A Comparative Study of Isozyme Patterns in Gampanularia Flexuosa and Cordylophora Lacustris

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A COMPARATIVE STUDY OF ISOZYME PATTERNS IN
CAMPANULARIA FLENUOSA AND CORDYLOPHORA LACUSTRIS

CAROL JEAN STANCHER

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE
SCHOOL OF LOYOLA UNIVERSITY IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

1973
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INTRODUCTION

In man and many other organisms, the powers of self-adjustment and self-maintenance decline with the passage of time, while the probabilities of disease and death increase (Comfort, 1970).

Studies concerning the biology of senescence seek the molecular basis of the phenomenon and its control mechanism - the so called "death messenger" that triggers the life long process of aging.

Lysosomes are the cytoplasmic particles which were first described by De Duve (1959). They are membrane-bound bodies which function as storage vesicles for many powerful (hydrolytic) enzymes (Novikoff and Holtzman, 1970). Since lysosomes appeared to be related to the age pigment inclusion which had a bearing on gradual parenchymal dysfunction and because the processes of cell death, resorption, and atrophy involved the malfunction of systems controlling lysosomal activity, Strehler (1961) emphasized the importance of basic studies of the lysosomes. Allison (1966) also suggested that the escape of hydrolytic enzymes from lysosomes seemed to be related to the process of aging.

Cnidarians have proven to be useful tools in studying biological basis of senescence. They have furnished simplified, parallel models of systems observed within more highly evolved metazoa such as man (Strehler, 1961 b). The Cnidarians chosen for this study were Campanularia flexuosa and Cordylophora lacustris. Both organisms are colonial Hydrozoans. In Campanularia, individual hydromedus progress through embryonic development, adult life, and regression in seven to eight days (Crowell, 1953). The
onset of regression is quite sudden; the dissolution, death, and replacement of the individual hydranths cataclysmic (Brock, 1968). In Cordylophora, however, although the cells of the hydranths age and are replaced, the individual hydranths survive indefinitely and do not demonstrate senescence (Fulton, 1962).

Thus the purpose of this investigation is to compare the isozyme patterns of Campanularia flexuosa, a "mortal" Cnidarian, and Cordylophora lacustris, an "immortal" Cnidarian, by means of polyacrylamide gel disc electrophoresis in search of further insight into the molecular dynamics of the biological phenomenon of senescence.
REVIEW OF THE LITERATURE

Many theories have been suggested as possible explanations of the molecular basis of aging. Prominent theoretical suggestions include:

1) a relationship between metabolic rate and life span (Pearl, 1928);
2) the wear and tear caused by stress (Selye, 1960);
3) the cross-linkage of collagen (Verzar, 1963);
4) the accumulation of pigment in fixed cells (Bjorkerud, 1964; Strehler, 1964);
5) the escape of enzymes from lysosomes (Allison, 1966);
6) the spontaneous mutations of an organism's somatic cells (Curtis, 1966);
7) the production of molecular cross-linkage caused by free radicals (Harmon, 1966);
8) the monotonically increasing cross-linkage of proteins and nucleic acids (Bjorksten, 1968);
9) the synthesis of complex molecules at a more rapid rate than they can be removed (Carpenter, 1969);
10) the release of "death" hormones which inhibit cells from utilizing other hormones (Denckla, 1972).

The most recent reviews of aging theories are those of Comfort (1970), Frolkis (1971) and Goldstein (1971). They are characterized by an attempt to synthesize the various aspects of aging phenomenon into an integrated whole.

The purpose of this research was to investigate the suggestion that enzymes, including hydrolytic enzymes, play a role in the mechanism of aging.

The pioneering work of Hunter and Markert (1957) and Vesell and Bearn (1957), and Markert and Moller (1959) demonstrated that enzymes previously considered "pure" were actually composed of isozymes - multiple molecular
forms. The considerable differences in isozymes from tissues of an organism and the fluctuations of levels of isozymes during the life style are important strengths inherent in the isozyme approach to senescence (Brewer, 1970).

