



W&M ScholarWorks

[Dissertations, Theses, and Masters Projects](#)

[Theses, Dissertations, & Master Projects](#)

1975

Changes in Amount and Composition of Gelatin from Development Stages of the Scyphozoan Jellyfish, *Chrysaora quinquecirrha*

Janet Murphy Quensen
College of William & Mary - Arts & Sciences

Follow this and additional works at: <https://scholarworks.wm.edu/etd>

 Part of the [Developmental Biology Commons](#)

Recommended Citation

Quensen, Janet Murphy, "Changes in Amount and Composition of Gelatin from Development Stages of the Scyphozoan Jellyfish, *Chrysaora quinquecirrha*" (1975). *Dissertations, Theses, and Masters Projects*. Paper 1539624890.

<https://dx.doi.org/doi:10.21220/s2-thhj-3q18>

This Thesis is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Dissertations, Theses, and Masters Projects by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.

CHANGES IN AMOUNT AND COMPOSITION OF GELATIN
FROM DEVELOPMENTAL STAGES OF THE SCYPHOZOAN
JELLYFISH, *CHRYSAORA QUINQUECIRRHA*

A Thesis

Presented to

The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of
Master of Arts

By

Janet M. Quensen

1975

ProQuest Number: 10625373

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10625373

Published by ProQuest LLC (2017). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Arts

Janet M. Quensen
Author

Approved, May 1975

Robert E. L. Black
Robert E. L. Black, Ph.D.

Carl W. Vermeulen
Carl W. Vermeulen, Ph.D.

Paul L. Zubkoff
Paul L. Zubkoff, Ph.D.

5

TABLE OF CONTENTS

| | Page |
|--------------------------------|------|
| ACKNOWLEDGMENTS. | iv |
| LIST OF TABLES | v |
| ABSTRACT | vi |
| INTRODUCTION | 2 |
| MATERIALS AND METHODS. | 6 |
| RESULTS. | 8 |
| DISCUSSION | 16 |
| BIBLIOGRAPHY | 21 |
| VITA | 24 |

ACKNOWLEDGMENTS

The author would like to express sincere appreciation to Dr. Robert E. L. Black, under whose guidance this study was conducted, for his contribution of ideas, enthusiasm, and understanding. Appreciation is also expressed to Dr. Paul Zubkoff and Dr. Carl Vermeulen for their helpful suggestions and review of the manuscript. Additional thanks are extended to Dr. Kenneth Webb, Mr. Edward Gardner, and Mrs. Amy Sung for their technical assistance and to Mrs. Linda Jenkins for her assistance in preparation of the manuscript.

LIST OF TABLES

| Table | Page |
|---|------|
| 1. Total protein and percent gelatin in various developmental stages. | 11 |
| 2. Molar percentages of amino acids in gelatin fractions extracted from whole <i>Chrysaora</i> in various life stages. | 12 |
| 3. Molar percentages of amino acids in gelatin from whole dialyzed adult and lyophilized, dialyzed, whole adult medusae | 13 |
| 4. Molar percentages of amino acids in gelatin from non-autoclaved, adult mesoglea and in adult mesogleal protein | 14 |
| 5. Molar percentages of amino acids in protein of podocyst cuticle. | 15 |
| 6. Molar percentages of amino acids in the hydrolyzed gelatin, collagen and mesoglea of cnidarians. | 20 |

ABSTRACT

A qualitative and quantitative analysis of collagen extracted in the form of gelatin from *Chrysaora quinquecirrha* polyps, polyp bases remaining after strobilation, ephyrae, young adult and adult medusae was undertaken. In all stages the contribution of gelatin to total protein is high, ranging from 43.5% in polyps to 80% in adult medusae. The amino acid composition of autoclaved soluble gelatin extracted from whole organisms is unusual in that glutamic acid is the predominant amino acid, ranging from 33.7% of the amino acids in polyp gelatin to 15.2% in adult medusae gelatin. Typical amino acid compositions, with glycine as the predominant amino acid, were observed for adult medusae mesoglea, lyophilized whole adults, and TCA insoluble mesoglea protein. The ratio of proline/hydroxyproline is high in all samples.

CHANGES IN AMOUNT AND COMPOSITION OF GELATIN
FROM DEVELOPMENTAL STAGES OF THE SCYPHOZOAN
JELLYFISH, *CHRYSAORA QUINQUECIRRHA*

INTRODUCTION

Although the Chesapeake Bay scyphozoan cnidarian, *Chrysaora quinquecirrha* (DeSor), is known locally as the sea nettle, it is widely distributed in warm waters of the Atlantic and Indian Oceans as well as along the western Pacific coast (Calder, 1972). In the Chesapeake Bay and its tributaries this species occurs in troublesome abundance, presenting itself as a menace because of its irritating, if not toxic, sting.

