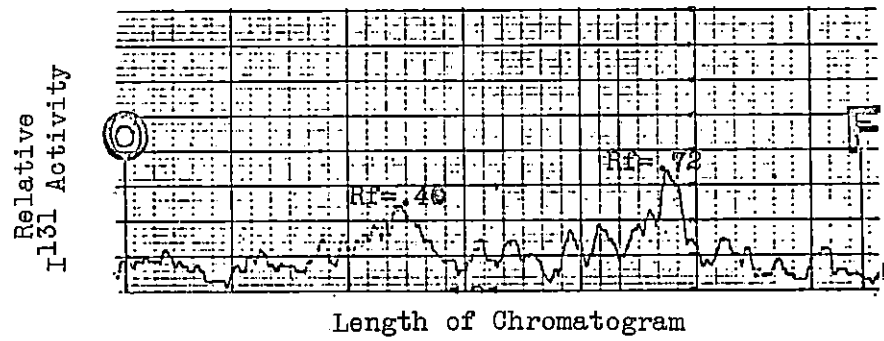


C: 28 hours of I^{131} exposure

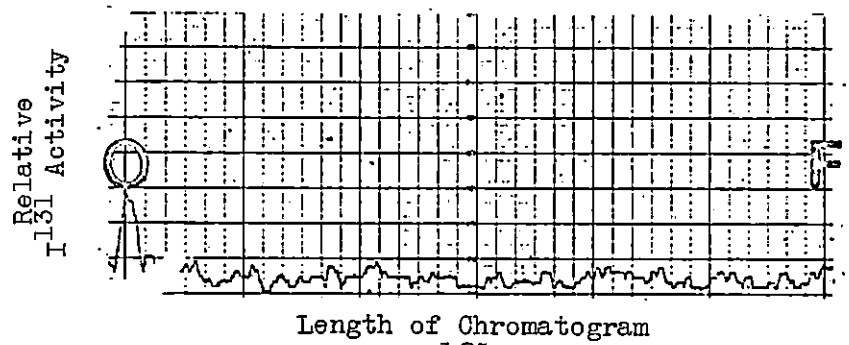


D: 32 hours of I^{131} exposure

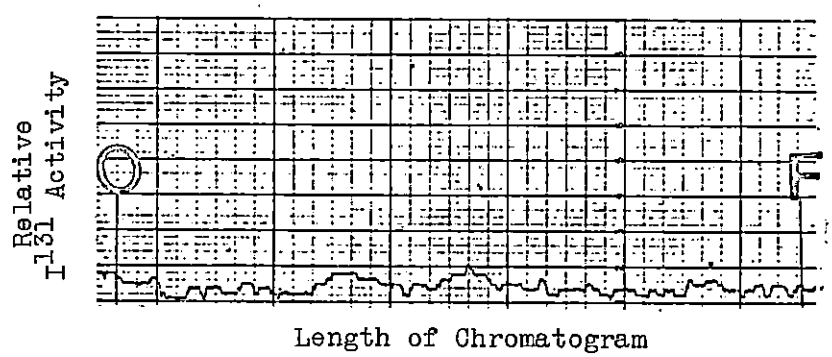
Figure 6 - Radiochromatographic strips indicating tracer peaks present in 20, 24, 28 and 32 hours exposure to I^{131} of A. aurita polyps. (O = origin, F = solvent front)



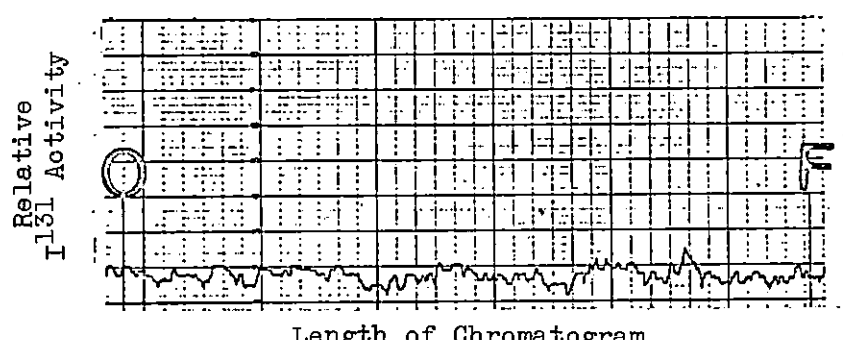
A: 40 hours of I^{131} exposure



B: 48 hours of I^{131} exposure



C: 72 hours of I^{131} exposure



D: 96 hours of I^{131} exposure

Figure 7 - Radiochromatographic strips indicating tracer peaks present in 40, 48, 72 and 96 hours of exposure to I^{131} of A. aurita polyps. (O = origin, F = solvent front)

Rf values of unknown peaks and when they appear after I^{131} exposure are presented in Tables 4 and 5. These tables represent both solvent systems that were utilized.