Tiselius (1937) first developed the moving boundary method of electrophoresis. Acrylamide gel electrophoresis was introduced by Raymond and Weintraub (1959), and Ornstein and Davis (1959).

The organisms that were chosen for this study were two colonial hydroids.

The earliest study of *Campanularia flexuosa* with a light microscope was reported by Thacher (1903). He described general organization and cells. Further cellular details were revealed by Lunger (1963). Recent studies (Brock and Strehler, 1968; Brock, 1970) have concentrated on the elucidation of the ultrastructure of various age levels, using electron microscopy.

The study of *Cordylophora lacustris* was begun by Allman (1953). Work on the reorganization of *Cordylophora* tissue masses was done by Beadle (1937). Fulton perfected culture techniques (1961) and studied environmental factors influencing the growth of the hydranths (1962, 1963). The most recent work on *Cordylophora lacustris* was done by mace and mackie (1971).
It dealt with Cordylophora's ecological role in estuarian waters.

The lysosomal enzyme investigated was acid phosphatase. The discovery, physiology, and morphology of lysosomes and their hydrolytic enzymes were described by De Duve (1959, 1963a, 1963b, 1964, 1965, 1970, 1971) and Novikoff (1961, 1963).

The work of Folley and Kay (1939) indicated that there were three types of acid phosphatases which could be differentiated by their optimum pH, their sensitivities to Mg$^{2+}$ ions, and their relative activities towards alpha and beta glycerophosphates.

More recent studies continue to support the idea that a number of separate acid phosphatases exist in single situations. Differences in the rate of acid phosphatases exist in single situations. Differences in the rate of acid phosphatase activity in *Tetrahymena pyriformis* towards several substrates (Connor and MacDonald, 1964) have been attributed to multiple enzyme forms.

A second enzyme investigated was cytochrome oxidase. Enrlich (1885) discovered the indophenol reaction. He observed that upon injecting alpha naphthol and dimethy-p-phenylenediamine into animals it formed in their tissues a blue substance, indophenol blue. This is called "nadi reaction" from the first two letters of naphthol and diamine. In 1895 Rohrmann and Spitzer showed that the indophenol reaction is given both by plants and animals. The enzyme concerned was called indophenol oxidase. Recent evidence indicated that indophenol oxidizes only cytochrome c,
which in turn oxidizes the nadi reagent. Hence the enzyme was renamed cytochrome oxidase.

The third enzyme studied was lactic dehydrogenase (LDH). This enzyme was chosen because it had been reported (Strehler, 1964) that there was an increase of LDH with age.

The heterogeneity of LDH was first recognized when Neilands (1952) demonstrated activity in each of the two electrophoretically distinct proteins. Weiland and Pfleiderer (1957) found that most organs contain up to five fractions each exhibiting LDH activity. Though the LDH isozyme pattern may vary considerably from species to species, it remains remarkably constant, in the absence of disease, between different individuals of the same species (Wilkinson, 1965).

The fourth enzyme studied was malate dehydrogenase (MDH). Vesell and Bearn (1957) used potato starch grains as an anticovalent and supporting medium for the zone electrophoresis of isozymes of human serum MDH. Unidimensional acrylamide gel electrophoresis was applied to the separation of MDH by Goldberg (1963).

The final enzyme investigated was catalase. Loew (1961) showed that the decomposition of hydrogen peroxide by plants and animals was caused by a specific enzyme. He named this enzyme catalase. Various studies of this enzyme have been undertaken since then. Unidimensional acrylamide gel electrophoresis was applied to the separation of catalase by Baumgarten (1963).
MATERIALS AND METHODS

Test Organisms

Cultures of *Campanularia flexuosa* and *Cordylophora lacustris* were obtained from the Woods Hole Biological Station, Woods Hole Mass. The species were identified following the keys and descriptions of Fraser (1944).