Chrysaora exhibits a life history which is characteristic of most scyphozoans, with alternation of asexual, sessile polyp (scyphistoma) and free-swimming, sexual medusa generations. Although the predominant mode of asexual reproduction in the polyp is the formation of podocysts (Herouard, 1911), budding can also occur. In addition, by means of the metamorphic process of strobilation, the polyp is capable of constricting and subsequently budding into several free-swimming ephyra larvae. After strobilating, it regenerates feeding tentacles and mouth and resumes its former mode of existence (Percival, 1923; Chuin, 1930). The young ephyra, measuring only a fraction of a millimeter in diameter, matures into an adult, with a diameter of several inches, in about two months (Calder, 1972). The adult is dioecious, and eggs, fertilized in the stomach cavity of the female, are released and develop into a planula larva within 16 to 20 hours (Littleford, 1939). Terminating its swimming existence in 3 to 5 days, the planula

settles onto a suitable substrate, most commonly the under-side of certain hard substances such as oyster shells (Cargo & Schultz, 1966). Within 3 days to 2 weeks after attachment, the planula develops into a full-sized polyp (Cargo & Schultz, 1967), thereby completing the life cycle.

Histologically *C. quinquecirrha* is a typical cnidarian in that it possesses a tissue level of organization, the principal tissues being epithelial, muscular, and nervous (Hyman, 1940). The tissues are organized into a solid body wall which surrounds a central digestive cavity. The body wall is composed of 3 basic layers: an outer epidermis of ectodermal origin, an inner gastrodermis of endodermal origin, and a middle mesoglea, a type of primitive connective tissue, with an uncertain origin. The mesoglea may or may not contain cells, their presence apparently depending on the species. Although Hyman (1940) believes that a cellular mesoglea is present in all classes except the Hydrozoa, G. Chapman (1953), upon observing the medusae of the scyphozoans *Cyanea lamarcki*, *Aurelia aurita*, and *Chrysaora mediterranea*, found cells only in *A. aurita*.

In recent years the study of cnidarian mesoglea has increased in intensity, mainly because a knowledge of its composition may shed light on the origin and composition of connective tissues in general. Although once thought to be a rather uninteresting, structureless entity, the mesoglea is now considered a structurally complex, functionally indispensable unit. Originally, the mesoglea was thought to function merely as a mesoskeleton (von Kock, 1887) acting as a cement to hold the two cellular layers together. More recently it has been found that the mesoglea of *Hydra* may play an important part in morphogenesis since

the state of the mesoglea is a factor in determining the location of cell sloughing, bud formation, and migratory pathways of epidermal and gastrodermal cells along the body column (Burnett & Hausman, 1969). In medusoid forms, it has been suggested that the mesoglea performs a number of functions, acting as an antagonist to the musculature, a flotation device, and a storage depot for metabolically useful material (G. Chapman, 1974; G. Chapman, 1966).

Structurally, the mesoglea of cnidarians varies depending upon the class and body type of the species in which it is located. According to G. Chapman (1966) the mesoglea may range from thin and featureless in the hydrozoan, *Hydra* to slightly fibrous in hydromedusae such as *Aequorea* to fibrous in the scyphozoan medusae *Pelagia* and *Aurelia* to very fibrous in the anthozoan *Calliactis*. The mesogleal fibers of the scyphozoan medusa are probably collagenous, since they exhibit the X-ray diffraction patterns typical of collagen (Grimstone et al, 1958; Piez & Gross, 1959), have a high content of hydroxyproline, hydroxylysine, glycine and proline, and contract when heated (see G. Chapman, 1966 for review). Although the mesoglea of the scyphistoma is also fibrous (D. Chapman, 1970; Bynum & Black, 1974), no evidence, other than a 600Å banding pattern and sensitivity to collagenase in *Aurelia* polyps (D. Chapman, 1970), exists as to the chemical nature of the fibers.

The protein collagen is known to be widely distributed throughout the animal kingdom occurring in all the major animal phyla except the protozoa (Lowther, 1963). In all cases, it functions primarily in extracellularly supporting and maintaining cells and organs, when present, in an orderly arrangement (Hunt, 1970). Physically collagen

has been defined by Astbury (1938) as a class of proteins of fibrous nature, having a characteristic, unique wide-angle X-ray diffraction pattern with a major axial repeat at approximately $2.8-2.9\overset{\circ}{\text{Å}}$ and an equatorial repeat of about $11\overset{\circ}{\text{Å}}$. It is characterized by high glycine and proline content and by the presence of hydroxyproline and hydroxylysine (Hunt, 1970; Lowther, 1963). Structurally it consists of 3 polypeptide chains which may be either different with respect to amino acid content i.e., vertebrate collagens or identical i.e., anemone collagen (Nowack & Nordwig, 1974). In vertebrates an intracellular precursor, pro-collagen, has been identified which contains amino acid sequences which are absent from extracellular collagen (Bornstein et al, 1972). The presence of procollagen in cnidarians has been suggested by Gosline and Lenhoff (1968).

The mesoglea is quantitatively an important component of the scyphozoan medusa, since by volume it constitutes the bulk of the organism. The extreme difference in size and body form in the scyphistoma suggests that the mesoglea may comprise a much smaller proportion of the polyp than of the medusa. Because the major protein component of the mesoglea in the medusa is collagen (G. Chapman, 1953), changes in mesoglea during strobilation and growth of the medusa might be reflected by changes in total amount and amino acid composition of collagen. The purpose of this study was to determine whether such changes occur.