Table 4 - Occurrence of Tracer Peaks, Expressed as Rf Values of Iodinated Compounds Obtained from Aurelia aurita Polyps after Various Periods of Exposure to I^{131}. Solvent System: Secondary-Butanol, Ammonia (3:1).*

Hours of I^{131} Exposure	Calculated Rf Values of Tracer Peaks .09	.20	.33	.48
4	+	+	+	-
8	+	+	+	-
12	+	-	+	+
16	-	-	+	+
20	-	-	+	+
24	-	-	+	+
28	-	-	+	+
32	-	-	+	+
40	-	-	+	+
48	-	-	-	-
72	-	-	-	-
96	-	-	-	-
120	-	-	-	-

*The symbols + and - represent the respective presence or absence of these peaks.

Rf values of unknown substances appearing after incubation periods of A. aurita with I^{131} correspond to monoiodotyrosine, diiodotyrosine, iodine and tetraiodothyronine (thyroxine). These data, presented in Table 6, are obtained in each of the solvent systems utilized.

Table 5 - Occurrence of Tracer Peaks, Expressed as Rf Values of Iodinated Compounds Obtained from Aurelia aurita Polyps after Various Periods of Exposure to I^{131}. Solvent System: N-Butanol, Ethanol, Ammonia (5:1:2).*

Hours I^{131} Exposure	Calculated Rf Values of Tracer Peaks .13	.33	.40	.72
4	+	+	+	-
8	+	-	+	-
12	+	-	+	+
16	-	-	+	+
20	-	-	+	+
24	-	-	+	+
28	-	-	+	+
32	-	-	+	+
40	-	-	+	+
48	-	-	-	-
72	-	-	-	-
96	-	-	-	-
120	-	-	-	-

*The symbols + and - represent the respective presence and absence of these peaks.

Table 6 - Rf Values for Known Iodinated Compounds in Two Solvent Systems

Iodinated Compounds	Solvent A*	Solvent B+
Diiodotyrosine	.13	.09
Moniodotyrosine	.33	.20
Iodine	.40	.33
Tetraiodothyronine (Thryoxine)	.72	.48

*N-Butanol:Absolute-Ethanol:2N Ammonia (5:1:2)
+Secondary-Butanol:3% Ammonia (3:1)

Total I^{131} uptake by A. aurita polyps after four hours of incubation was small in relation to later time periods (Figure 5-A). Acitivity appears at Rf values of .13, .33 and .40 in the solvent N-Butanol-Ethanol-Ammonia (solvent A), and .09, .20 and .33 in the solvent sec-Butanol-Ammonia (solvent B). Graphs for solvent A have been illustrated and the results from both solvent systems were used in evaluating data. Rf values for monoiodotyrosine (MIT) are .33 and .20 in solvents A and B respectively. Rf values for diiodotyrosine (DIT) are .13 and .09 in solvents A and B. These values coincide with activity peaks present at four hours. The Rf value .40 in solvent A coincides with that of iodine.

Iodine^{131} uptake, after eight hours of exposure, increased, but was still relatively small (Figure 5-B). The overall profile was similar to that of the four hour sample.

At twelve hours of exposure, tracer peaks corresponding to MIT and DIT were greatly diminished (Figure 5-C). A new peak appeared with a Rf of .72 in solvent A, and Rf of .48 in solvent B. These values correspond to values for thyroxine in each solvent. This compound labelled with I^{131} tracer is identified as thyroxine. Another peak which occurred with a Rf of .40 is considered to be free iodine.

Peaks at 16 hours of I^{131} exposure are similar to those peaks at 12 hours (Figure 5-D). Activity was increased with respect to previous samples.

Activity at 20 hours of exposure was also higher than that at 16 hours (Figure 6-A). This increase was noticeable,

especially at the thyroxine peak. Other peaks noted were one corresponding to iodine, and one at the origin. The origin peak is apparently residual material containing I^{131}, that was not transported by the solvent system. The form of I^{131} at the origin remains unknown.

The major portion of I^{131} tracer after 24 hours of exposure appeared as a thyroxine peak (Figure 6-B). From comparisons of time periods that were utilized, 24 hours was determined as the I^{131} incubation period that contained the greatest thyroxine levels. The level begins to diminish during later incubation periods.

