THE AMINO ACID COMPOSITION OF THE ORGANIC MATRIX OF SOME RECENT AND FOSSIL SHELLS OF SOME WEST COAST SPECIES OF *MYTILUS*

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ABSTRACT

The organic matrix from each structural unit of Mytilus californianus has a distinctive amino acid composition. Systematic variations in composition occur within the outer prismatic layer and the periostracum. In small shells there are profound changes in the periostracum composition as the animal grows during the first year or so.

There is a relationship between the amount of organic material present in the original shell unit and the relative amounts of the basic and acidic amino acids in the organic matrix. The matrix from calcitic shell structures has a larger net excess of acidic residues than does the matrix from aragonitic structures. Temperature changes appear to be reflected in compositional changes in the nacreous aragonite layer with increasing temperature favoring a higher number of net acidic amino acid residues. Thus, as temperature increases, the differences in matrix composition between the nacreous aragonite layer and the outer prismatic calcite layer become progressively smaller. Decreasing salinity seems to have a similar effect to increasing temperature.

From a study of a series of radiocarbon-dated shell materials, it is found that the composition of the organic matrix undergoes a significant amount of change within a few thousand years under a rather dry environment. The oxidation products of certain amino acids are present in progressively larger amounts in older materials, suggesting oxidation as one of the possible mechanisms involved in fossilization.

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INTRODUCTION

The demonstration of the presence of intact or only slightly altered organic material in the geologic record (Keilin, 1953; Abelson, 1954; Erdman, et al., 1956; Gregoire, 1959a, b; Jones and Vallentyne, 1960) has provided a tool that is potentially useful in attacking a variety of geologically interesting problems.

One area of potential application of the study of paleobiochemistry is in the study of biochemical evolution. Distinct biochemical changes possibly underlie observed morphological changes, and it may be possible to elucidate certain paths and mechanisms of evolution by a study of the various biochemical products preserved in fossils of extinct organisms. In addition, it may be possible to detect ecologically controlled differences in the organic components of certain organisms and thereby contribute to the paleocology of a particular fauna in which the original organic material has been preserved.

Also in the study of the diagenetically altered sediments the study of the composition of the preserved organic material may contribute useful information, since biochemical compounds are geologically stable only under certain restricted conditions of temperature, bacterial activity, pH, oxidation-reduction potential, ground-water action, and perhaps other factors involved in the diagenesis of sediments.

A study of preserved biochemical compounds in coal and petroleum has already given, (see Hanson, 1959) and will continue to give, information on the parent materials as well as the mechanisms of formation.

A knowledge of present-day biochemical products and processes is essential to an understanding of the types of biochemical materials one finds in the geologic record.

Organic Material Associated with Mineralized Tissue

One type of organic material potentially useful in paleobiochemistry is the organic matrix associated with the mineralized skeletal parts of certain organisms. This organic matrix is involved in the mineralization process, and for the mollusks, the group which will be considered in this study, the matrix is completely separated from the secreting mantle after mineralization and is therefore nonliving material in the calcified shell (Wilbur, 1960). The organic matrix is thus an integral part of the shell and is capable of preservation for geologically significant times. Gregoire (1959a and 1959b) has demonstrated by electron microscopy that the structurally intact organic matrix of certain mollusks can be detected and taxonomically classified to a certain extent in specimens as old as Ordovician.

Gregoire's studies on fossil materials did not include chemical characterization of the organic matrix, and it would be interesting to know to what extent the original chemical composition has been preserved. It is possible that further significant data on taxonomic relationships of some of the long-extinct forms could be obtained by chemical analysis of this "fossil" organic matrix.

The shell-organic matrix system provides a useful model for the study of the effects of diagenesis on organic material. Potentially destructive agents such as bacteria, moisture, and oxygen must

penetrate the shell before acting on the organic material. In contrast, the organic material disseminated in sediments is affected by such processes in varying degrees during sedimentation and early diagenesis. Knowledge of present-day shell-organic matrix systems should make it possible to determine how much of the original shell organic material is still intact and to what extent migration of organic material to or from the enclosing sediments has taken place.

Purpose and Scope

The present study is an investigation of the chemical composition of the organic components of a group of modern and sub-fossil calcareous shells of close genetic affinity from a variety of habitats. The objective is to determine the extent of variations in the organic matrix composition with shell mineralogy, ecology, and taxonomy. Only when present-day variations in matrix composition are known is it possible to interpret the results from the fossil record and thereby get information on the stability of the components of the organic matrix to diagenetic processes of sedimentation.

The shells for this study were selected for their shell mineralogy, ecological distribution, and representation in the fossil record. The two following West Coast species of the pelecypod family Mytilidae were used: Mytilus californianus and Mytilus edulis diegensis.

The sub-fossil materials studied were specimens of Mytilus californianus supplied by Dr. C. L. Hubbs. These specimens were collected from radiocarbon-dated horizons in archeological sites along the West Coast (Hubbs, et al., 1960).

The shell structure of Mytilus has been reviewed and described by Boggild (1930) and Dodd (1961). Mytilus californianus has four recognized layers in its shell; periostracum, outer prismatic layer, nacreous layer, and inner prismatic layer. In addition to these structural units, there is the ligament and the ligamental ridge. Usually some byssus fibers are attached to the shell. The outermost layer, the periostracum, is uncalcified organic material, while the prismatic layers are both calcite and the nacreous layer is aragonite. Dodd (1961) found a marked temperature effect on the development of the inner prismatic calcite layer with cooler temperatures favoring increased development of this unit. For this reason, the specimens used for the study of the organic matrix of the various structural units of contemporary shell material were taken from the Sonoma Coast State Park in northern California ($38^{\circ}23'$ N), a moderately cool water environment in which the inner prismatic layer of Mytilus californianus is well developed.

Specimens of Mytilus californianus from two localities east and south of Punta Banda ($31^{\circ}45'$ N) and also Corona del Mar ($33^{\circ}36'$ N) were compared to the Sonoma Coast specimens for possible ecological differences. Only the outer prismatic and the nacreous layers were sampled from these as well as the specimens described below.

A specimen of Mytilus edulis diegensis from Corona del Mar was compared to the specimen of Mytilus californianus from the same location to define possible genetic differences.

Two specimens of Mytilus edulis diegensis from near San Francisco (Cliff House, $37^{\circ}47'$ N and Glen Cove, $38^{\circ}3.5'$ N, $122^{\circ}12.6'$ W) were compared for possible salinity effects. Cliff House is on the open

coast with near normal salinity (~34 o/oo) while Glen Cove, near Benecia, had a salinity of only 20 o/oo.

Five specimens of sub-fossil Mytilus californianus were sampled. One specimen was from Oregon, a relatively moist environment at present. The other specimens came from Southern California and Baja California, a relatively dry environment at the present time.

THE NATURE OF THE ORGANIC MATRIX IN THE SHELLS OF MOLLUSKS

Literature Review

One of the objectives of this study is to determine, if possible, what chemical differences, if any, exist in the organic matrices associated with aragonite and calcite. Is the organic matrix in any way responsible for the determination of the crystallographic form which is deposited? If so, one might expect chemical differences in the respective organic matrices. Roche, et al. (1951) attempted to show such chemical differences in the organic components of shell aragonite and calcite, but his analyses were too incomplete to be at all definitive. They did, however, show significant species differences. Ranson (1952) considered the organic matrix to be the determining factor in aragonite and calcite formation. He showed that the organic matrices from the prismatic and nacreous layers in certain pelecypods react differently to histochemical stains. However, this does not demonstrate a cause-effect relationship.

Gregoire, et al. (1955, 1957, 1960, 1961a) have demonstrated significant genetically-controlled structural differences in the organic matrices associated with aragonite in the shells of three classes of mollusks (gastropods, pelecypods, and cephalopods). The organic matrix in nacreous aragonite is in the form of thin ($<1\mu$) parallel sheets which alternate with lamellae of aragonite. These are held together by transverse bridges of organic material which are apparently identical to the thin organic sheets. These transverse bridges of the organic matrix cut through the aragonite sheets at right angles and thus produce

the typical "brick wall" pattern so often seen pictured in electron micrographs of etched aragonite cross-sections (Gregoire, 1957).

When nacreous aragonite is decalcified, the residue of organic material consists of soft, almost transparent membranes. Gregoire (1955) used ultrasonic vibrations to further break up the organic residue into thin, lacelike reticulated sheets. After appropriate preparation, the structure of these thin films of organic material was studied by electron microscopy. In general, the surface of the pellicles is characterized by a pattern of pores. The size, shape, and distribution of these surface pores is characteristic of the taxonomic class of the animal investigated. For example, the organic matrix of aragonite from pelecypods is characterized by smaller-sized pores than is the matrix from gastropods. The average number of pores per unit area is slightly higher in pelecypods, and the fraction of surface area occupied by the pores in the pelecypod matrices is approximately one-half that found in the gastropod matrices. The cephalopod pattern is distinct from either of the above patterns (Gregoire, 1959a,b).It is characterized by even larger pores than are found in gastropods and has distinctive elongate to broadly rounded openings.

The organic matrix associated with calcite shell structures has been studied (Gregoire, 1961ab) with some interesting and significant observations. The organic residue from the decalcification of the calcitic prismatic shell structures is in general more resistant to ultrasonic vibrations than is the aragonitic shell matrix. Ultrasonic vibration produces opaque fragments of randomly-broken organic matrix together with some fibrils. The fibrils appear similar to those

derived from the matrix of aragonitic structures. In specimens of Mytilus edulis the organic matrix surrounding the prisms of calcite is extremely thin and can be fragmented into thin reticulated sheets which appear identical to those derived from the aragonitic portion of the same shell. This seems to show that the organic matrices of calcite and aragonite are structurally similar. It is conceivable, however, that minor chemical differences exist, which in turn may influence shell mineralogy.

Role of the Organic Matrix in Mineralization

Mollusk shell formation, in common with all mineralization processes, involves the two-step sequence of nucleation and subsequent crystal growth. Just how and to what extent the organic matrix participates in one or both of these steps is at present not completely clear (Wilbur, 1960).

Observations on the repair of an injured shell of a sea scallop indicate that for this organism the mantle first secretes the organic matrix upon which droplets of aragonite are deposited (Merrill, 1960). In the nacreous layer of many mollusks, similar unoriented droplets grow, eventually become oriented, and merge into a thin crust of aragonite (Wilbur, 1960). A vertical section through the nacreous region of most mollusks reveals thin alternating layers of aragonite and organic material (Gregoire, 1957), indicating that the secreting mantle periodically provides organic matrix and mineral material. The bulk of the mineral material is provided by the mantle in dissolved form with subsequent crystallization taking place in or on the organic matrix (Wilbur, 1960). Bevelander (1953) reported the presence of optically-

recognizable $CaCO_3$ crystals in the organic matrix as it is secreted.

In the inner layer of the oyster Crassostrea virginica (calcitic in this organism) (Watabe, et al, 1958) unoriented crystal seeds are deposited directly on the organic matrix. After these have grown and become oriented they merge into a single layer of crystals, after which further crystal seeds appear to be deposited directly on these larger underlying crystals. This second type of crystal seed is oriented from the start and seems to develop independently of the organic matrix upon which the initial seeds developed. (See Murray, 1954, on seed effects.)

Bevelander and Benzer (1948) reported that the initial crystal granules deposited on the organic matrix of mollusks were calcium phosphate; however, later work by Bevelander (1952) using the radioactive isotopes Ca^{45} and P^{32} showed that phosphate was present in the organic matrix surrounding the crystals but not in the crystal phase itself.