MATERIALS AND METHODS

The investigation of changes in amount and composition of gelatin was based on the presumption that collagen is unique among proteins in that it can be solubilized at high temperatures, forming gelatin (Veis, 1964). The procedure for extracting gelatin was a modification of that described by Gosline and Lenhoff (1968). Polyps, ephyrae, or young adults were homogenized in a hand-operated glass homogenizer in 80% ethanol, and the insoluble residue was recovered after centrifugation at about 2,000 g for 10 minutes in an International clinical centrifuge. Adults were homogenized in a Waring blender and centrifuged at about 10,000 g in a Sorvall RC2-B refrigerated centrifuge. All material was extracted three times in about 20 volumes of 80% ethanol, following which it was autoclaved for 18 hours at 15 p.s.i. in about 10 volumes of distilled water. The soluble gelatin fraction was separated from the insoluble non-collagenous residue by repeated centrifugation (2,000-16,500 g) and the gelatin precipitated in 80% ethanol.

The extraction procedure was performed on batches of 500 polyps, from which the bases and podocysts had been removed. Batches of 100 polyps were allowed to strobilate completely, and about 1000 ephyrae were obtained from each batch. The ephyrae and polyp bases were extracted separately. Young adult medusae, measuring 2.5 to 3.5 cm in diameter, were extracted in pairs, and older adults, averaging

about 13 cm, were treated singly. The ethanol-insoluble residue from one such adult was dialyzed against distilled water for 6 days prior to autoclaving. Gelatin was also prepared from one-gram batches of lyophilized adult medusae, after the material had been dialyzed against distilled water for 2 days.

Pieces of adult mesoglea were excised for determination of amino acid composition. The mesoglea was obtained from 4 adults which had been preserved in 80% ethanol for several months. The epidermis was peeled off and pieces of the mesoglea were excised with scissors and then were homogenized, extracted twice in 80% ethanol, once in 100% ethanol, once in diethyl ether, and dried.

For comparison with whole mesoglea, adult mesogleal protein was prepared by extracting lyophilized, sonified mesoglea six times in 5% TCA. The protein containing residue was washed once with 95% ethanol, three times with ethyl ether and dried to constant weight.

For determination of amino acid composition, 1 to 10 mg of dry material was placed in a glass ampoule with 1 to 2 ml of 6N HCl, the ampoule was sealed *in vacuo*, and hydrolysis was carried out in an oven at 105-110°C for 18 hours. The hydrolysates were evaporated either with a stream of nitrogen or in a vacuum dessicator over NaOH pellets. The amino acid compositions were determined using a semi-automatic ion exchange analyzer (Technicon Auto-Analyzer).

Total protein was determined on aliquots of extracted gelatin and on dissolved non-collagenous protein from the autoclaved residue by use of the Lowry procedure (Lowry et al, 1951).

RESULTS

The values for total protein and autoclave-soluble protein are presented in Table 1. The remarkable increase in size during development from polyp to medusa is reflected by the change in total protein, which is about 5×10^6 times greater in the medusa than in the polyp. This is roughly equivalent to the change in volume, if the polyp is assumed to be a cylinder and the medusa is considered to be one-half sphere.

The contribution of protein extracted as gelatin to total protein is not greatly different in the scyphistoma and the ephyra, but increases remarkably during growth from young to adult medusa (Table 1). Surprisingly, a high proportion of total protein in the polyp base after strobilation is also found in the gelatin fraction. The protein in the gelatin fraction is probably mostly collagen, and the differences observed may reflect differences in the ratio of collagen to cellular protein in the various developmental stages.

The amino acid compositions of the gelatin fractions of whole organisms are given in Table 2. Glutamic acid is the predominant component of this fraction in all stages; however, it drops from 33% of the amino acids in the polyp to 16% in the adult. In the polyp the next most abundant acid is chromatographically identified as ornithine, which is not a normal component of protein. Only trace amounts of this amino acid are found in the gelatin fraction of later stages. Aspartic acid

9

makes up 10% of the residues in the polyp and 12% in the ephyra; reduced percentages of this amino acid are found in the later stages. In the whole medusa alanine is next to glutamic in abundance, but it makes up a lower proportion of the polyp gelatin fraction. Glycine, which comprises about 30% of most collagens, is low in abundance in all stages.

The proline/hydroxyproline ratios in the gelatin fractions vary from 3 in the ephyra to 6 in the medusa. These are higher than those in vertebrate collagens, which are generally around 1 (Piez & Gross, 1959), but similar to the value of 6 for the anemone *Aiptasia* (Gosline & Lenhoff, 1968).