Radioactive levels in the polyps were reduced at 28 hours of I^{131} exposure (Figure 6-C). No new iodinated compounds appeared, although two previously defined peaks remained. A gradual reduction continued after 32 hours of I^{131} exposure (Figure 6-D). There was no other recorded change in overall profile of the graphic representation of the chromatographic strip.

Activity levels for peaks corresponding to iodine and thyroxine were greatly diminished at 40 hours of I^{131} exposure (Figure 7-A). This level was the last in which these or any peaks appeared. There were no tracer peaks recorded after 48, 72 and 96 hours of I^{131} exposure (Figures 7-B, 7-C, 7-D).

Thyroxine levels coincide with overall activity values prior to chromatographic separation. Figure 8 shows relative radioactive levels, before chromatographic separation of time samples used.

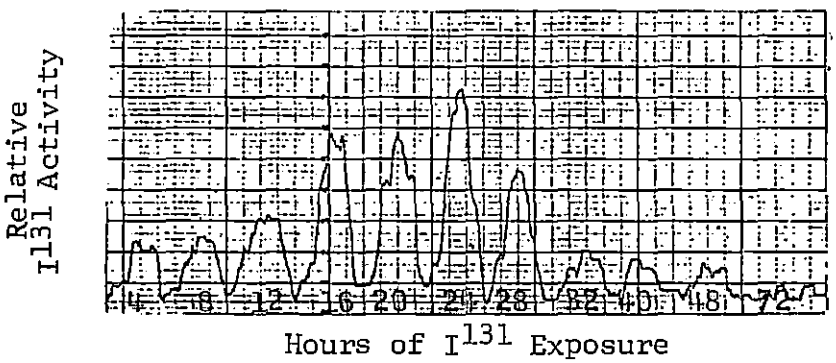


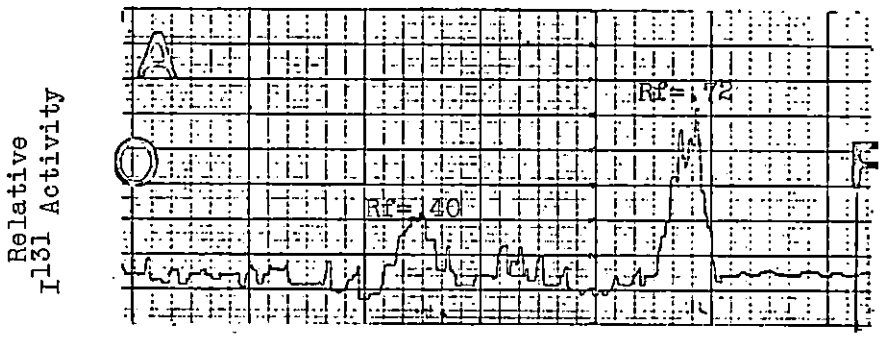
Figure 8 - Total radioactive peaks of A. aurita polyps after various I^{131} exposure times and before chromatographic separation.

When each group of organisms was removed from the tracer-ASW medium and rinsed, 10 extra polyps were also removed. These extra polyps were rinsed and put into fresh ASW media, without an iodine source. These polyps were observed for any morphological changes that indicated strobilation, and observations were recorded. All organisms removed at both four hours and eight hours remained as scyphistomae. All organisms removed at 12 hours and later, strobilated. Several strobilae were present in 96 and 120 hour samples prior to removal. This sequence of strobilation response indicates that whatever factor was derived from the iodine was present between 8 and 12 hours, but not before 8 hours.

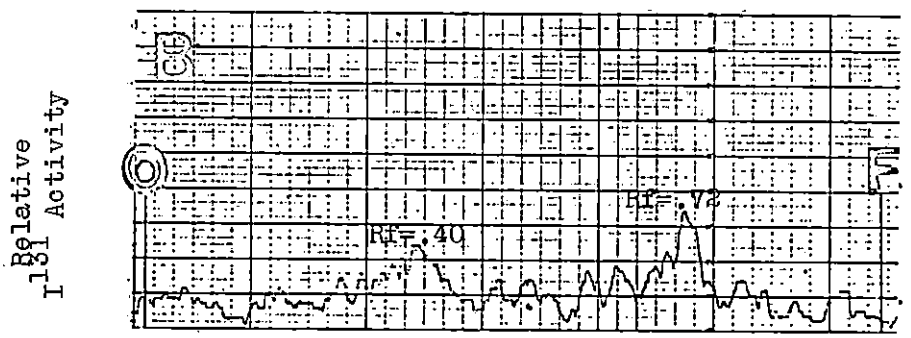
Experimentation involving iodine inhibitors was performed with respect to the same time sequence used in the I^{131} tracer study. Inhibitors and tracer were added to ASW medium, and polyps were exposed to the same time schedule as previously described. Chromatographic samples of organisms incubated with I^{131} and potassium thiocyanate (PTC) displayed a pattern similar to the previously described tracer results.