Culture Techniques

*Campanularia* were cultured following the technique of Crowell (1953). They were maintained in a twenty-five gallon Instant Ocean Culture Systems, model CS-25. The aquariums were thirteen inches deep by twenty-nine inches wide by fourteen inches high and were equipped with a refrigeration unit.

Instant Ocean synthetic sea salt was used. The medium contained:

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<tr>
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<td>Ca</td>
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<tr>
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<td>140</td>
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<td>20</td>
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<td>Sr</td>
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The specific gravity was 1.025. This synthetic sea water was checked daily to ensure that the temperature was 20°C; the pH was 7.9; and the salinity was 33.31/1000. Also, the medium was analyzed every third day as to levels of ammonia, nitrogen, dissolved oxygen, carbon dioxide, nitrite, sulfide, and iron. These tests were carried out by employing LaMotte colorimetric test kits.

The *Campanularia* were attached to microscope slides by means of clear nylon thread. Five such slides were kept in each slide box.
Twenty-four slide boxes were maintained in each aquarium. *Campanularia* cultures were subcultured on the first and third Mondays of the month.

*Cordylophora* were cultured following the technique of Fulton (1962). They were maintained in a constant temperature incubator, outfitted with a Sargent Welsh model 505 incubator control system. The temperature was maintained at 20°C.

The medium employed was brachish water (CCS-5). It was prepared in five gallon quantities. Per five gallons, it contained:

- 1.2 g Versene
- 58.4 g NaCl
- 2.0 g KHCO₃
- 15.8 g CaCl₂
- 20.4 g MgCl₂

Versene was always added first and the other chemicals were added in the order shown. The medium was checked daily to ensure the temperature was 20°C and the pH was 6.8.

The *Cordylophora* were attached to microscope slides by means of clear nylon thread, three quarters of an inch from the bottom edge of the slide. Two slides were placed back to back in a 100 ml beaker, the slide standing on its long axis. Each beaker contained 75 ml of CCS-5 medium.

**Feeding Procedure**

Both *Campanularia* and *Cordylophora* were fed daily with newly hatched brine shrimp, six to eight hours old.

Dried brine shrimp eggs were obtained from Sternco Industries, Harrison, N.J. One quarter teaspoon of eggs was added to a gallon plastic jug con-
aining three quarters of a gallon of tap water and six tablespoons of sodium chloride (technical grade). The temperature of the water was maintained at $34^\circ$C through use of a goose-necked lamp equipped with hundred watt bulb. Two gallon jugs were placed in each holding rack and were aerated by silent giant aquarium pumps. The eggs hatched in approximately forty-eight hours. The gallon jugs were emptied into holding pans. A lamp was placed at one end of the pan and the hatched shrimp would gather near the light source.

The shrimp were collected and strained from the medium by use of a suitably meshed net and a funnel. They were rinsed with tap water. Half of the shrimp were then placed in a 100 ml beaker containing 800 ml synthetic sea water. The slide boxes which contained the Campanularia were taken from the aquarium and placed in the beaker for five minutes. The slide boxes were then rinsed in a second beaker of clean synthetic sea water and returned to the aquarium. The other half of the shrimp were rinsed in tap water and placed in CCS-5 medium. Some of this shrimp mixture was added to each beaker which contained Cordylophora. After the Cordylophora had fed, each slide was rinsed clean in CCS-5 and placed in a fresh beaker of CCS-5.

**Protein Concentration Determination**

In order to validate a comparison of general proteins and specific isozymes, equivalency of Campanularia and Cordylophora samples had to be ensured. An adaptation of Lowery's procedure for the determination of
protein concentration was employed (Bradshaw, 1966). This method worked well with the size and dilutedness of the samples studied. It was not however, a precise quantitative measure. Rather it provided a means by which the samples of the two organisms could be compared to a known standard. It was learned that each Cordylophora hydranth contained as much protein as 2.76 Campanularia hydranths (Graph I). Based on these findings, all electrophoretic runs were set up using seven hundred fifty Campanularia hydranths per 0.5 ml of extraction medium and two hundred fifty Cordylophora hydranths per 0.5 ml of extraction medium. Both the number of hydranths and the wet weight of each sample were recorded for each run to ensure standardization.