Because of the unusual composition of the gelatin fractions, it was felt that calcium salts of free amino acids or large oligopeptides might have been incompletely extracted in 80% ethanol and would thus be present as contaminants of the gelatin fraction. Accordingly, an adult medusa preparation was extensively dialyzed against distilled water after the extraction with ethanol and before autoclaving. In addition, lyophilized small females from the Ware River, Virginia, taken in 1972, were dialyzed, and the gelatin fraction was hydrolyzed in the autoclave for 14 hours. The results of these treatments are shown in Table 3. The gelatin from fresh adults, obtained after dialysis, has essentially the same composition as the preparation from non-dialyzed fresh adults. The gelatin from lyophilized medusae is quite different in composition and resembles collagens from sources (Piez & Gross, 1959). No explanation is available for this difference.

It was of interest to determine the amino acid composition of whole mesoglea which had not been autoclaved, as well as of mesogleal protein after removal of most of the carbohydrate material with TCA.

These results are presented in Table 4. The compositions of these preparations, as well as that of the gelatin from whole lyophilized, dialyzed medusae, bear a reasonable similarity to the compositions of collagens from other sources (Piez & Gross, 1959). Glycine is the most abundant amino acid, followed by glutamic and alanine. The proline/hydroxyproline ratios are still higher than those of vertebrate collagens.

One possible explanation for the differences in composition between the polyp and medusa gelatin fractions is that the polyp might possess unusual proteins not present in the medusa. One such protein is that of the podocyst covering, which is synthesized by basal epidermal cells of the polyp. It was reasoned that, even after removal of the polyp base, some cells might still possess quantities of this protein and that it might be extracted in the gelatin fraction. Podocyst coverings were prepared and hydrolyzed, and the amino acid composition is shown in Table 5. Glycine is by far the predominant amino acid followed by arginine and lysine; glutamic acid is not especially abundant. Hydroxyproline is fairly abundant, and the proline/hydroxyproline ratio is about 1.0. Because of the possibility of interactions between amino acids and podocyst chitin during hydrolysis, these results may not reflect the actual amino acid composition. Since this protein is obviously not unusually high in glutamic acid, it probably could not have contributed to the high glutamic content of polyp gelatin.

TABLE 1

TOTAL PROTEIN AND PERCENT GELATIN
IN VARIOUS DEVELOPMENTAL STAGES

| Life Stage | Total Protein/Animal (µg) | s | n | %Gelatin | s | n |
|-------------|---------------------------|---------|------------------|----------|-----|------------------|
| Polyp | 17.70 | .00385 | 10* ^a | 43.5 | 5.5 | 10* ^a |
| Polyp Base | 2.34 | .00069 | 10* ^b | 72.2 | 9.8 | 10* ^b |
| Ephyra | 2.06 | .00046 | 10* ^c | 58.5 | 6.8 | 10* ^c |
| Young Adult | 1.618 x 10 ⁶ | .31961 | 10* ^d | 55.2 | 4.0 | 10* ^d |
| Adult | 4.890 x 10 ⁸ | 128.518 | 6† | 80.0 | 0.8 | 6† |

*Each sample of 10 consists of (a) 500 organisms, (b) approx. 100 organisms, (c) approx. 1000 organisms, (d) 2 organisms.

†Each sample of 6 consists of 1 organism.

TABLE 2

MOLAR PERCENTAGES OF AMINO ACIDS IN GELATIN
FRACTIONS EXTRACTED FROM WHOLE *CHRYSAORA* IN
VARIOUS LIFE STAGES

| Amino Acid | <u>Life Stage</u> | | | |
|----------------|-------------------|--------|----------------|-------|
| | Polyp* | Ephyra | Young Adult | Adult |
| Cysteic acid | 0.4 | 0.4 | 0.3 | 0.3 |
| Taurine | 0.3 | 1.6 | 0.4 | 1.1 |
| Hydroxyproline | 0.9 | 0.8 | 1.0 | 1.3 |
| Aspartic acid | 10.3 | 12.0 | 6.7 | 6.2 |
| Threonine | 5.5 | 5.4 | 7.2 | 8.8 |
| Serine | 5.5 | 3.6 | 3.6 | 3.7 |
| Glutamic acid | 33.7 | 17.7 | 23.6 | 16.2 |
| Proline | 4.0 | 2.7 | 6.1 | 7.8 |
| Glycine | 8.2 | 9.5 | 7.9 | 11.5 |
| Alanine | 7.2 | 8.4 | 9.8 | 12.8 |
| Valine | 4.2 | 6.6 | 6.3 | 7.7 |
| Cystine | | | | |
| Methionine | 2.7 | | 2.3 | 0.1 |
| Isoleucine | 2.8 | 4.4 | 3.4 | 4.7 |
| Leucine | 4.4 | 5.7 | 3.6 | 3.0 |
| Tyrosine | 1.6 | 1.1 | 1.1 | 0.5 |
| Phenylalanine | 2.2 | 2.6 | 1.7 | 1.2 |
| Hydroxylysine | 0.2 | 0.3 | 1.5 | 1.8 |
| Ornithine | 11.8 | 0.4 | 0.3 | 0.2 |
| Lysine | 1.8 | 9.9 | 7.3 | 5.7 |
| Tryptophan | | | | |
| Histidine | 0.5 | 1.7 | 1.3 | 0.8 |
| Arginine | 5.5 | 5.1 | 4.6 | 4.5 |

*Values are the means obtained from duplicate amino acid analyses.