Bevelander (1952, 1953) described the physiological process of mollusk shell deposition. Different epithelial cells of the mantle and the mantle folds secrete two distinct types of organic material. That associated with the inner shell layer is somewhat more basic to histochemical stains than is the organic matrix associated with the prismatic layer.

The source of the crystals deposited on the organic matrix, according to Bevelander (1953), is the numerous mucus glands located in the mantle. These crystals apparently originate in the glands in an environment containing polysaccharides, ribonucleic acid, glycogen, and phosphate. Alkaline phosphatase was shown to be absent.

It may be that the function of the organic matrix is to control the extent of mineralization. In the present study, it was observed that when Mytilus shells are decalcified in a weak acid solution, the remaining organic residue (before drying) resembles the megascopic morphological detail of the original shell structures.

Chemical Components of the Organic Matrix of Mollusks

The chemical composition of the organic matrix in shell materials has not been investigated as thoroughly as has its physical submicroscopic structure. The reason for this is the lack, until recent years, of techniques to isolate and detect minute amounts of organic materials. The development of the technique of paper chromatology for the analysis of amino acids by Consden, Gordon, and Martin (1944) and the later development of ion-exchange methods by Moore and Stein (1951) enabled investigators to separate and detect microgram quantities of organic compounds.

The predominant component in the organic matrix is protein, and consequently most studies have been concerned only with this fraction. Roche, et al. (1951) applied paper chromatography to the study of the composition of the organic matrices associated with a variety of mollusk and echinoderm shells. The study included only ten amino acids (see Appendix I), and though incomplete showed interesting variations between different species. In the proteins studied, Roche showed that the amino acids glycine and tyrosine were found in greater concentration in the organic matrices of calcite than in the matrices from aragonite shell materials.

In a later study, Gregoire, Duchateau, and Florkin (1955) divided the matrix protein into three components: (1) a fraction soluble in borate buffer at pH 9.2 , (2) a fraction soluble in 5 per cent sodium carbonate, and (3) an insoluble fraction. The distribution of the three protein fractions was distinct from one species to another. A total of 15 amino acids were identified, but no systematic comparisons were possible because of the limited number of samples.

Abelson (1956) identified 15 amino acids in the matrix from Mercenaria mercenaria. In an investigation of a wide variety of mollusk shells, Abelson detected protein material in each case, varying in amount from 0.1 to over 0.5 per cent of the intact shell.

Beedham (1958) studied the qualitative distribution of 11 amino acids in various layers of shells of Anodonta, Mytilus, and Ostrea. Significant differences between layers as well as between species were noted.

Piez (1961) has analyzed the protein from the matrix of a snail (Australorbis globratus). The analysis includes 19 amino acids varying in concentration from one residue per thousand for methionine to 265 residues per thousand for glycine (see Appendix II-C).

Components other than proteins present in the organic matrix of mollusk shell material include pigments (Comfort, 1951) and, according to Beedham (1958), small amounts of lipoid material and polysaccharides. Bevelander (1953) reports the presence of polysaccharides in several species of mollusks.

Comfort (1951) has reviewed the distribution of pigments in mollusk shells. Some of these pigments are acid-soluble and therefore are dissolved from the organic matrix during decalcification. Among

the identified pigments of this group are the porphyrins and pyrroles. The acid-insoluble pigments appear to be firmly bound to the protein components and seem to be similar to the pigment-protein complex of insect cuticles (Brown, 1950). These are apparently melanin-like pigments characterized by a continuous near-linear spectrum between 4000 A and 6000 A (Schaeffer, 1953).

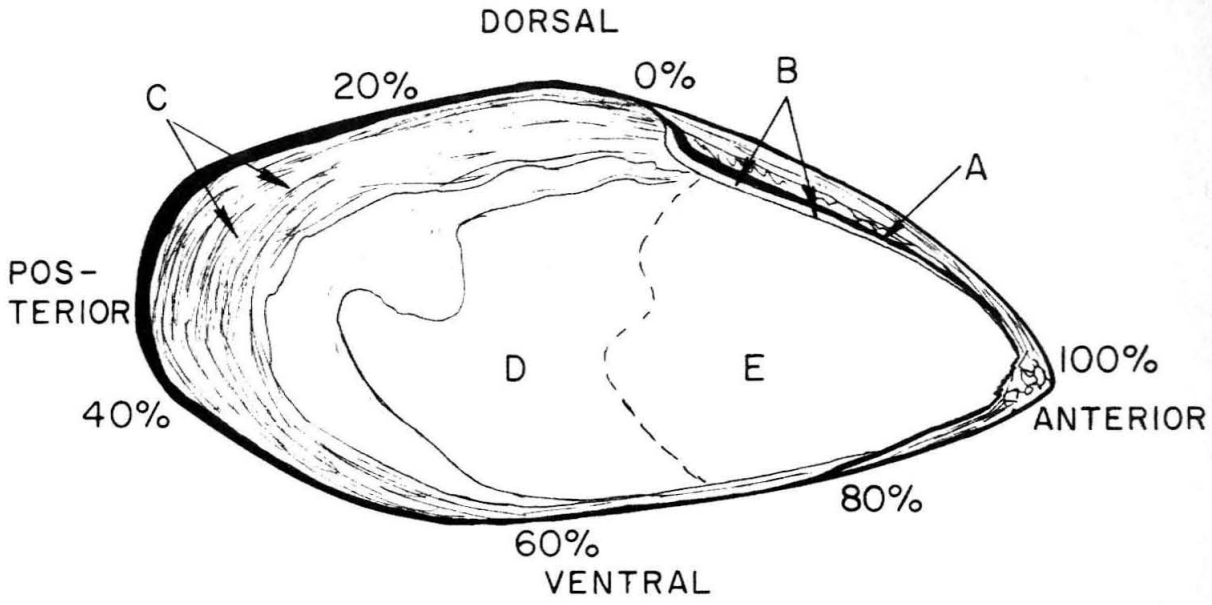
Analytical Methods and Sample Selection

In the present study, portions of each structural unit of the shells of Mytilus californianus were decalcified (if necessary) and then hydrolyzed in 6N HCℓ for 22 hours at 110°C. Amino acid determinations were made on a Beckman Model 120 amino acid analyzer.

For subsequent studies of the effects of ecological and genetic differences, only portions of the nacreous and outer prismatic layers were sampled.

In the course of this study, it became apparent that systematic differences exist in the organic matrix composition of both the outside prismatic layer and in the periostracum (see Figure 1). In order to correlate samples from one shell to another, the position at the growing edge of the shell is arbitrarily designated as a percentage of the peripheral distance starting at the ligamental ridge - outer prismatic layer contact and continuing around the posterior edge of the shell to the beak area. This is illustrated in Figure 1.

The gross distribution of organic material in the shell units of Mytilus californianus is given in Table III-1, page 94. The trace amounts of lipids, carbohydrates, and pigments present were not studied in detail.



Percentages refer to arbitrary designation of positions around periphery of growing edge.

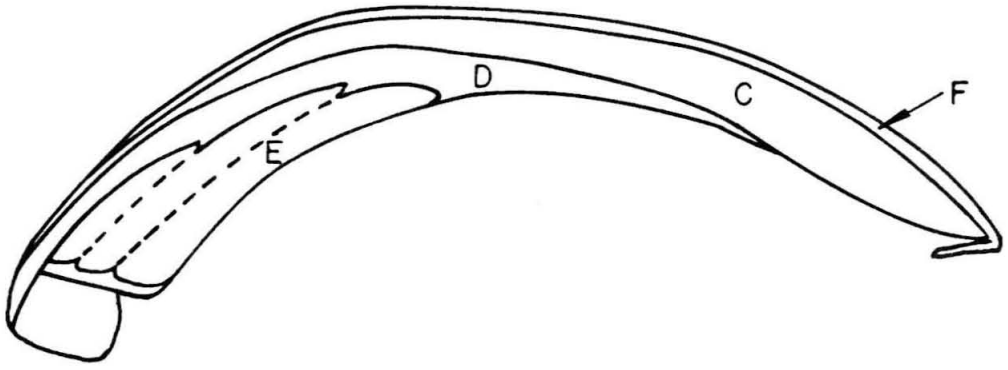


FIGURE 1. Drawing showing relationships of structural units in shells of Mytilus californianus. Lower section after Dodd (1961). A. Ligament. B. Ligamental Ridge. C. Outer Prismatic Layer. D. Nacreous Layer. E. Inner Prismatic Layer. F. Periostracum.

THE AMINO ACID COMPOSITION OF THE ORGANIC MATRIX PROTEINS FROM EACH STRUCTURAL UNIT OF MYTILUS CALIFORNIANUS

In order not to superimpose any possible environmental or size-age effects, the shells for this phase of the study were mature, full-grown specimens of nearly the same size taken from a single environment on the same day. The shells were collected in February 1961 from Portuguese Beach on the Sonoma Coast, about sixty miles north of San Francisco, California.

Reproducibility and the Effects of Variations in Sample Treatment

To ascertain the significance of the variations encountered in the amino acid composition of proteins, it is necessary to determine how closely the results agree when two identical or nearly identical samples are analyzed. It is also useful to determine any possible effects due to unavoidable variations in sample preparation. For these purposes, corresponding samples from the outside prismatic layers of the two valves of a single specimen were used. Here, any microenvironmental effects or biological variability should be essentially absent, since both valves are formed by the same animal under identical conditions. Figures 2 and 3A, B show the results on two separate pairs of samples treated identically. The results from the two samples in each pair agree to better than 2 per cent for all the amino acids except histidine, proline, cystine, methionine, and phenylalanine. In general, the accuracy of the method for a given amino acid decreases as the concentration of the amino acid drops below 0. 2 micromoles, and it will

be noticed that the above amino acids are generally in low concentrations. The maximum deviation from the mean was just over 3 per cent for proline.

The effect of varying the treatment of a sample is illustrated for pairs 3 and 4, also shown in Figs. 2 and 3 A, B. The samples are prepared using a dental cutting tool, and often during the course of the preparation the sample is heated so that the odor of burning protein may be detected. The sample from the left valve in the 0 - 5 per cent position was chipped without heating, while the corresponding sample from the right valve was heated far more than normally. The sample was too hot to touch. Most of the amino acid results are still better than 2 per cent. The deviation in threonine is just over 3 per cent, while that for cystine is 5 per cent. One may conclude that short-term heating probably does not affect the results seriously.