Collection Procedure

Samples of Campanularia and Cordylophora were collected no sooner than twenty hours after their last feeding of brine shrimp. Additionally, an electrophoretic run was made on a sample of brine shrimp to determine their banding pattern and this was used as a check that shrimp bands were not confounding the results.

Analysis of the two organisms was carried out on hydranths only. the hydranths were cut from the stalks one eighth inch from the base of the hydranth as this allowed greater uniformity of sample.

Extraction Procedure

Extractions were accomplished using tissue grinders. Each sample
of tissue was ground for ten minutes, eighty strokes per minute.

Three extraction media (distilled water, 0.05% Triton X detergent, and Tris buffer, pH 8.2) were tested with each specific enzyme study. A second consideration was that of pH. The pH of the buffer had to coincide with the optimum pH for the extraction of the enzyme. As enzymes are heat labile, the factor of temperature had to be dealt with. Tissue extraction was carried out in an ice bath and the gel tubes were run at 0 to 5°C to minimize denaturation and destruction of the enzymes by heat.

**Disc Electrophoresis**

In attempting to select an electrophoretic medium both starch gel and polyacrylamide gel were considered as both of these media are high resolution electrophoretic media.

As the concentration of polyacrylamide is varied between 5% and 30%, the pore size of the gel also varies. This property allowed the construction of the gels with a varying sieving effect. While it was also possible to obtain this effect with starch gel, the total range of variation in pore size was less than that with acrylamide (Brewer, 1970). Acrylamide gel was selected because it was not as fragile as starch gel, was easier to work with and handle, had relatively better keeping qualities, and could be used for densitometric analysis (Brewer, 1970).

In each electrophoretic run, twelve tubes were used. Five tubes (tubes one to five) contained *Campanularia* sample. The next five tubes (tubes six to ten) contained *Cordylophora* sample. The remaining tubes
(tubes eleven and twelve) contained samples of human serum. These last tubes served as controls in each run. Results of the serum controls verified that in any particular run, the equipment functioned normally and the solutions and procedures were correct. Samples of *Campanularia* and *Cordylophora* were processed together in each run to minimize variation in results because of flukes such as temperature fluctuations, variations in running times, or discrepancies in gel prep.

Canalco's research disc standard reagent kit (RDS) and the quick disc kit for the analysis of very dilute proteins (QDD) were used. The gels and tubes were prepared according to the procedures detailed in each kit's instructions. The directions were followed exactly with the exception of the following modifications. The catalyst of the separating gel was prepared every second day. The sucrose solution was prepared every fourth day. The buffer was prepared daily and was never reused. The gel components were not brought up to room temperature before they were combined in order to retard decomposition.

Ten microliters of hydranth sample was run in each experimental tube and three microliters of serum was run in each control tube when the RDS kit was used. When the QDD kit was used, one ml of hydranth sample was run in each experimental tube and 0.25 microliters of serum was run in each control tube.

The electrophoretic run procedure followed the specific kit directions. The instructions recommended the tubes be run at three to five milliamps per
tube. All samples were therefore run at 4 ma per tube. The run was terminated when the tracking dye was one half inch from the posterior end of the tube.

**General Protein Analysis**

This comparative study of *Campanularia* and *Cordylophora* was begun with a general protein comparison. The purpose of these runs was to determine if there were species differences in the presence of proteins—the number of protein bands, the size of the bands, and the intensity of the bands.

For the analysis of general proteins the samples were extracted in 0.5 ml Tris buffer, the buffer used in the electrophoretic run. Electrophoresis was carried out in usual manner. The gels were stained with Aniline Blue Black as per the directions included in the Canalco RDS kit. Destaining was accomplished electrophoretically according to the RDS instructions. The process was terminated when the sections of the gels containing no separated fractions were clear of stain (approximately thirty minutes).