TABLE 3

MOLAR PERCENTAGES OF AMINO ACIDS IN
GELATINE FROM WHOLE DIALYZED ADULT
AND LYOPHILIZED, DIALYZED,
WHOLE ADULT MEDUSAE

| Amino Acid | Whole Adult | Lyophilized* Whole Adults |
|----------------|-------------|---------------------------|
| Cysteic acid | 0.2 | 0.3 |
| Taurine | 1.9 | 0.6 |
| Hydroxyproline | 1.5 | 2.7 |
| Aspartic acid | 3.7 | 6.6 |
| Threonine | 12.4 | 7.1 |
| Serine | 3.0 | 3.5 |
| Glutamic acid | 14.2 | 11.5 |
| Proline | 9.2 | 6.7 |
| Glycine | 9.8 | 22.1 |
| Alanine | 15.0 | 11.0 |
| Valine | 10.4 | 5.4 |
| Cystine | | |
| Methionine | 0.1 | 0.7 |
| Isoleucine | 5.4 | 3.0 |
| Leucine | 2.4 | 2.8 |
| Tyrosine | 0.4 | 0.5 |
| Phenylalanine | 1.0 | 1.2 |
| Hydroxylysine | 1.6 | 3.4 |
| Ornithine | 0.3 | 0.2 |
| Lysine | 3.9 | 4.5 |
| Tryptophan | | |
| Histidine | 0.5 | 0.7 |
| Arginine | 3.4 | 5.7 |

*Values are the means obtained from duplicate amino acid analyses.

TABLE 4

MOLAR PERCENTAGES OF AMINO ACIDS IN
GELATIN FROM NON-AUTOCLAVED, ADULT
MESOGLEA AND IN ADULT MESOGLEAL
PROTEIN

| Amino Acid | Adult Mesoglea | Protein Of* Adult Mesoglea |
|----------------|----------------|-------------------------------|
| Cysteic acid | 0.3 | 0.3 |
| Taurine | 0.5 | 0.1 |
| Hydroxyproline | 2.5 | 2.7 |
| Aspartic acid | 7.6 | 8.8 |
| Threonine | 4.5 | 5.6 |
| Serine | 3.3 | 4.7 |
| Glutamic acid | 11.8 | 10.7 |
| Proline | 7.8 | 7.9 |
| Glycine | 25.9 | 17.7 |
| Alanine | 10.1 | 9.9 |
| Valine | 4.3 | 5.0 |
| Cystine | | |
| Methionine | | 1.4 |
| Isoleucine | 3.0 | 3.6 |
| Leucine | 3.5 | 5.3 |
| Tyrosine | 0.7 | 1.9 |
| Phenylalanine | 1.4 | 2.9 |
| Hydroxylysine | 3.7 | 2.0 |
| Ornithine | 0.1 | |
| Lysine | 3.9 | 5.7 |
| Tryptophan | | |
| Histidine | 0.5 | 1.1 |
| Arginine | 4.8 | 2.7 |

*Protein insoluble in TCA extraction.

TABLE 5

MOLAR PERCENTAGE OF AMINO ACIDS
IN PROTEIN OF PODOCYST CUTICLE

| Amino Acid | Mole % |
|----------------|--------|
| Cysteic acid | 3.2 |
| Taurine | |
| Hydroxyproline | 5.0 |
| Aspartic acid | 5.2 |
| Threonine | 2.5 |
| Serine | 3.0 |
| Glutamic acid | 2.6 |
| Proline | 5.1 |
| Glycine | 35.2 |
| Alanine | |
| Valine | 3.1 |
| Cystine | |
| Methionine | 0.1 |
| Isoleucine | 3.3 |
| Leucine | 2.5 |
| Tyrosine | 4.0 |
| Phenylalanine | 1.7 |
| Hydroxylysine | |
| Ornithine | 0.3 |
| Lysine | 7.1 |
| Tryptophan | |
| Histidine | 2.8 |
| Arginine | 13.4 |

DISCUSSION

The total protein per animal increases remarkably during development (Table 1), roughly paralleling the increase in volume. The latter may be calculated if the polyp is assumed to be a cylinder of 0.25 mm radius and 1.0 mm length, giving a volume of approximately 0.2 mm^3 , while the medusa is calculated as a hemisphere with a radius of 6.5 cm and having a volume of $1.45 \times 10^6 \text{ mm}^3$. The increase is therefore about 7.2×10^6 , compared with an increase in protein content of 5×10^6 fold. It may be concluded that an increase in water binding per unit protein does not contribute appreciably to the growth of the medusa.

The contribution of gelatin to total protein is high in all stages in comparison to values observed for other organisms. Gelatin extracted from the sea anemone *Aiptasia* contributes only about 10% to total protein (Gosline & Lenhoff, 1968). Slightly higher values of 25-35% of the total body protein are estimated for collagen of most mammals (Neuberger, 1955).