Another source of variation in the procedure for sample preparation occurs during the decalcification step. Generally, the samples remain in acid solution under a partial vacuum until they will no longer evolve CO_2 bubbles. This means that larger samples take longer to decalcify, and consequently there may be a tendency for the more soluble components to be leached away from the organic matrix residue. In the fourth pair (Figures 2, 3), the sample from the right valve was left in the decalcifying solution for 30 hours after the samples ceased to evolve CO_2. The only significant change observed was a 15 per cent decrease in cystine. Since the length of time involved was much greater than normally encountered, it would certainly seem that except for cystine the length of time for decalcification is not a significant

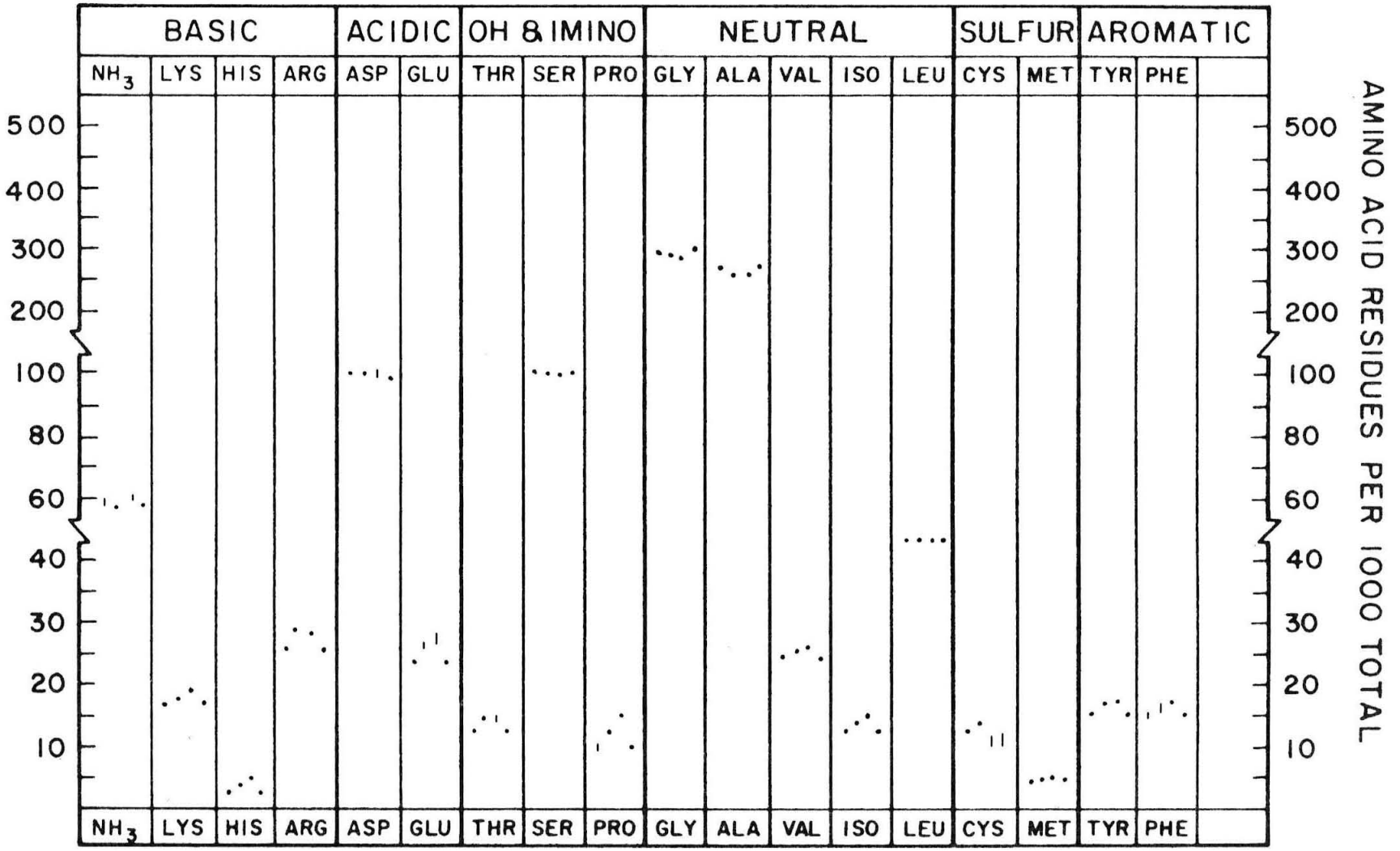


FIGURE 2. Reproducibility of 4 pairs of nearly identical samples. Vertical extent of lines indicates difference between values for each pair. Left to right pairs 1, 2, 3, and 4. Data in Tables III-2, 3. p. 95, 96.

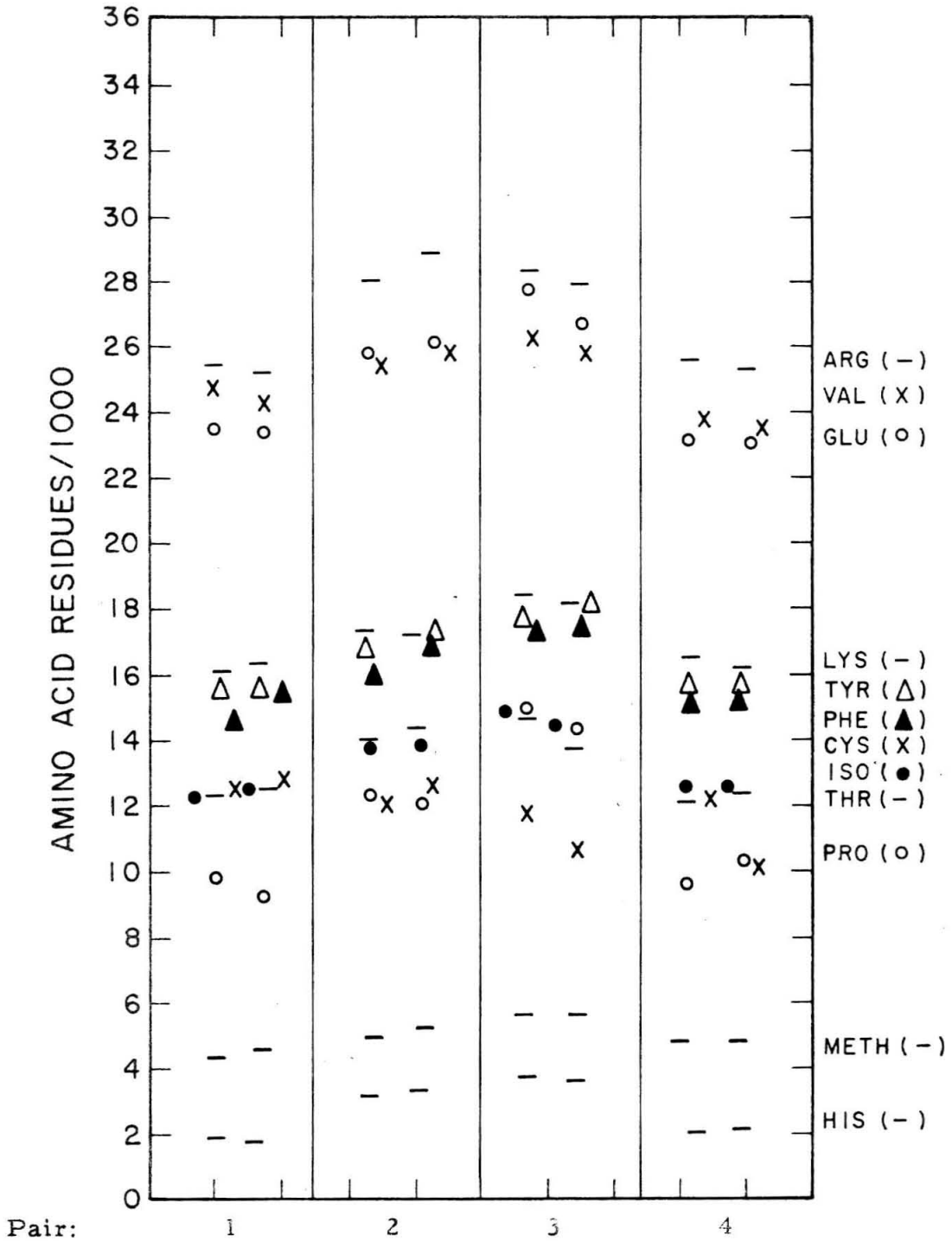


FIGURE 3A. Reproducibility and effect of variations in treatment of sample.
Data from Tables III-2, 3. p. 95, 96.

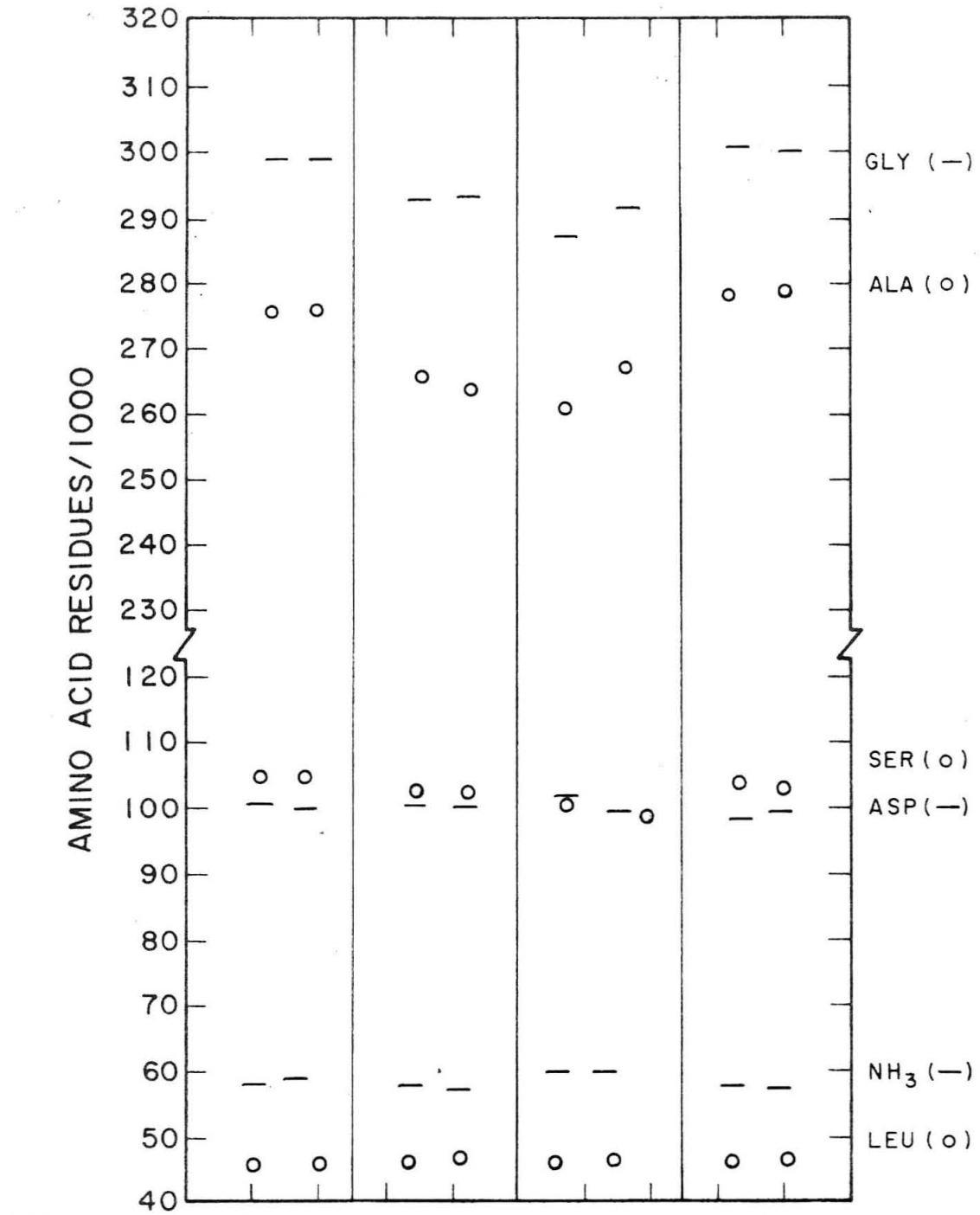


FIGURE 3B. Reproducibility and effect of variations in treatment of sample.
Data from Tables II-2, 3. p. 95, 96.

factor.