For purposes of identification each band was labeled (Plate 1). The numerical values were determined by dividing the distance each protein band traveled through the separating gel (measurement taken from the center of the band to the anterior tip of the separating gel) by the distance the tracking dye traveled.
Isozyme Analysis

Acid Phosphatase

In order to study acid phosphatase isozyme patterns, samples of Campanularia and Cordylophora were collected as they were for protein runs. They were extracted in 0.5 ml of 0.05% Triton X detergent solution. It not only increased the rupture of the lysosomal membrane, but it was a medium in which the isozymes were soluable (Pearse, 1968). Electrophoresis was carried out in the usual manner. The gels were cut longitudinally using a dressmaker’s pattern wheel. They were then stained for thirty minutes.

The stain was prepared using Sigma kit 104 - Histochemical Demonstration of Acid Phosphatase. The procedure was completely detailed in the kit instructions. Results showed that prior to staining, it was not necessary to "fix" the gels as per the Sigma directions.

For a colorimetric study, samples of Campanularia and Cordylophora were collected and extracted as described above. Using Sigma kit 108 - Colorimetric Demonstration of Acid Phosphatase, they were tested for the presence and concentration of acid phosphatase using a Bausch and Lomb Spectronic 20. The procedure for running the test was detailed in the kit directions.

Cytochrome Oxidase

Cytochrome oxidase tests were run on the usual sample sizes.
Extraction was carried out in 0.5 ml of distilled water. Electrophoresis was carried out in the usual manner. The gels were cut longitudinally, and stained for one hour using the staining technique of Brewer (1967). The staining mixture was made up just prior to its use.

Lactic Dehydrogenase

In the electrophoretic study of LDH isozymes, samples of the two organisms were collected as previously described. They were extracted in one half ml of distilled water. The gels were cut longitudinally and stained for three hours using Wilkinson's stain procedure (Wilkinson, 1965). The staining mixture was made shortly before it was used as some of the reagents were not very stable.

Malate dehydrogenase

Malate dehydrogenase tests were run on the usual sample sizes. Extraction was carried out in 0.5 ml of distilled water. A modification in the usual preparation procedure for the catalyst of the separating gel was adopted (Brewer and Sing, 1969). In addition to 0.14 gm of ammonium persulfate being added to 100 ml of distilled water, DPN (0.00015M) was also added to the solution. Electrophoresis was carried out in the usual manner. The gels were cut longitudinally and stained for three hours using the staining technique of Brewer (Brewer and Sing, 1969). After the gels were stained, they were placed in 50% ethanol for twenty-four hours. The ethanol was then replaced by distilled water for storage.
Catalase

Catalase tests were run on the usual sample sizes. Extraction was carried out in 0.5 ml of 0.05% Triton X detergent. A variation in the preparation of the separating gel catalyst was employed as suggested by Thorup (et al., 1961). Three grams of starch were added along with the 0.14 gm of ammonium persulfate to one hundred ml of distilled water. Electrophoresis was carried out in the usual manner. The gels were cut longitudinally and stained according to the method of Thorup (et al., 1961).

Photographic Procedure

Gels were photographed immediately after staining to minimize diffusion of the bands. Pictures were taken with the Canalco Phoret-PHOTO unit, outfitted with a polaroid Land Camera model 450 and a model 563 close up kit. No flash was used and the lighting selector was set at the position closest to the "light" position on the dial.
RESULTS

Staining of electrophoretic gels with Aniline Blue Black demonstrated that in *Campanularia* and *Cordylophora*, general proteins banded in patterns which were species specific (Plate 1). The consistency and uniqueness of the protein banding patterns indicated that such electrophoretic "fingerprints" could be used as a method of species identification.