Iodotyrosines appeared early (4-12 hours) and thyroxine appeared at 12-16 hours and had its highest level at 24 hours. Total amounts were less than the I^{131} test without PTC, described earlier. Chromatographic analysis of thiourea samples differed from the PTC inhibition in that no tracer peaks appeared at any time period.

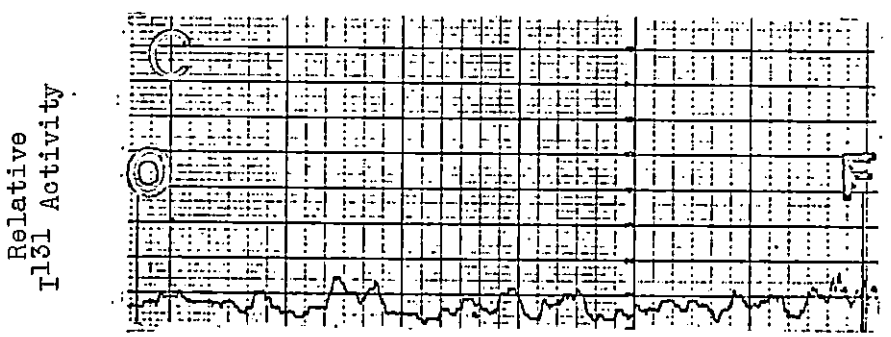
Organisms removed for observations did not strobilate within the 14 day test period, when incubated with either thiourea or potassium thiocyanate. Both substances inhibited initiation of strobilation. Figure 9 illustrates the tracer peak relationship of the two inhibitors at 24 hours of exposure, compared to the 24 hour sample of the previous tracer test. This time level was used for illustration because it represents the period of greatest thyroxine levels, as determined in previous tests.



A: 24 hours of I^{131} exposure



B: 24 hours of I^{131} and potassium thiocyanate exposure



C: 24 hours of I^{131} and thiourea exposure

Figure 9 - Radiochromatographic strips indicating tracer peaks present in 24 hour samples of A. aurita polyps, comparing two inhibitors with a control I^{131} sample. (O = origin, F = solvent front)

Discussion

The life cycle of Aurelia aurita represents a complex system of morphological development. The most unique aspect of this cycle is the strobila. Development of the strobila represents the change of scyphistoma to the alternate generation, medusa. Apparently there exists certain factors that control the occurrence of strobilation. One of these factors, the requirement of iodine, has led to this study which involves elucidation of an iodine dependent mechanism that may be used to explain the morphological changes that occur at strobilation. Other factors, such as light, temperature and nutrition, play a complementary role that may be of more importance in the natural environment, but are less important under these chemically controlled laboratory conditions.

The study of an iodine mechanism first involved the determination of iodine containing molecules present relative to strobilation. These determinations were accomplished experimentally by introduction of the radioisotope I^{131}. The results indicate that iodinated compounds are present immediately prior to strobilae formation. Comparison of chromatographed unknown compounds obtained from polyps to known anticipated compounds yielded identification of the unknown compounds. Three compounds are thus identified: monoiodotyrosine, diiodotyrosine and tetraiodothyronine (thyroxine). These compounds appear in the organism at

distinctive time periods after exposure to the iodine tracer. Iodotyrosines appear within eight hours after exposure (Figure 5-B). Which of the iodotyrosines, MIT or DIT, appear first is unknown. However radiochromatograms of 8 and 12 hour samples exposed to I^{131} show that diiodotyrosine is still present and the monoiodotyrosine level is no longer present. (Figure 5-C). The recorded disappearance of MIT and DIT after 16 hours of incubation is accompanied by the appearance of thyroxine (Figure 5-C). This strongly implies that sequential formation of MIT, DIT and thyroxine are required for the initiation and completion of strobilation. This is illustrated in Table 7.