Summarizing, it seems reasonable to assume that for all the amino acids except histidine, proline, cystine, methionine, and phenylalanine, the results are meaningful to within ± 2 per cent. For the others (except cystine) probably 4 or 5 per cent would be realistic; and for cystine, certainly the reproducibility would be better than ± 15 per cent. These limits are shown as ranges in Figure 2.

Non-Calcified Components of the Shells of Mytilus californianus

The non-calcified units include the periostracum and the byssus fibers by which the shells are attached to a substrate. Because these units are entirely organic material and are not protected by calcification, they are generally not preserved in the fossil record. Jones and Vallentyne (1960) record the presence of intact periostracum material of Mercenaria mercenaria in mid-Pleistocene deposits. Interestingly enough, the periostracum material still had a greater variety of its original amino acids than did the calcified shell materials of the same specimens. Nevertheless, because it is not calcified, the periostracum is rarely preserved. For this reason, only a limited number of periostracum samples were analyzed, and only a single sample of byssus fiber was included for comparative purposes. Details of sample treatment, analysis, and expression of results are given in Appendix II. Non-calcified fractions such as the periostracum and byssus fiber were hydrolyzed directly.

The results given in amino acid residues per 1000 total are shown in Figures 4 and 5. This method of expressing the amino acid composition of a protein is used extensively in comparative biochemical

studies (Piez and Likins, 1960). It is independent of sample size and also eliminates the diluting effect of non-protein components such as lipids and carbohydrates, which may be present in various amounts. The total number of micromoles of amino acids recovered is arbitrarily considered as 1000 total residues. The concentration of each amino acid is then calculated as the number of residues which are present per 1000 total residues.

The results for the byssus fiber include the amino acid hydroxyproline, which is not found in any other of the organic fractions of the shell units studied. This amino acid is considered to be typical for the class of structural proteins termed collagens (Gross and Piez, 1960). Another chemical characteristic of collagens is the glycine content of approximately one-third, which is well matched by the 326 residues per 1000 present in the byssus fiber sample. Hydroxylysine, which is usually but not always present in collagen, seems to be completely absent in the sample analyzed. A comparison of the composition of byssus fiber with the range of typical invertebrate collagens (Gross and Piez, 1960) is given in Figure 4.

In Figure 6, the byssus fiber is compared to the range of values found in the unpigmented, most recently deposited periostracum at various positions near the periphery of the shell. The glycine content of approximately 500 residues is far higher than in byssus fiber; and further, there is no hydroxyproline in periostracum material. The very large amount of tyrosine is typical for periostracum secreted by full-grown specimens.

There is considerable variation in the physical appearance and

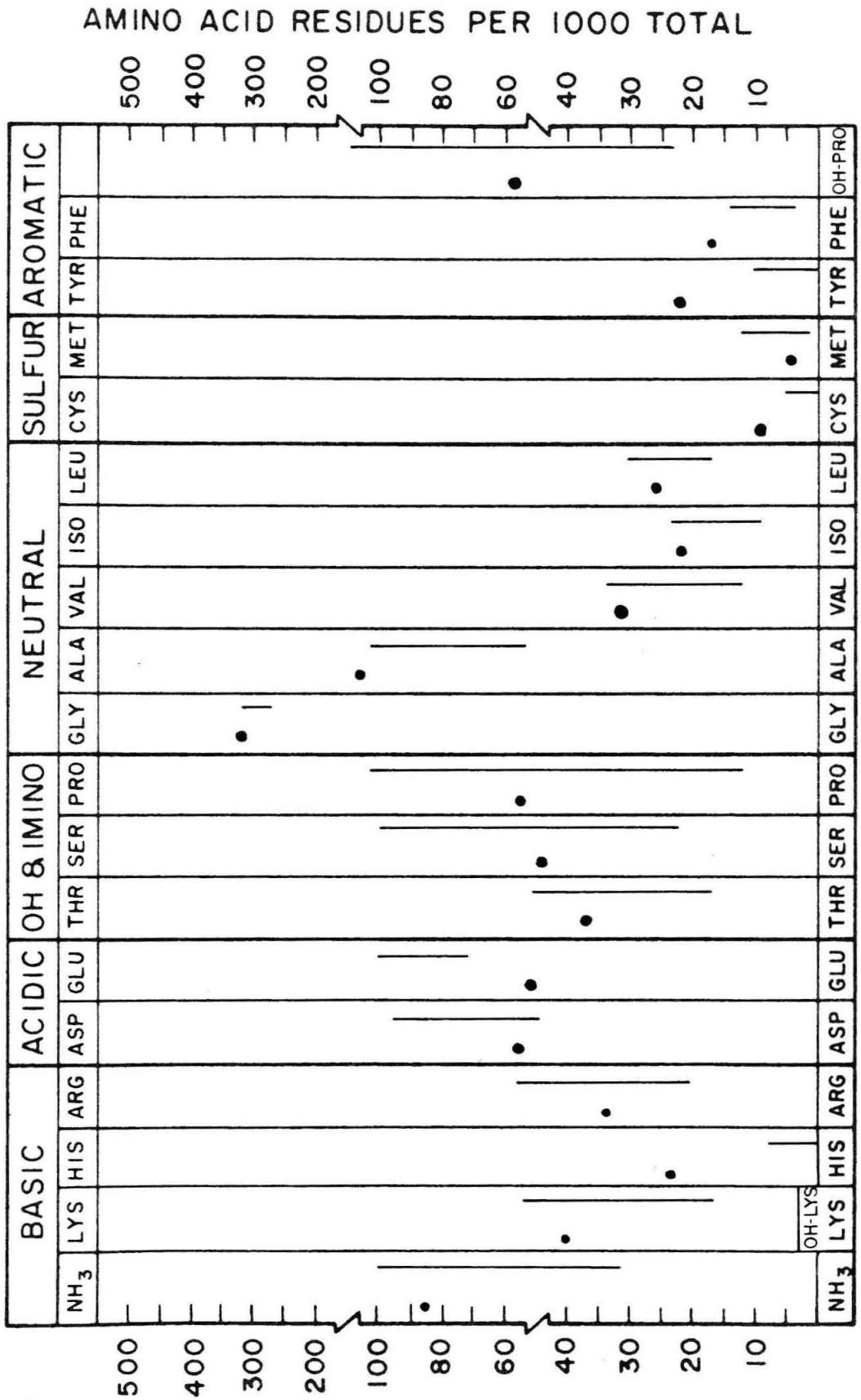


FIGURE 4. Amino acid composition of byssus fiber (●) from Mytilus californianus from Sonoma coast, California (Data from Table III-4, p. 97) compared to range of compositions reported for several invertebrate collagens (Data from Gross and Piez, 1960).

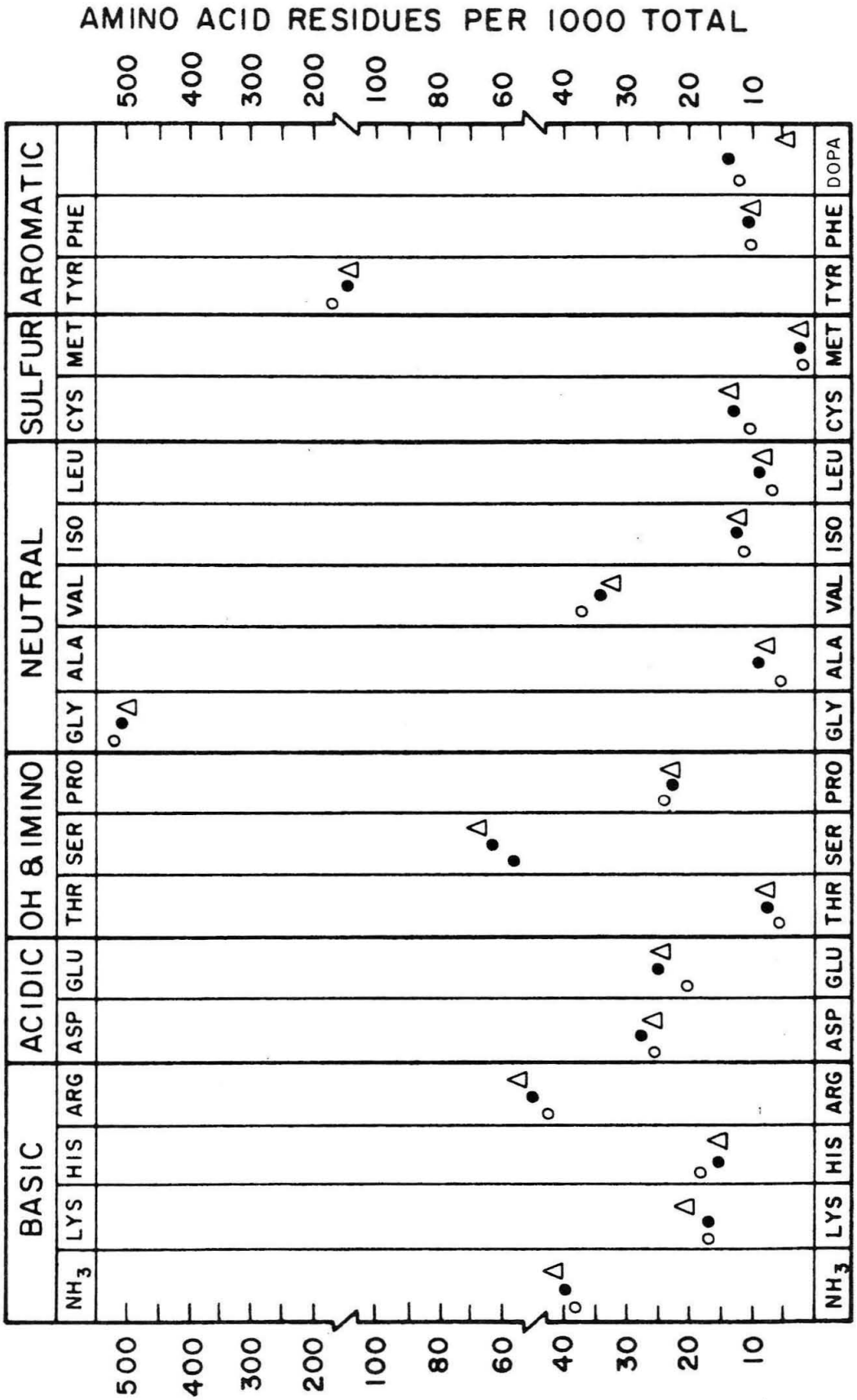


FIGURE 5. Amino acid composition of transparent periostracum from three different positions in Mytilus californianus from Sonoma coast, California. Data from Table III-4, p. 97.