The *Campanularia* sample produced ten discrete bands. These bands, while they were quite consistent, were not very dark - indicating the presence of very dilute proteins.

The sample of *Cordylophora* produced sixteen very discrete and distinct bands. These bands were consistent and were darker than those of the *Campanularia* sample - indicating the presence of more concentrated proteins.

Acid phosphatase proved to be one of two enzymes for which the two organisms showed the greatest discrepancy. Acid phosphatase isozyme levels in *Campanularia* (the mortal form) greatly exceeded those of *Cordylophora* (the "immortal" form).

Colorimetric analysis of acid phosphatase concentration showed that *Campanularia* contained slightly more than twice the level of acid phosphatase demonstrated by *Cordylophora* (Graph II). These findings were supported by subsequent electrophoretic analysis (Plate 2).

*Campanularia* produced four isozyme bands. These bands were distinct and indicative of the concentration of the enzyme that was present. More
than half of the acid phosphatase present in the sample was included in the band .87 complex which was the most rapidly migrating band.

The _Cordylophora_ sample produced four distinct isozyme bands. All of these bands ( .04, .17, .31, and .47) migrated slowly. None of them reached even half of the distance traveled by the tracking dye.

Cytochrome oxidase was the enzyme for which the largest variance between the two organisms was found. Cytochrome oxidase isozyme levels were substantially lower in the aging system (_Campanularia_) than in the non-aging system (_Cordylophora_).

Electrophoretic analysis of cytochrome oxidase isozymes (Plate 3) revealed that _Campanularia_ produced only one isozyme band, possessing one third the concentration of cytochrome oxidase isozymes contained in _Cordylophora_'s three bands.

Electrophoretic studies of LDH isozyme patterns showed that _Campanularia_ possessed four LDH isozymes while _Cordylophora_ had three (Plate 4). What was most interesting was the fact that three of the four isozymes displayed by _Campanularia_ were the same three isozymes which were demonstrated by _Cordylophora_. Also not only was there a nineteen per cent increase in LDH levels in _Campanularia_ as compared to _Cordylophora_ but the bands were darker.

The results of electrophoretic analysis of patterns of MDH isozymes followed the trend set by the LDH isozymes. _Campanularia_ demonstrated a sixteen per cent increase in levels as compared to _Cordylophora_ (Plate 5).
Not only were the *Campanularia* bands more numerous, they were also darker and better defined than those of *Cordylophora*.

Electrophoretic studies of catalase isozymes in *Campanularia* and *Cordylophora* were inconclusive. Attempts to adapt staining procedures for catalase isozymes from starch gel to polyacrylamide gel were not successful. Work had progressed to the point where banding for both organisms occurred. However, the banding was extremely variable and inconsistent not only between the two organisms, but between samples of the same organism in any given run. Thus the data was inconclusive. Further work on adapting the staining procedure is needed before any meaningful comparison could be made or any conclusions drawn regarding catalase isozyme levels in *Campanularia* and *Cordylophora*. 
DISCUSSION

Several strengths are inherent in an isozyme approach to the study of the biology of senescence. For example, studies can be carried out on crude extracts. Purification of the enzyme is not required (Brewer, 1970). Also, the technique enables one to visualize enzymes directly. This allows for the detection of multiple gene products catalyzing the same reaction (Brewer, 1970). Then too, the commercial availability of high quality substrates lends many of the isozyme methods considerable specificity (Maurer, 1971). A large number of such specific reactions are catalyzed by multiple forms of enzymes and an understanding of the mechanisms by which this great isozymic diversity is brought about and of the functions it subserves is important to the study of senescence.

Using the standard Canalco electrophoresis gel kit (RDS) results on Campanularia were negligible. Canalco's kit for analysis of very dilute proteins (QDD) was then employed. Campanularia bands became clearer and somewhat more pronounced. Yet the results obtained from such Campanularia samples were not as clear or well resolved as those of Cordylophora samples.