In the largest *Chrysaora* medusa, gelatin reaches 80% of the total protein (Table 1). This must reflect the increased percentage of body protein present in the mesoglea, in which the major protein appears to be collagen (see below). In many organisms the proportion of collagen to total protein increases with age. This has been observed for collagen in the tendon, sclera, and skin of

cattle (Smits, 1957), the lung, liver, and kidney of rats (Chvábil, 1957), and cartilage of the embryonic chick (Jackson, 1958).

Harkness (1961) has proposed that the increase in collagen during growth is an adaptation to the mechanical requirements of increased size. The concentration of gelatin in the polyp bases after strobilation is also nearly 80%; this surprisingly high figure is not explainable by Harkness' hypothesis.

The gelatin fractions derived from fresh organisms of all stages, especially polyps, are highly unusual in amino acid composition (Table 2). The high ornithine content in the polyp gelatin is noteworthy, since this acid is not a normal constituent of proteins. One possible way to account for its presence would be the incomplete extraction of free amino acids prior to autoclaving; however, the gelatin fraction was precipitated in 80% ethanol after autoclaving. This should have eliminated any remaining free amino acids not extracted from the polyp. Ornithine might have been derived from arginine during hydrolysis (Piez & Likins, 1957); if this were the case, then the original gelatin fraction must have had an extremely high arginine content. Another possibility is that ornithine is a definite component of *Chrysaora* gelatin. Assuming that collagen turnover and the urea cycle are in operation in polyps, ornithine, freed in high concentrations from degraded polyp collagen, could be converted to arginine, a highly concentrated component of podocyst cuticle protein (Table 5). Another possibility is that ornithine was incorrectly identified in the chromatographic effluent. Cysteic acid and taurine, which are present in all gelatin fractions, have not been reported

in gelatins from other sources. Cysteic acid could be derived from cystine, but the possible source of taurine, which is generally found in the free amino acid fraction (Webb et al, 1972) is not clear.

Glutamic acid is the predominant residue in the gelatin fraction from polyps, and it is unusually high in all stages (Table 2). This must result from contamination of the gelatin fraction with non-collagenous protein. In the adult, such protein must be cellular in origin, since glutamic is much lower in mesogleal hydrolysate than in gelatin from the whole organism. The contaminating cellular protein must be exceedingly rich in glutamic acid, to account for the large amount of this acid in the gelatin fraction. One possibility was that in the polyp the protein contaminant might be derived from cuticular protein of the podocyst, which would be present in basal cells; however, analysis of a hydrolysate of podocyst cuticle (Table 5) did not give a high glutamic acid content. Proteins known to contain glutamic as the predominant acid are the most toxic protein found in *Chrysaora quinquecirrha* nematocysts (Blanquet, 1972) and adult *Chrysaora* tentacle hydrolysates (Stone et al, 1970); however, polyps have not been examined in these respects.

In contrast to the unusual composition discussed above, one gelatin fraction, prepared from lyophilized adults, proved to be generally similar to gelatins and collagens obtained by other workers from *Physalia*, *Metridium*, and *Actinia* and from *Hydra* mesoglea (Table 6). The gelatin from *Chrysaora*, like the mesoglea of *Hydra*, has a glycine content of 22 moles per cent, which is considerably below the range of 28.6 to 32.4 moles per cent reported for other invertebrate collagens

(Gross, 1963). *Chrysaora* gelatin also has a higher ratio of proline to hydroxyproline than gelatins of other cnidarians (Table 6) or of mammals. The ratio in *Chrysaora* is about 2.5, compared to about 1.9 for *Hydra*, 1.0 for *Physalia*, 1.3 for *Metridium*, 0.8 for *Actinia*, and about 1.0 for mammals (Neuberger & Richards, 1964). Hydroxylation of collagen proline occurs in the microsomes of the chick embryo after the incorporation of proline into polypeptide (Udenfriend, 1966). In the scyphozoan the rate of hydroxylation may be lower than the rate of collagen synthesis, or the specific sequences recognized by the hydroxylation enzyme may be lower in number than in other organisms.

The amino acid composition of whole mesoglea of adult *Chrysaora* (Table 4) does not differ appreciably from that of adult gelatin, or from gelatin from other cnidarians (Table 6). The major protein of mesoglea must therefore be collagen. When mesogleal protein is extracted with TCA prior to hydrolysis, the glycine content of the residue is considerably decreased. This may mean that exhaustive extraction with TCA has removed some protein, having a high glycine content, from the mesoglea, leaving a residual protein which is low in glycine. Mammalian collagen is known to be soluble in acid, particularly when it is not heavily cross-linked (see Veis, 1964, for a review). It would be of interest to investigate the heterogeneity of mesogleal protein, as it may contain differing types of collagen.