○ 0-10%
● 25-36%
△ 60-80%

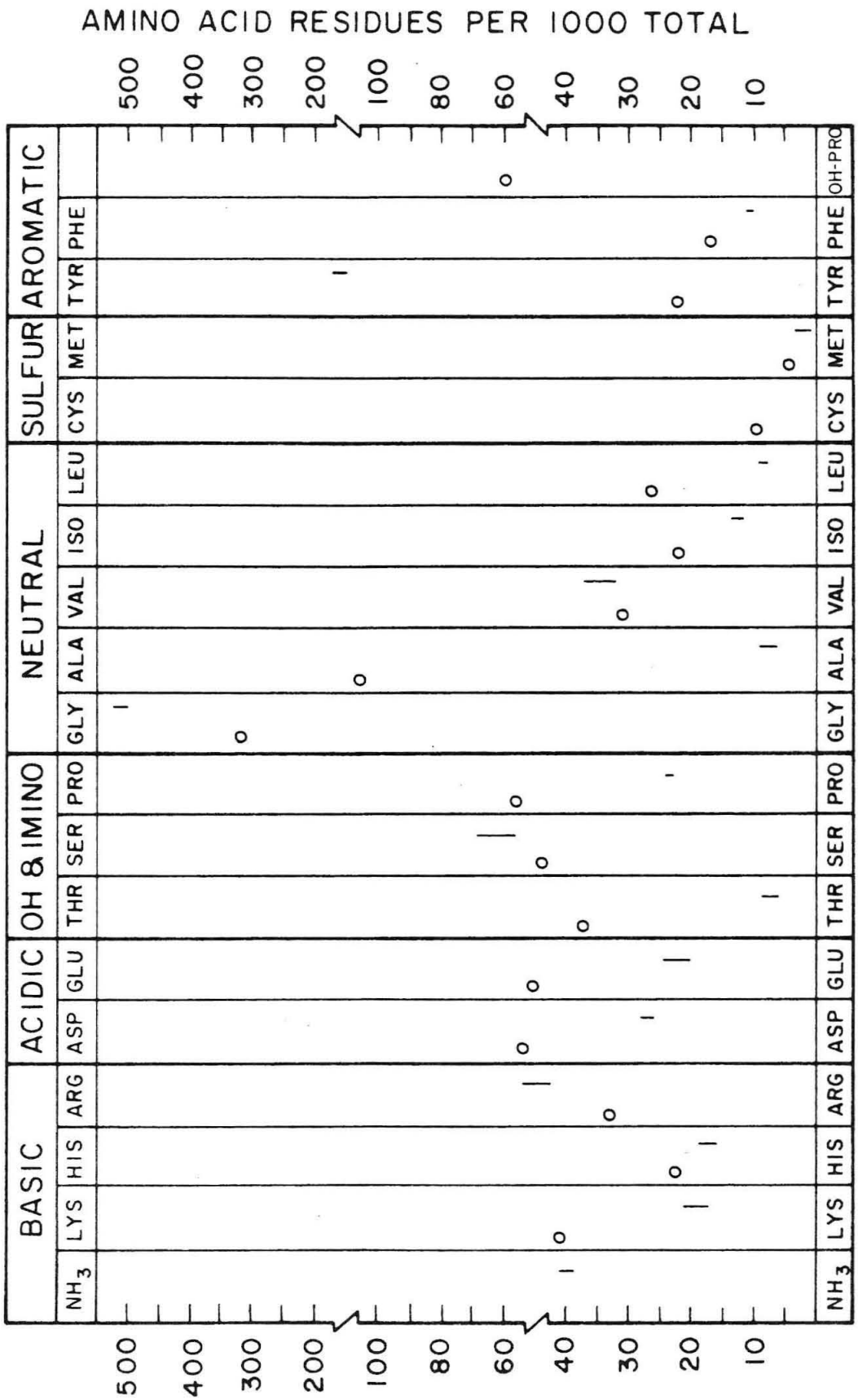


FIGURE 6. Amino acid composition of non-calcified components of Mytilus californianus shells from Sonoma coast, California. Data from Table III-4, p. 97.

o Byssus fiber

| Periostracum (range from Fig. 5)

amino acid composition of recently secreted periostracum. On the ventral side of the shell (70 - 80 per cent position in Figure 1) the recently secreted periostracum is typically thicker and more of a greenish color. On the posterior edge (25 - 35 per cent) of the shell, the periostracum is often typically reddish in appearance and generally thinner. Amino acid compositions varying with position are shown in Figure 5.

Calcified Components -- Ligament and Ligamental Ridge

Like the uncalcified components considered above, the ligament and ligamental ridge are poorly preserved in most fossil specimens. The ligament superficially appears to be entirely organic material but is actually approximately two-thirds aragonite and one-third organic matrix (mineralogy determined by x-ray diffraction). This large amount of organic material accounts for the preservation problem. Although the ligamental ridge has only a little over one per cent organic material, it has a porous structure and is often found recrystallized in older fossil materials.

Figure 7 shows the amino acid composition of the ligament and ligamental ridge compared to byssus fiber. Both proteins have distinctive compositions. By comparison, ligament matrix material has extremely high amounts of methionine (327 residues per 1000), proline (71 residues), and lysine (92 residues) and very low amounts of arginine (7.8 residues) and tyrosine (not detected). The organic material from the ligamental ridge has more nearly equal amounts of the various amino acids rather than the extreme values found in periostracum and

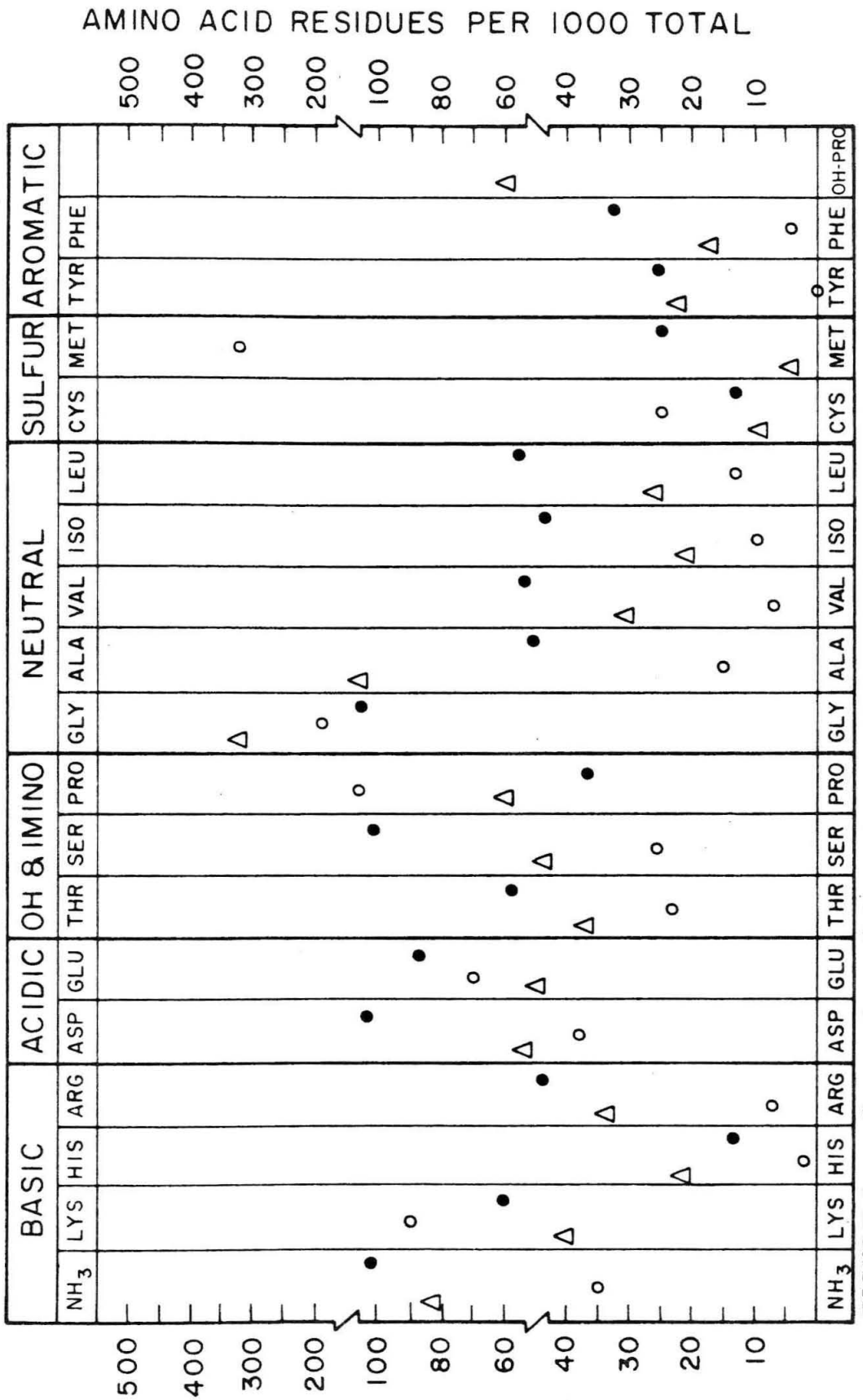


FIGURE 7. Comparison of the amino acid composition of the proteins from byssus fiber, ligament, and ligamental ridge, Mytilus californianus. Location: Sonoma coast, California. Data from Table III-4, p. 97.

Δ Byssus fiber

o Ligament matrix

● Ligamental ridge matrix

ligamental materials.

Outside Prismatic Layer

Morphologically, there are probably greater variations in the outside prismatic layer than in any of the other structural units of Mytilus californianus, with the possible exception of the periostracum. The outside prismatic layer is thicker on the dorsal side near the ligamental ridge. In most specimens, the outside layer is somewhat thinner on the ventral side and much less pigmented, possibly due to lack of incident light. There are large variations in shell thickness, shape, and color in specimens from the same locality. If the organic matrix composition is a factor in mineralization, one may expect to find systematic compositional differences in various parts of the outer prismatic layer reflecting morphological variations. Such seems to be the case.

Figure 8 shows the maximum range of amino acid concentrations found in the outside calcite layer of a single specimen. Figure 9A, B shows some of the amino acids and their variations in concentration with position in the outside layer starting at 0 per cent at the contact with the ligamental ridge and extending to 100 per cent at the anterior end of the shell (see Figure 1, page 13). Only the growing edge was included in this particular comparison because it was desired to compare only samples which were contemporaneously deposited.

In Figure 10 A, B , similar data for a second specimen is included. The curve derived from the previous specimen is superimposed to show correlation. Certain amino acids like aspartic acid and leucine show very little variation. In general, the trend of variations