The QDD kit differed from the standard electrophoretic kit. It did not require stacking or loading gel. Thus a larger volume of sample was able to be run per tube. A medium support system (replacing the loading gel) gave support to the sample but took up negligible space, also allowing more sample to be run per tube. Finally, the QDD kit utilized tubes which were one half inch longer than those employed in the standard kit and this again provided an opportunity for more sample per tube.
It seems likely that the gel pore size had been reduced in order to concentrate the dilute protein. However, the QDD process is under patent and additional information as to gel and buffer ingredients or pore size are at present unavailable. When such information becomes available, additional manipulation could further improve the results obtained using Campanularia samples.

It must be remembered that Campanularia are marine hydroids. Even though samples are rinsed in distilled water before extraction, the normal ionic and electrolyte environment of Campanularia will subtly effect the results of the electrophoretic run.

Acid phosphatase was chosen for study because it is considered one of the most important lysosomal enzymes (Pearse, 1968). It was often used as a marker of lysosomal activity (Braudhuin, 1969).

The colorimetric testing indicated that Campanularia possessed almost twice as much acid phosphatase as Cordylophora (Graph II). These results were supported by further evidence from a very simple experiment. Entire colonies of untreated Campanularia and Cordylophora were placed in the staining mixture at the same time as the gels. The Campanularia culture turned (in approximately ten minutes) very deep red, becoming almost black. The Cordylophora after the half hour elapsed, had turned pink but showed nothing approximating the reaction of the Campanularia colony.

The electrophoretic patterns of acid phosphatase isozymes in
Campanularia and Cordylophora showed species differentiation (Plate 2). Most important however, is the fact that there was a one hundred per cent increase in the level of acid phosphatase in the mortal form this would have seemed to show that a correlation might have existed. The exceptionally high concentration of acid phosphatase in the Campanularia indicates a high probability of correlation and surely warrants more intense study of the levels of acid phosphatase throughout the life cycle.

The increase in the acid phosphatase level of Campanularia agrees with results obtained by Erlanger and Gershon (1970). In studying isozymes of Tubatrix acetil, they found an increase in acid phosphatase activity in older populations.

Future research concerning the role of lysosomes in aging will have to employ techniques which can differentiate between membrane bound, latent acid hydrolases and hydrolases which have been released from the lysosome and are active in the cytoplasm. This indeed will prove to be a most challenging undertaking but its consequences will be far reaching in senescence research.

Cytochrome oxidase was chosen for study because it was often used as a marker for mitochondrial activity (Baudhuin, 1969).

Previous work with hydroids and poly-phenol oxidases indicated that poly-phenol oxidases increased with age. Results of this investigation of cytochrome oxidase produced opposite results.

Campanularia populations demonstrated a single, rather diffuse band,
*Gordylophora* populations showed three sharp bands. There is a pronounced species differentiation. The two organisms do not share a common polyphenol oxidase isozyme. The great decrease in concentration of this isozyme in *Campanularia*, the mortal form, was unexpected. The size of the variation indicates a promising new avenue for further studies. The reason for such a decrease in cytochrome oxidase among aging systems will no doubt shed light on the total phenomenon of aging.

Lactic dehydrogenase was chosen for study because it has been reported that LDH increases with age (Strehler, 1961).

When gels of each of the two organisms were compared it became evident that they shared common LDH isozymes. The two fastest moving isozymes (0.77 and 0.88) and the slowest (0.23) were present in both species. However, only *Campanularia* possessed the intermediate band (0.65).

Malate dehydrogenase was chosen for study because it had been reported by some investigators to increase with age (Fairbairn) and to decrease with age by other workers (Erlange and Gershon, 1970).

Results of this investigation showed that MDH isozymes increased sixteen per cent in the aging system. Further investigation is required in order to determine if there is a definite pattern between MDH levels and age or if MDH levels follow no definite pattern and are a species specific factor.