Table 7 - Proposed Sequence of Iodinated Compounds Present in Aurelia aurita after Various Time Periods of I^{131} Exposure with Respect to Formation of Strobilae*

Hours of I^{131} Exposure	Iodinated Compounds			Formation of Strobilae
	MIT	DIT	T_4	
4	+	+	-	-
8	+	+	-	-
12	-	+	+	-
16	-	-	+	-
20	-	-	+	-
24	-	-	+	-
28	-	-	+	-
32	-	-	+	-
40	-	-	+	-
48	-	-	-	-
72	-	-	-	+
96	-	-	-	+
120	-	-	-	+

*+ represents presence; - represents absence

This sequential formation of iodinated compounds in Aurelia aurita can be compared to the classical model of thyroxine synthesis in vertebrates. The model illustrates that iodides are concentrated by the thyroid gland and then enzymatically added to tyrosine molecules to form first 3 - monoiodotyrosine and then 3, 5 - diiodotyrosine. The enzymatic condensation of two molecules of 3, 5 - diiodotyrosine yields 3, 5, 3′, 5′- tetraiodothyronine and alanine.

The system responsible for this metabolism in Aurelia aurita takes up iodine and iodinates tyrosine molecules within 4 hours. Diiodotyrosine condensation to thyroxine occur within 12 hours, since thyroxine levels can be determined then. The highest level of thyroxine appears at 20 to 24 hours and is apparently initiating a response by the organisms, because the levels diminish soon after 24 hours. Although strobilation is not observed until 72 to 96 hours after exposure to iodine thyroxine formation evidently is needed to initiate the morphological changes and then is no longer required. Results of the mechanism are not visible until thyroxine has disappeared.

The mechanism by which thyroxine initiates the changes in Aurelia aurita is just as unclear as in vertebrate systems. Several actions of thyroxine have been postulated, but none have yet been accepted as definitive. One theory is that thyroxine has some role in enhancing the presence of oxidases in cells, which would expedite oxidative phosphorylation for increased energy supplies. Another theory states that

thyroxine's effect is to increase permeability of the cell or the mitochondrion membrane which then increases availability of reactants. This problem obviously could not be included into this investigation and therefore will not be elaborated further.

The radiochromatograms (Figure 5-A) also show a fourth peak that matches Rf values with the free iodine tracer. This peak must be assumed as iodine, since no iodinated organic compounds match that value. There is no easy explanation for its presence, since all free and inorganic iodides were presumably removed by the preparation procedure of protein-bound material. There is no pattern to this peak, other than appearing in every sample that took up I^{131}. This lack of pattern indicates that the iodine peak is not directly the result of the biological metabolism of the organism. The enzymatic action during incubation or the solvent may have effected the deiodination of the organic compounds. Also a possibility is that all of the extracellular free iodine was not removed by the preparation method. This peak is not considered significant in the effect on strobilation, other than perhaps as a degradation product of the organic compounds.

The lack of further iodinated compounds after the disappearance of thyroxine indicates that thyroxine is not being degraded within the cell by any previously described mechanism. Triiodothyronine and iodotyrosines would be expected by-products of degradation but do not appear in

the protein portion of the organism. This indicates that the molecules may be discharged directly out of the cell. Detection of thyroxine in the media was not feasible due to the dilution factor. This proposed action does not coincide with the enzymatic deiodination, storage and recycling of thyroxine, tyrosines and iodine that occur in the vertebrate thyroid gland. However there is apparently no further use for thyroxine after strobilation is initiated and therefore no mechanism for recycling and conservation of thyroxine is present in Aurelia aurita.

The iodine dependency for strobilation in Aurelia aurita is further strengthened by the effect of the iodine inhibitors. Thiourea and potassium thiocyanate prohibit strobilation when the organism is exposed simultaneously with the I^{131} tracer. Strobilation was observed only in the control organisms which were devoid of inhibitors. Thiourea prohibits the uptake of iodine in the mammalian thyroid gland, therefore no new iodotyrosines are formed. The chromatograms (Figure 9-C) show that no iodocompounds are formed in Aurelia aurita when thiourea is introduced into the I^{131} medium. Therefore the effect of thiourea is similar in both Aurelia aurita and mammalian systems. Potassium thiocyanate inhibits thyroid function of mammals at a different level. This level is considered to be inhibition at some point of the thyroxine effect, rather than the synthesis (Gorbman 1963). Chromatograms (Figure 9-B) show that the same basic iodinated compounds (MIT, DIT, T_4) are synthesized by Aurelia

aurita exposed to potassium thiocyanate as those without the inhibitor. This effect of potassium thiocyanate shows a similar effect in mammals (Gorbman 1963). These effects by both inhibitors therefore indicate there are similar mechanisms for the uptake of iodine and utilization of synthesized iodocompounds in both mammalian tissue and Aurelia aurita. Also the use of these inhibitors strengthen the conclusion that iodocompounds are required for strobilation.