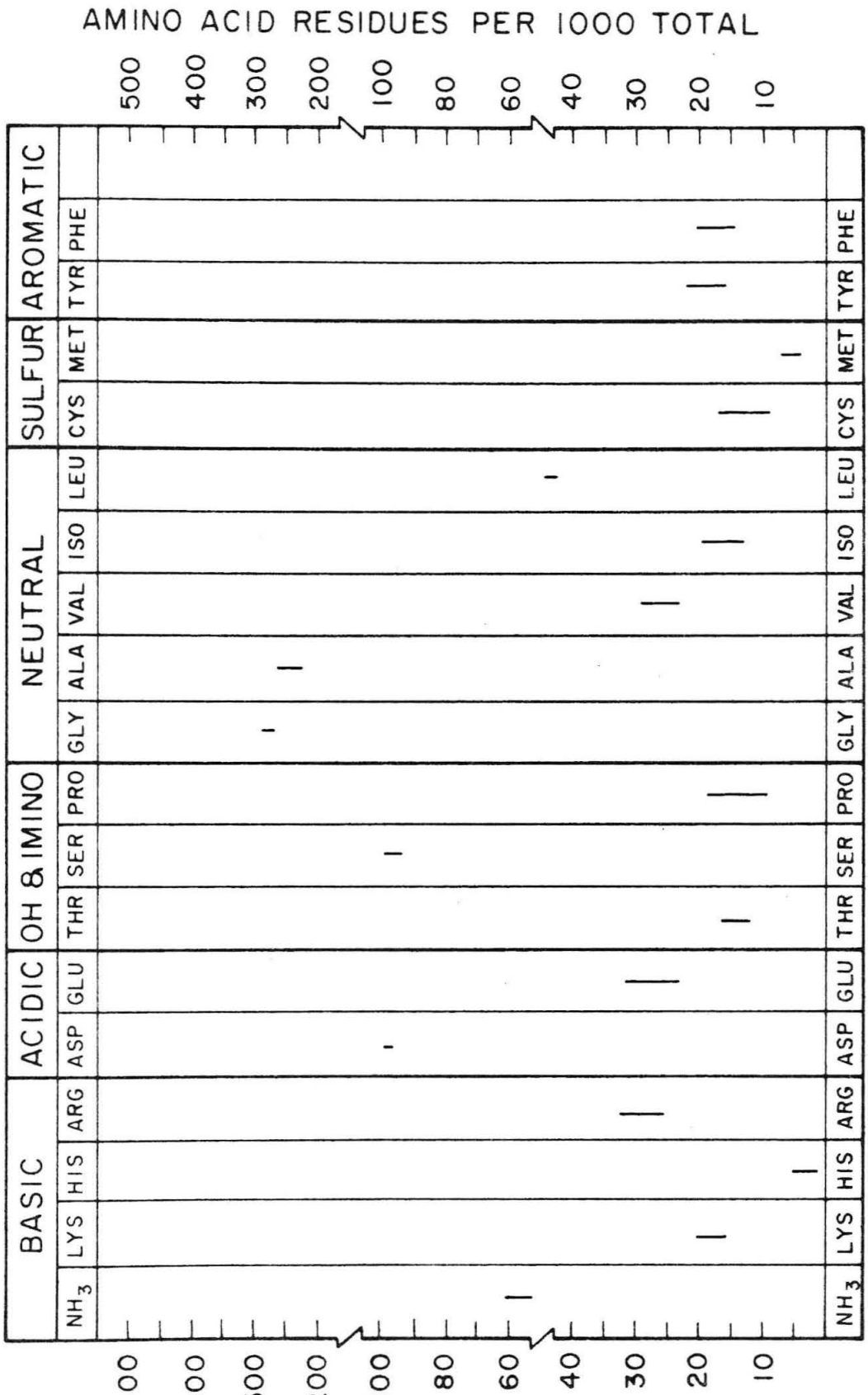


FIGURE 8. Maximum range of amino acid composition found in various samples of the organic matrix from the outer prismatic layer (calcite) of Mytilus californianus from Sonoma coast, California. Data from Tables III-5, 6, pp. 98, 99.

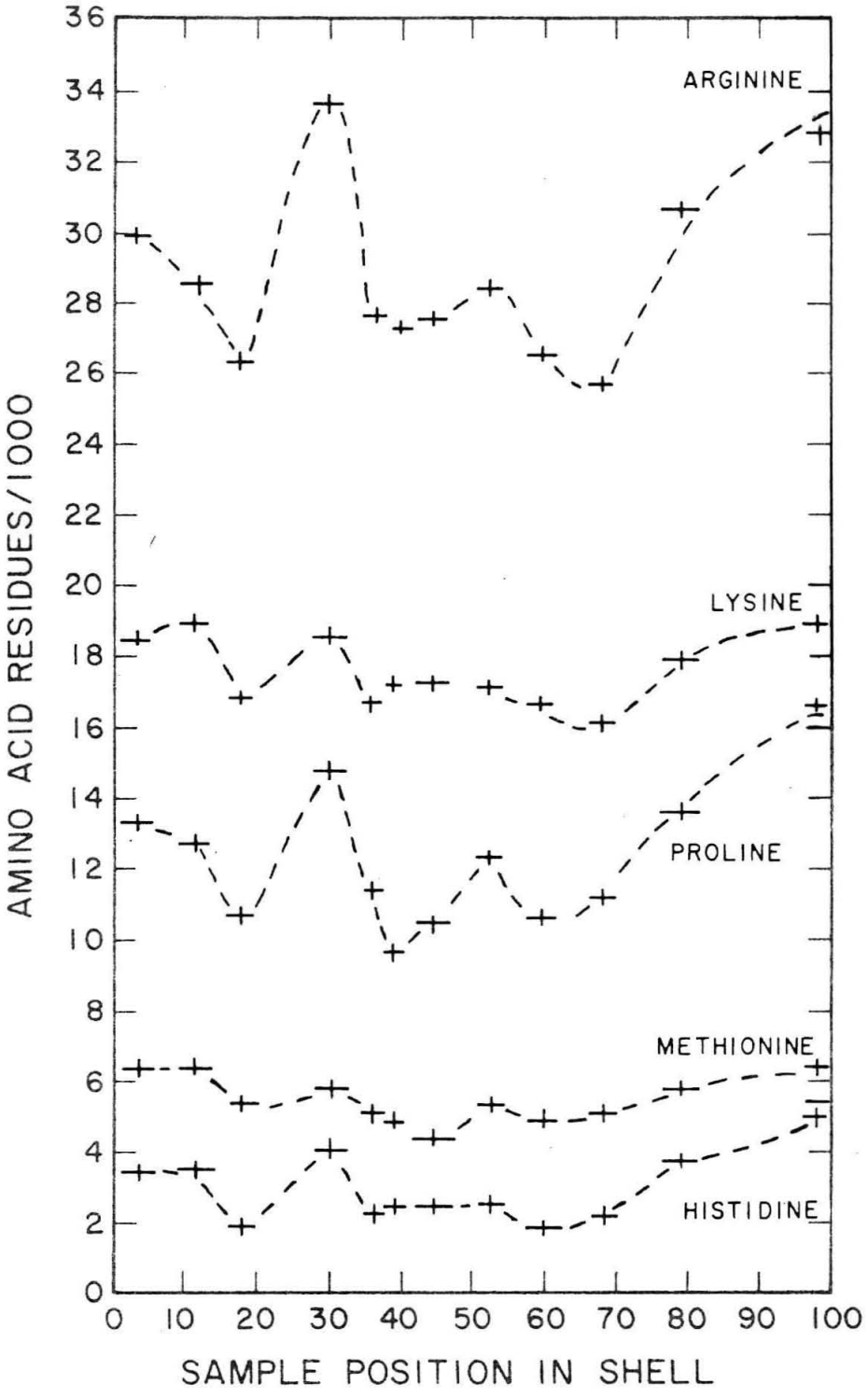


FIGURE 9A. Variations in certain amino acids with position in outer prismatic layer of specimen 1 (Mytilus californianus, Sonoma coast, California). Data from Table III-5, p. 98.

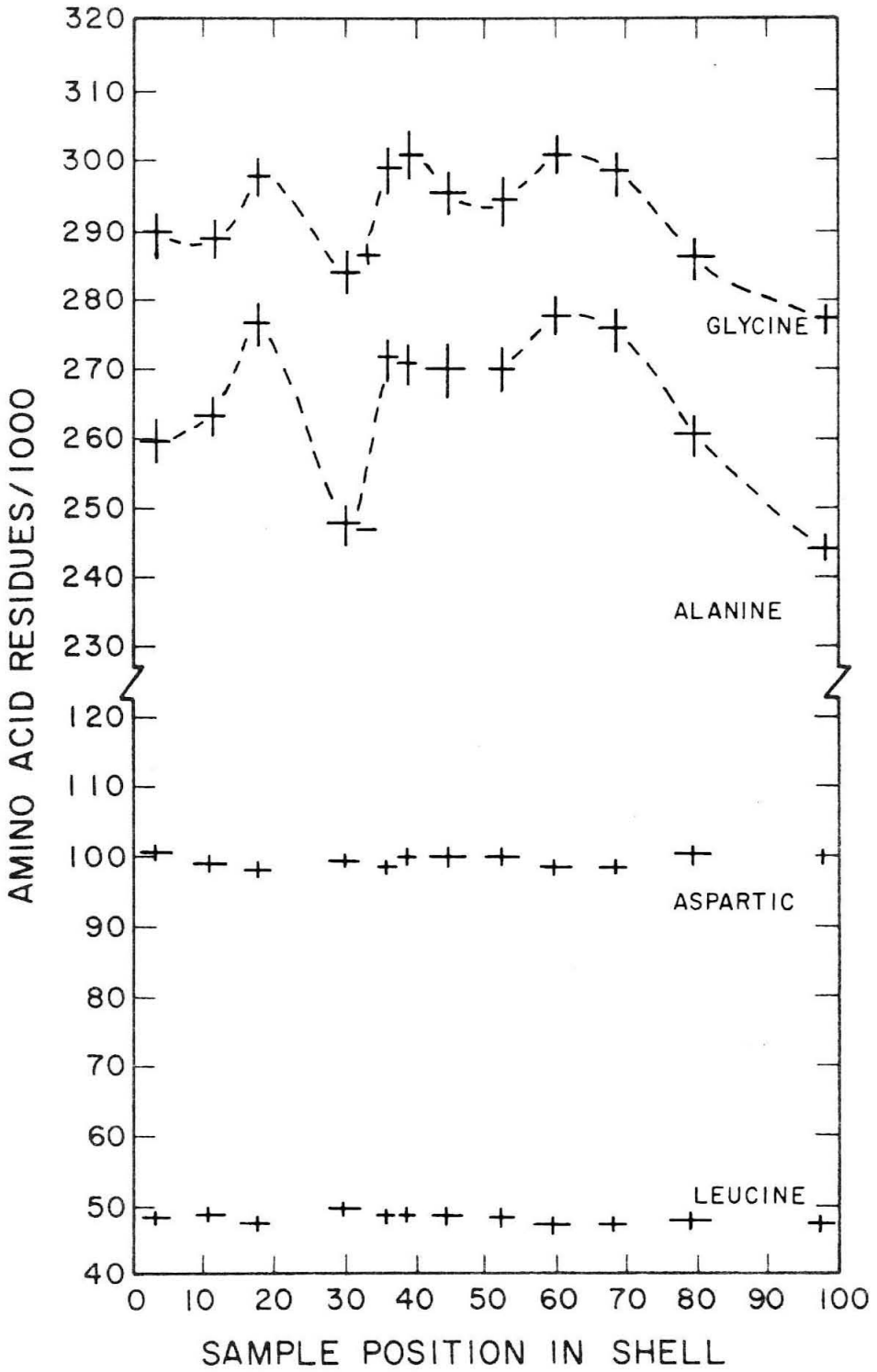


FIGURE 9B. Variations in certain amino acids with position in outer prismatic layer of specimen 1 (Mytilus californianus, Sonoma coast, California). Data from Table III-5, p. 98.