Catalase was chosen for study as it was often used as a marker for peroxisomal activity.
There seem to be two factors to explain the erratic results obtained from the catalase isozymes. Peroxisomes have been shown to have a lower sensitivity as compared with lysosomes to detergents and to repeated freezing and thawing (Baudhuin, 1969). Extraction procedures that worked well with other isozymes failed with catalase. Attempts at modification and variation of the basic extraction method were not successful. Secondly, the starch that was added to the separating gel (in order to facilitate the staining procedure) did not meld well with polyacrylamide. The starch seemed to diffuse to random locations on the gel surface and confounded the results.

Further isozyme work is necessary. Adaptations for catalase and lysosomal enzymes such as arylsulfatase from starch to acrylamide gel electrophoresis is required before a complete investigation can be undertaken.

Present work is now under way to use the gels which have provided the data for this project with a densitometer to detect the subtle species differences between the two organisms.

Future work indicated is that of doing isozyme studies on *Campanularia* samples of specific age groups to determine fluctuation of various isozymes during the life cycle. Perfection and adaptation of micro-electrophoresis procedures that would require only one hydranth per gel tube would greatly refine the data and help further the synthesis of knowledge that studies of senescence seek.
SUMMARY

1.) The results of the protein - nitrogen tests showed that each Cordylophora hydranth contained as much protein as 2.76 Campanularia hydranths (Graph I).

2.) General protein staining demonstrated that the protein banding patterns of these two colonial Hydrozoans were species specific (Plate I).

3.) There was a 100% increase in acid phosphatase levels in the model aging system, Campanularia, as compared to Cordylophora, the model non-aging system (Graph III).

4.) There was a 66% decrease in cytochrome oxidase levels in Campanularia as compared to Cordylophora (Graph III).

5.) There was a 19% increase in LDH levels in Campanularia as compared to Cordylophora. This data agreed with results obtained by earlier investigators (Graph III).

6.) There was a 16% increase in MDH levels in Campanularia as compared with Cordylophora. This data also agreed with that obtained by previous workers (Graph III).


Brock, M.A. and B.L. Strehler. 1968. Ultrastructural studies on the life
cycle of short lived Metazoan, *Campanularia flexuosa*. J. Ultra-
structural Res. 21: 281 - 312.


1965. The separation and characterization of sub-cellu-
lar particles. Harvey Lectures Ser. 59: 49 - 68.

1970. Association of lactoferrin with specific gran-


Fraser, C. M. 1944. Hydroids of the Atlantic Coast of North America. Toronto.


Pearl, R. 1928. The Rate of Living. A.A. Knoff, New York.


Sample size - 100 hydranths

Graph 1

mg protein per ml sample

Absorbance at 400 mu

Standard curve

X Cordylophora

X Campanularia

Protein concentration
Graph II

Optical density at 400 m

1.6

1.4

1.2

1.0

0.8

0.6

0.4

0.2

0.5 1.5 2.5 3.5 4.5 5.5

Acid phosphatase Sigma units per ml

\( \times \) Campanularia

\( \times \) Cordylophora
Graph III

- **Campanularia flexuosa**
- **Cordylophora lacustris**

Y-axis: Protein contained in isozyme bands

X-axis: Enzymes

- Acid Phosphatase
- Cytochrome Oxidase
- Lactic Dehydrogenase
- Malate Dehydrogenase
General proteins

Campanularia

Cordylophora
Acid phosphatase

Campanularia

Cordylophora
Cytochrome oxidase

Campanularia

Cordylophora
Lactic dehydrogenase

Plate 4

Campanularia

Cordylophora
Malate dehydrogenase

Plate 5

Campanularia

Cordyllophora
The thesis/dissertation submitted by Carol Jean Stancher has been read and approved by members of the Department of Biology.

The final copies have been examined by the director of the thesis/dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis/dissertation is now given final approval with reference to content and form.

The thesis/dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

May 18, 1973

DATE

Edward E. Palenese

ADVISOR'S SIGNATURE