TABLE 6
MOLAR PERCENTAGES OF AMINO ACIDS IN THE HYDROLYZED
GELATIN, COLLAGEN AND MESOGLEA OF CNIDARIANS

| Amino Acid | <i>Chrysaora</i> | | <i>Physalia</i> † Float Gelatin | <i>Metridium</i> † Body Wall Gelatin | <i>Actinia</i> + Mesoglea Collagen |
|----------------|---------------------------------------|----------------------------|---------------------------------------|--|--|
| | Lyophilized Whole Adult Gelatin | <i>Hydra</i> * Mesoglea | | | |
| Cysteic acid | 0.3 | | | | |
| Taurine | 0.6 | | | | |
| Hydroxyproline | 2.7 | 3.0 | 6.1 | 4.9 | 9.2 |
| Aspartic acid | 6.6 | 9.6 | 8.3 | 8.1 | 7.9 |
| Threonine | 7.1 | 3.8 | 3.3 | 3.9 | 3.8 |
| Serine | 3.5 | 6.1 | 4.7 | 5.4 | 3.8 |
| Glutamic acid | 11.5 | 12.1 | 10.4 | 9.5 | 8.8 |
| Proline | 6.7 | 5.7 | 6.3 | 6.3 | 7.5 |
| Glycine | 22.1 | 22.9 | 30.7 | 30.8 | 30.9 |
| Alanine | 11.0 | 5.7 | 6.6 | 11.3 | 6.6 |
| Valine | 5.4 | 2.7 | 2.6 | 3.4 | 3.1 |
| Cysteine | | 1.5 | 0.2 | 0.3 | |
| Methionine | 0.7 | 1.2 | 0.6 | 0.9 | 0.5 |
| Isoleucine | 3.0 | 2.4 | 2.2 | 2.3 | 2.3 |
| Leucine | 2.8 | 5.6 | 3.1 | 3.7 | 3.4 |
| Tyrosine | 0.5 | 2.0 | 0.6 | 0.8 | 0.4 |
| Phenylalanine | 1.2 | 2.3 | 1.1 | 1.2 | 0.8 |
| Hydroxylysine | 3.4 | 4.0 | 3.0 | 2.5 | 2.8 |
| Ornithine | 0.2 | | | | |
| Lysine | 4.5 | 4.3 | 2.7 | 2.7 | 1.9 |
| Tryptophan | | | | | |
| Histidine | 0.7 | 1.3 | 0.5 | 0.2 | 0.3 |
| Arginine | 5.7 | 3.8 | 5.4 | 5.7 | 6.2 |

*Data from Barzansky *et al*, 1975.

†Data from Piez and Gross, 1959.

+Data from Nordwig and Hayduk, 1969.

BIBLIOGRAPHY

- Astbury, W. T. 1938. Trans. Faraday Soc. 34: 377.
- Barzansky, B., H. M. Lenhoff, and H. Bade. 1975. Hydra mesoglea: similarity of its amino acid and neutral sugar composition to that of vertebrate basal lamina. Comp. Biochem. Physiol. 50B: 419-424.
- Blanquet, R. 1972. A toxic protein from the nematocysts of the scyphozoan medusa, *Chrysaora quinquecirrha*. Toxicon 10: 103-109.
- Bornstein, P., K. von der Mark, A. W. Wyke, H. P. Ehrlich, and J. M. Monson. 1972. Characterization of the pro- α 1 chain of procollagen. Jour. Biol. Chem. 247(9): 2808-2813.
- Burnett, A., and R. E. Hausman. 1969. The mesoglea of Hydra (pseudolegactis). II. Possible role in morphogenesis. J. Exp. Zool. 171(1): 15-23.
- Bynum, M. A., and R. E. Black. 1974. Ultrastructure of the mesoglea in strobilae of *Chrysaora quinquecirrha* (Scyphozoa). Jour. Exp. Zool. 187(3): 323-333.
- Calder, D. 1972. Tentative outline for the inventory of planktonic Cnidaria: *Chrysaora quinquecirrha* (stinging nettle). Ches. Sci. 13(supp.): 5179-5181.
- Cargo, D. G., and L. P. Schultz. 1966. Notes on the biology of the sea nettle, *Chrysaora quinquecirrha*, in the Chesapeake Bay. Ches. Sci. 7: 95-100.
- Cargo, D. G., and L. P. Schultz. 1967. Further observations on the biology of the sea nettle and jellyfishes in Chesapeake Bay. Ches. Sci. 8: 209-220.
- Chapman, D. M. 1970. Reextension mechanism of a scyphistoma's tentacle. Can. J. Zool. 48: 931-943.
- Chapman, G. 1953. Studies on the mesoglea of coelenterates. I. Histology and chemical properties. Quart. J. Microscop. Sci. 94: 155-176.
- Chapman, G. 1966. The structure and functions of the mesoglea, p. 147-168. In W. J. Rees (ed.) The Cnidaria and Their Evolution. Symp. Zool. Soc. Lond. No. 16. Academic Press, London and New York.