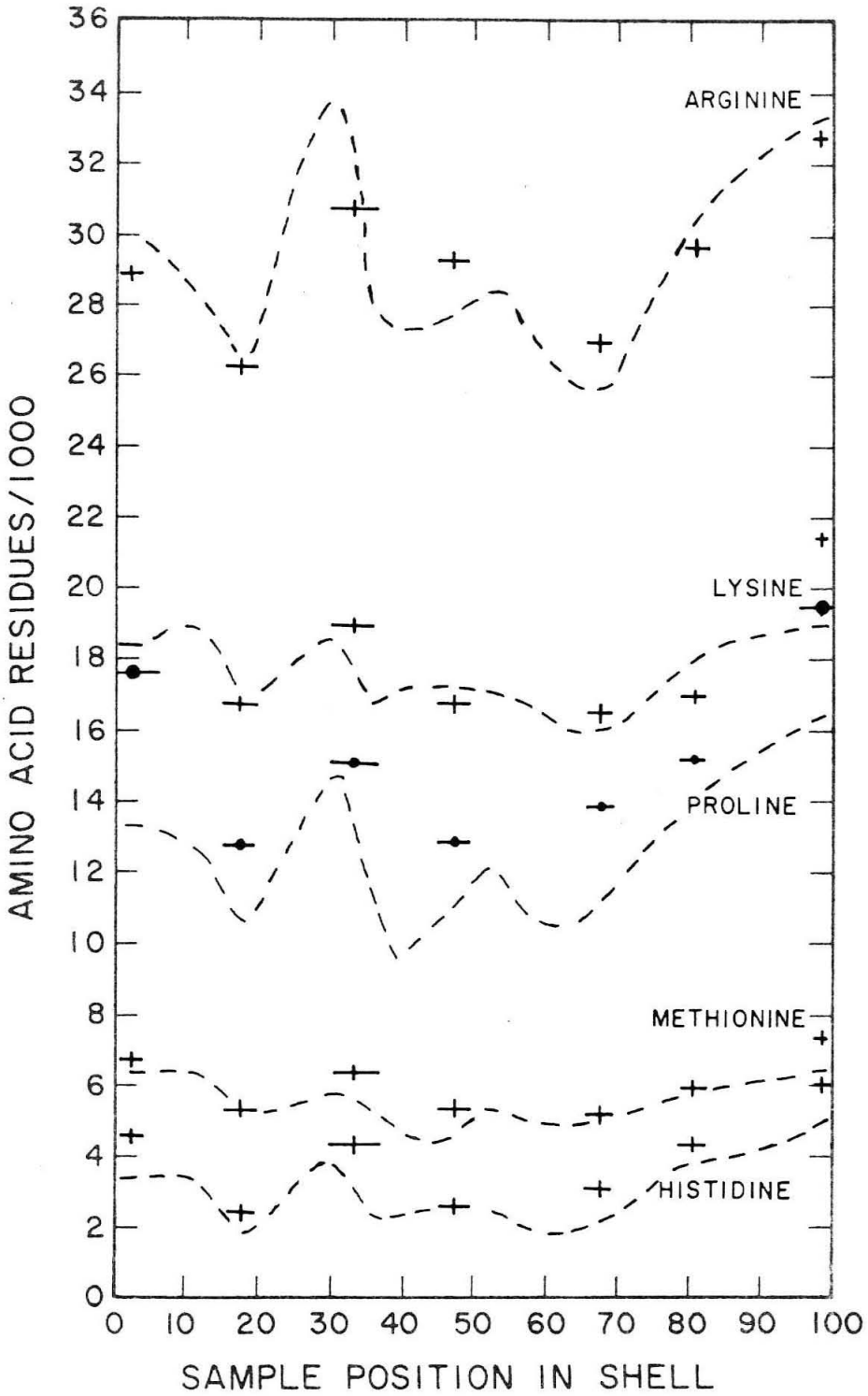


FIGURE 10A. Variations in certain amino acids with position in outer prismatic layer of specimen 2 (Mytilus californianus, Sonoma coast, California).

Curve is drawn from Figure 9A and is based on data for specimen 1.
Data from Table III-6, p. 99.

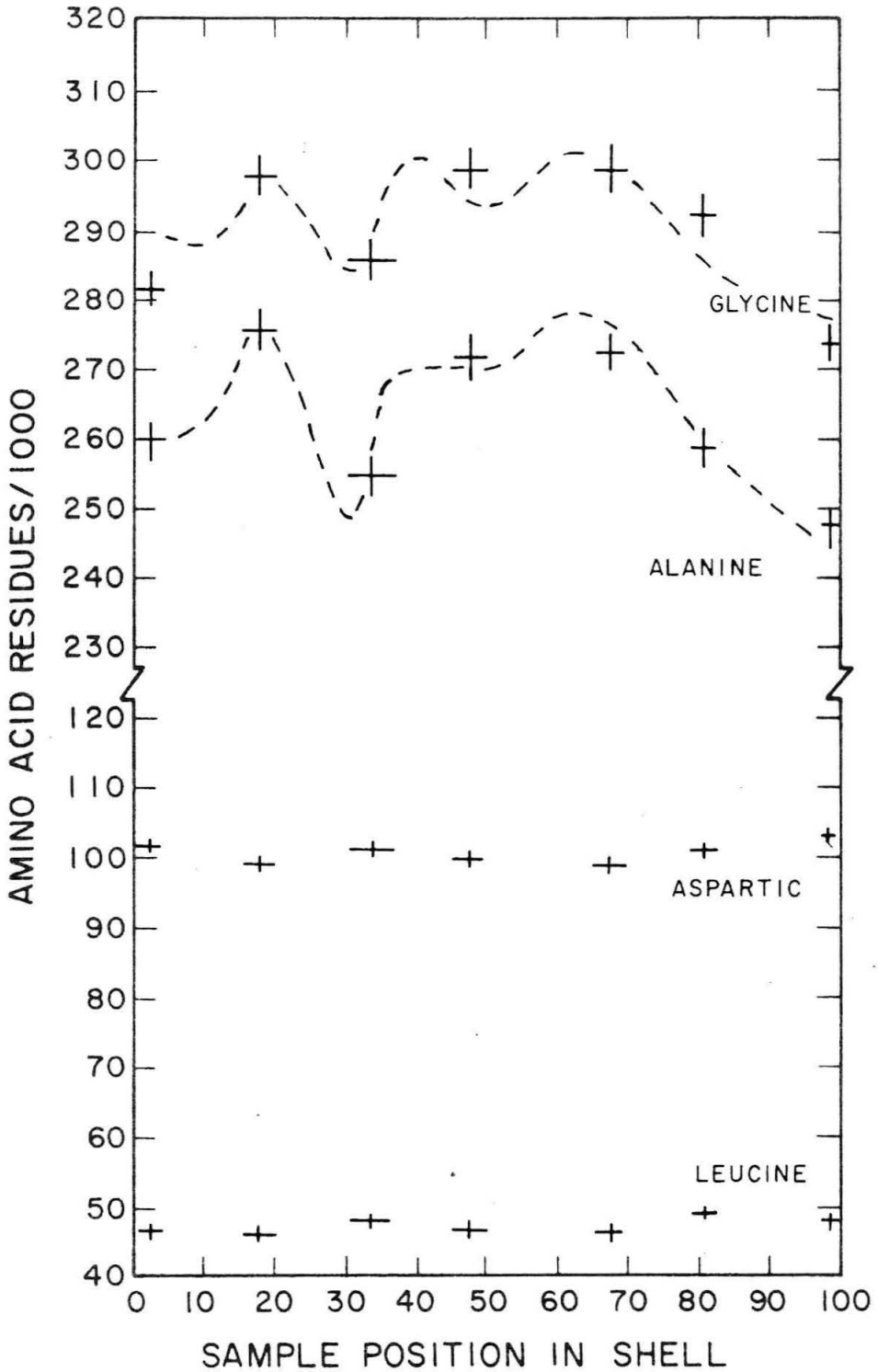


FIGURE 10B. Variations in certain amino acids with position in outer prismatic layer of specimen 2 (Mytilus californianus, Sonoma coast, California).

Curve is drawn from Figure 9B and is based on data for specimen 1.
Data from Table III-6, p. 99.

is similar in the two specimens. Deviations from ideal correlation are probably due to sampling errors, biological variation, and perhaps variations in the microenvironment.

Calcified Components -- Nacreous Layer and Inner Prismatic Layer

The nacreous layer of aragonite and the inner prismatic layer of calcite are intricately related. From the study of Dodd (1961), it appears that seasonal variations in the environment cause the organism to deposit inner prismatic and nacreous layers in differing amounts. This implies that certain cells in the mantle which normally secrete material forming the nacreous aragonite layer change to secrete material for the inside calcitic prismatic layer. Cooler temperatures favor relatively large amounts of inner prismatic material to form; consequently, since the specimens were from northern California, there are well-developed inner prismatic layers present.

Figures 11, 12A, B compare the amino acid composition of the matrix of the outer and inner calcite layers and the nacreous aragonite layer. There is actually very little apparent difference in the composition of the matrix associated with the three layers. The observation of Roche (1951) that glycine and tyrosine are predominant in calcite matrices seems to be confirmed for glycine but not for tyrosine. No Mytilus specimens were included in Roche's study.

The most clear cut distinction seems to be in the concentration of lysine and ammonia. The nacreous aragonite matrix has from 25-29 lysine residues and 66 - 70 ammonia residues, while the values for the outer and inner prismatic calcite matrices are somewhat lower

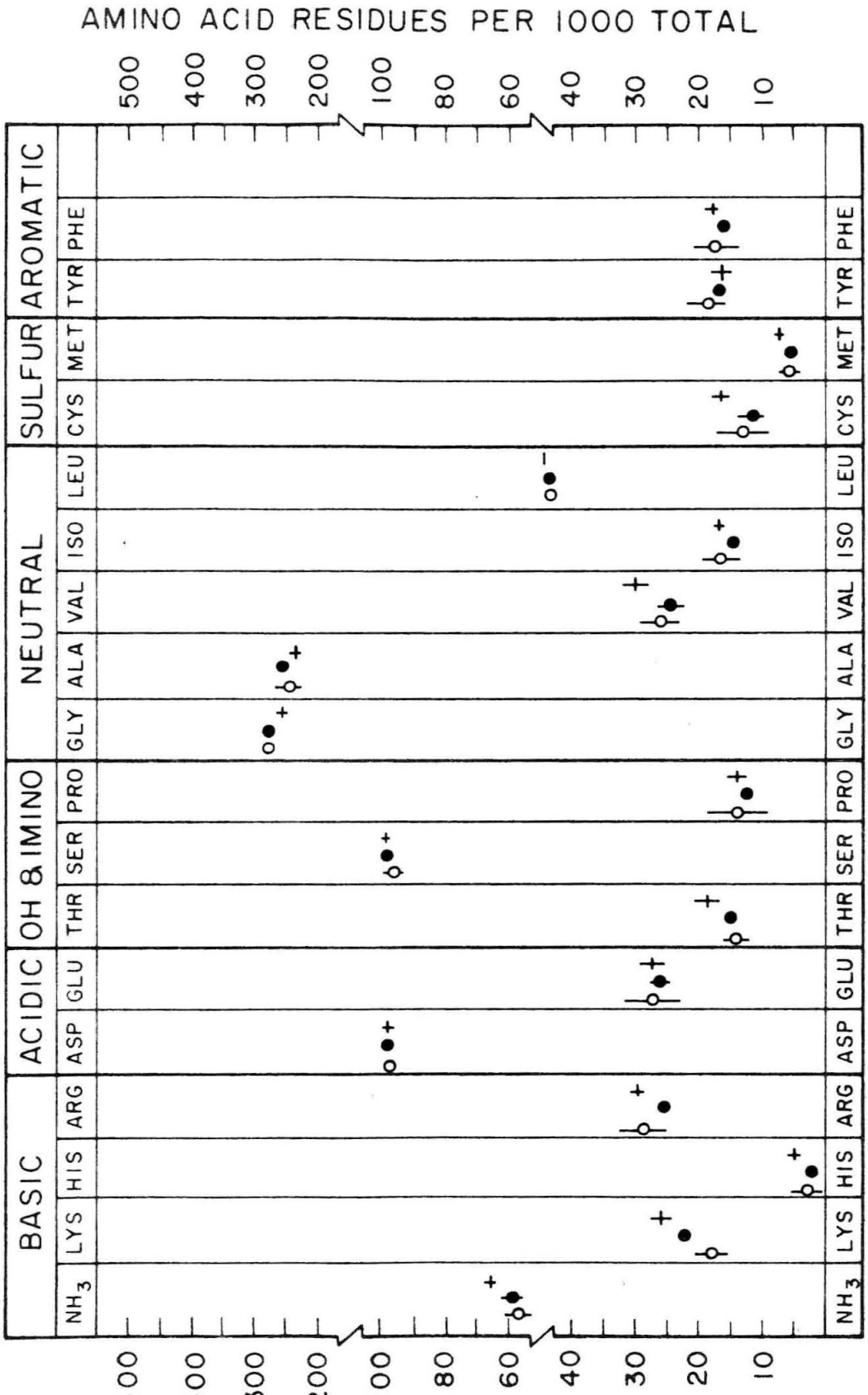


FIGURE 11. Comparison of amino acid compositions for outer prismatic layer, inner prismatic layer and nacreous layer for Mytilus californianus from Sonoma coast, California. Data from Tables III-5, 6, 7, 8, pp. 98-101.