A proposed overall view of iodine metabolism in Aurelia aurita scyphistomae and its effect on the life cycle at strobilation is presented. The organism is exposed to a constant level of iodine in the natural environment, but it strobilates during the coldest temperatures of the year. The laboratory maintained organisms are exposed to a constant temperature (19°C) and kept free from iodine. These organisms then respond to iodine and strobilate. Iodine is made available to the scyphistomae and uptake begins immediately. Iodination of tyrosines to monoiodotyrosine and diiodotyrosine occurs within 4 hours. The level of diiodotyrosine increases to 8 hours after exposure. Thyroxine is formed apparently by condensation reactions of two diiodotyrosine molecules between 8 hours and 12 hours. The twelve hour level is the least exposure time to iodine that will result in strobilation. Withdrawal of polyps after 4 and 8 hours of iodine exposure prohibits strobilation. This inhibition indicates that the presence of high levels of iodine may act as a positive modulator for the synthesis of

thyroxine. Removal from iodine exposure at 12 hours does not prohibit strobilation, indicating that once thyroxine is formed the high levels of iodine are not required. This is possibly due to the fact that thyroxine has already begun to exhibit its effect on scyphistomae after 12 hours of exposure. The level of thyroxine increases from 12 hours until 24 hours after exposure to iodine. This 12 hour period is considered the critical time within which thyroxine manifests its effect on the life cycle of Aurelia aurita. The levels of thyroxine decline after 24 hours and is completely absent by 48 hours after exposure. There are no metabolites that appear, which indicates that the molecule is being discharged directly into the environment. The visual signs of strobilation do not appear until 72 to 96 hours after iodine exposure. These visual signs are gross changes and do not include microscopic morphological changes which are probably occurring previous to the 72 hour level.

The proposed mechanism is presumed to occur in nature, since such a direct relationship between thyroxine synthesis after introduction of iodine and strobilation has been shown experimentally. As previously stated other factors may provide the stimulus for the initiation of this mechanism in nature. Temperature seems to be such a controlling factor. Therefore this work is not meant to be comprehensive with respect to defining the natural controls of the life cycle, but instead presents a model of sequential synthesis of iodinated compounds related to strobilation. Temperature

relation to thyroid function in vertebrates has no defined pattern because the thyroid reacts to higher temperatures in some animals in the same way the gland responds to lower temperatures in other animals (Turner 1971). There may exist a yet undefined relationship between temperature and thyroxine synthesis in Aurelia aurita.

Further investigation concerning this problem would involve defining the relationship of the iodine metabolism to temperature and other factors that influence the organism in its natural environment. Identification of iodocompounds by spectrophotometric analysis would add to greater acceptance of this theory of iodine metabolism. Radioautographic analysis of cell types to determine the existence of specialized cells capable of iodocompound synthesis would also be desirable. Finally, understanding the mechanism for thyroxine action perhaps could be assisted by studies of the hormone at this relatively simple biological level. Use of thyroxine in the metabolism of Aurelia aurita is presumably limited to the initiation of strobilation, therefore fewer factors may be involved than with the vertebrate thyroxine metabolism. The author hopes such advances can be made with respect to this investigation.

Summary

1) Strobilation of Aurelia aurita scyphistomae can be induced by introduction of iodine, monoiodotyrosine, diiodotyrosine and thyroxine to organisms that have been pre-conditioned to absence of iodides.
2) Aurelia aurita scyphistomae take up iodine and synthesize three iodinated compounds with Rf values corresponding to monoiodotyrosine, diiodotyrosine and thyroxine.
3) The appearance of monoiodotyrosine, diiodotyrosine and thyroxine prior to strobilation suggests a sequential synthesis similar to a model proposed for thyroid hormone synthesis in vertebrates.
4) Strobilation is dependent on production of an iodinated compound with an Rf value that corresponds to thyroxine. Exposure to I^{131} for 12 hours is necessary for strobilation. The first detection of thyroxine synthesis is also at 12 hours.
5) Production of these three iodinated compounds is inhibited by thiourea, which results in inhibition of strobilation.
6) The action of these three iodinated compounds is inhibited by potassium thiocyanate, also resulting in strobilation inhibition.

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