- Chapman, G. 1974. The skeletal system, p. 93-128. In L. Muscatine and H. M. Lenhoff (ed.) *Coelenterate Biology, Reviews and New Perspectives*. Academic Press, New York, San Francisco, and London.
- Chuin, T. T. 1930. Le cycle evolutif du scyphistome de *Chrysaora*. *Trav. Sta. Biol. Roscoff* 8: 1-179.
- Chvapil, M. 1957. Studies in fibroplasia. IV. Sclero-proteins in the lungs, liver, kidneys, and myocardium during ontogeny in rats. *Csl. Fysiol.* 6: 102-110.
- Gosline, J. M., and H. M. Lenhoff. 1968. Kinetics of incorporation of C¹⁴-proline into mesogleal protocollagen and collagen of the sea anemone *Aiptasia*. *Comp. Biochem. Physiol.* 26: 1031-1039.
- Grimstone, A. V., R. W. Horne, C. F. A. Pantin, and E. Robson. 1958. The fine structure of the mesenteries of the sea-anemone *Metridium senile*. *Quart. J. Microsc. Sci.* 99: 523-540.
- Gross, J. 1963. Comparative biochemistry of collagen, p. 307-346. In M. Florkin and H. S. Mason (ed.). *Comparative Biochemistry a Comprehensive Treatise Vol. 5*. Academic Press, New York and London.
- Harkness, R. D. 1961. Biological functions of collagen. *Biol. Rev.* 36: 399-463.
- Herouard, E. 1911. Sur la progenèse parthénogénésique à longue échéance de *Chrysaora*. *C. r. hebd. Seanc. Acad. Sci., Paris* 153: 1094-1095.
- Hunt, S. 1970. *Polysaccharide Protein Complexes in Invertebrates*. Academic Press, New York and London. 329 p.
- Hyman, L. H. 1940. *The Invertebrates: Protozoa Through Ctenophora*. McGraw-Hill, New York. 726 p.
- Jackson, S. F. 1958. Composition of developing bone rudiments of the embryonic fowl. *Fed. Proc.* 17: 78.
- Littleford, R. A. 1939. The life cycle of *Dactylometra quinquecirrha*, L. Agassiz in the Chesapeake Bay. *Bio. Bull.* 77: 368-381.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- Lowther, D. A. 1963. Chemical aspects of collagen fibrillogenesis, p. 63-119. In D. A. Hall (ed.) *International Review of Connective Tissue Research Vol. 1*. Academic Press, New York and London.

- Neuberger, A. 1955. Metabolism of collagen under normal conditions. Symp. Soc. Exp. Biol. 9: 72-84.
- Neuberger, A., and F. F. Richards. 1964. Protein biosynthesis in mammalian tissues. Studies on turnover in the whole animal, p. 243-296. In H. N. Munro and J. B. Allison (ed.) Mammalian Protein Metabolism. Academic Press, New York and London.
- Nordwig, A., and U. Hayduk. 1969. Invertebrate collagens: Isolation, characterization and phylogenetic aspects. J. Mol. Biol. 44: 161-172.
- Nowack, H., and A. Nordwig. 1974. Sea-anemone collagen: Isolation and characterization of the cyanogenbromide peptides. Eur. J. Biochem. 45(2): 333-342.
- Percival, E. 1923. On the strobilation of *Aurelia*. Quart. Jour. Micros. Sci. 67: 85-100.
- Piez, K. A., and J. Gross. 1959. The amino acid composition and morphology of some invertebrate and vertebrate collagens. Biochem. Biophys. Acta 34: 24-39.
- Piez, K. A., and R. C. Likins. 1957. The conversion of lysine to hydroxylysine and its relation to the biosynthesis of collagen in several tissues of the rat. J. Biol. Chem. 229: 101-109.
- Smits, G. 1957. Quantitative interrelation of the chief components of some connective tissues during foetal and post-natal development in cattle. Biochem. Biophys. Acta 25: 542-548.
- Stone, J. H., J. W. Burnett, and R. Goldner. 1970. The amino acid content of sea nettle (*Chrysaora quinquecirrha*) nematocysts. Comp. Biochem. Physiol. 33: 707-710.
- Udenfriend, S. 1966. Formation of hydroxyproline in collagen. Science 152: 1335-1340.
- Veis, A. 1964. The Macromolecular Chemistry of Gelatin. Academic Press, New York and London. 433 p.
- von Koch, G. 1887. "Fauna and Flora des Golfes von Neapel. XV. Die Gorgoniden." Friedländer, Berlin.
- Webb, K. L., A. L. Schimpf, and J. Olmon. 1972. Free amino acid composition of scyphozoan polyps of *Aurelia aurita*, *Chrysaora quinquecirrha* and *Cyanea capillata* at various salinities. Comp. Biochem. Physiol. 43B: 653-663.

VITA

Janet Murphy Quensen

Born in Boston, Massachusetts, 27 August 1947. Graduated from Girls' Latin School, Boston, Massachusetts, June, 1965. Laboratory technician, Moleculon Research Corporation, Cambridge, Massachusetts, 1967-1970. Summer student, Harvard University, Cambridge, Massachusetts, 1969. B.A. (Biology), Boston State College, Boston, Massachusetts, June, 1970. Graduate student in oceanography, Johns Hopkins University, Baltimore, Maryland, 1970-1971. Laboratory technician, Virginia Institute of Marine Science, Gloucester Point, Virginia, 1971-1974. Entered the Department of Biology of the College of William and Mary, September, 1973. Teaching assistant in the Department of Biology, January, 1974-August, 1975.