o Outer prismatic layer (calcite)
● Inner prismatic layer (calcite)
+ Nacreous layer (aragonite)

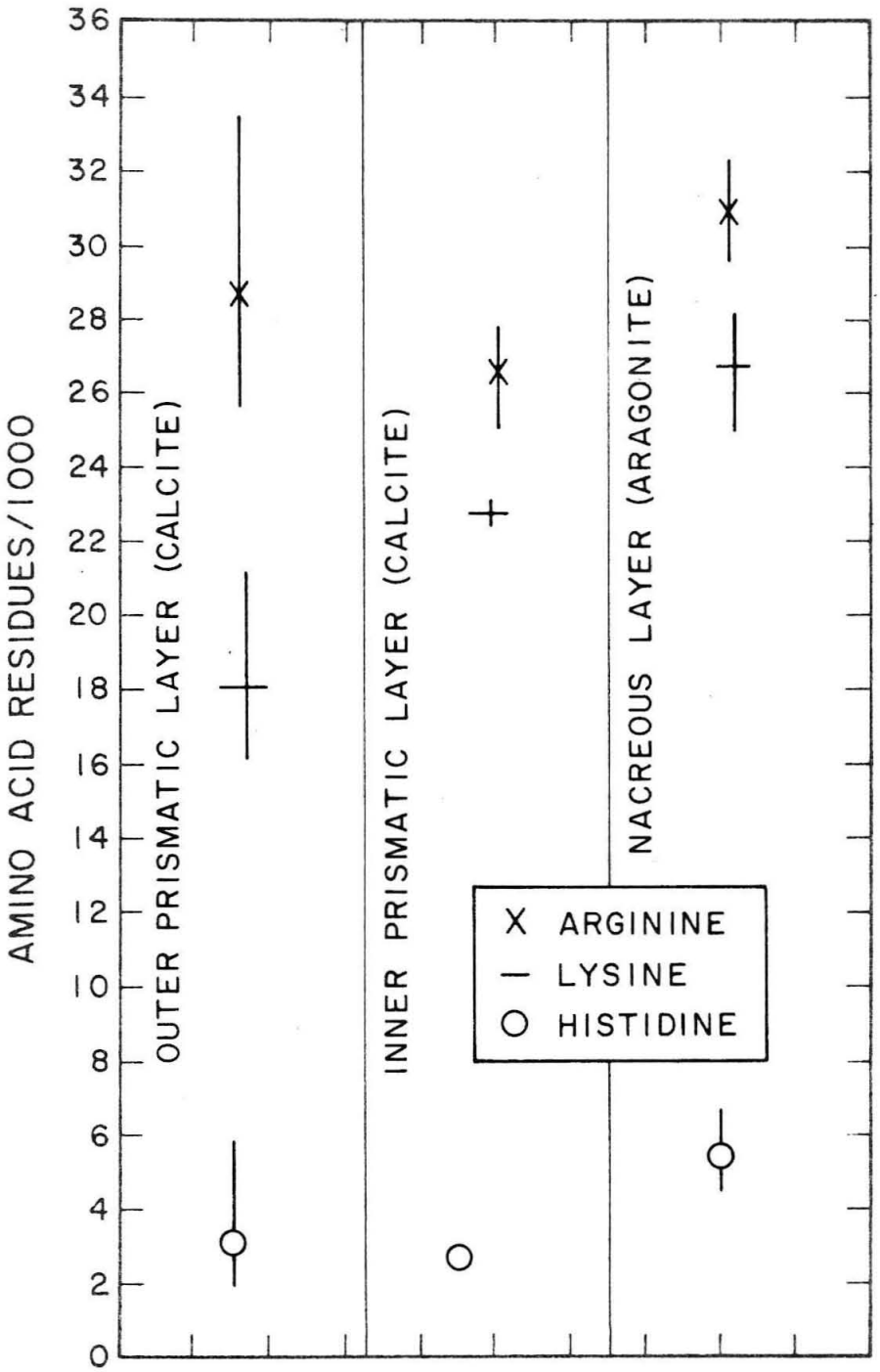


FIGURE 12A. Comparison of certain amino acids in aragonite and calcite layers of shells of Mytilus californianus from Sonoma coast, California.
Data from Table III-5, 6, 7, 8.

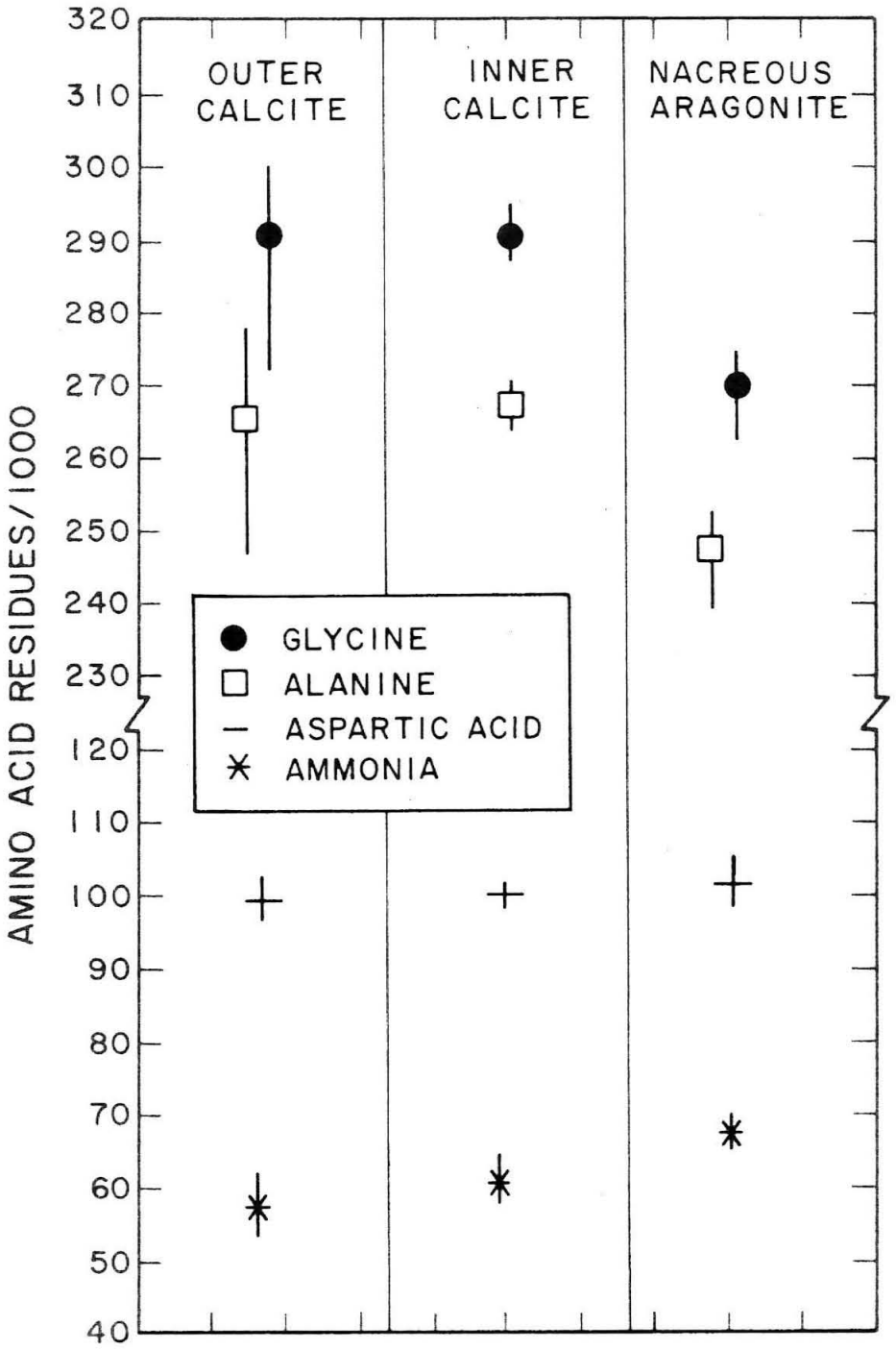


FIGURE 12B. Comparison of certain amino acids in aragonite and calcite layers of shells of Mytilus californianus from Sonoma coast, California.
Data from Table III-5, 6, 7, 8.

(16 - 23 for lysine and 55 - 65 for ammonia).

To understand the significance of the ammonia content, it is necessary to determine its source. As indicated in Appendix I, when asparagine and glutamine (both neutral amino acids) are subjected to acid hydrolysis, they yield quantitatively ammonia and the corresponding acidic amino acids -- aspartic or glutamic acid. This is probably the source of most of the ammonia. Some ammonia probably results from amino acid decomposition during hydrolysis, but since longer hydrolysis times increase the amount of ammonia only slightly, probably only a small fraction of the ammonia comes from the decomposition of amino acids other than glutamine and asparagine.

The ammonia present in the reagents used in hydrolysis and sample preparation was analyzed and subtracted from the analysis of each protein. This correction was of the order of 1 - 4 per cent of the total. The consistent ammonia results from the large number of samples run, suggest that the ammonia is coming from a definite constituent of the sample -- most probably glutamine and asparagine.

The presence of consistently larger amounts of lysine and ammonia in the matrix associated with aragonite suggests that perhaps the balance between the acidic and basic amino acids may be significant. The sum of the aspartic and glutamic acid residues less the amount of ammonia would give the number of actual acidic amino acids present in the protein. The residues of the basic amino acids lysine, histidine, and arginine are then summed up and compared to the number of acidic amino acids. The net number of acidic or basic amino acid residues/1000 is then calculated.

The results of such an analysis are plotted on Figure 13. The vertical axis is a representation of the degree of calcification. In the data in the appendix, the total number of micromoles of amino acid recovered per gram of total shell material is given. For most of the proteins involved in this study, there are approximately 10 micromoles of amino acids per milligram of protein. A value of 9600 micromoles recovered per gram of byssus fiber indicates approximately 960 milligrams per gram, or about 96 per cent of the material, is amino acid material. A value of 100 micromoles per gram of shell material would indicate approximately one per cent of the total shell material was amino acid material.

It is interesting to note that the proteins associated with calcite definitely have a higher proportion of acidic amino acids than do the aragonite matrices. There seems to be no overlapping. Considering the data from the outside layer alone, there seems to be a suggestion of a trend toward a higher percentage of organic matrix with an increase in the basic amino acids. For example, the calcite samples with the highest percentage of organic material generally have a lower proportion of acidic amino acids.

A similar trend seems to hold for aragonite. The greater the percentage of organic material, the higher the net number of basic amino acids is. In the extreme case of the non-calcified byssus fiber and periostracum, there is an extremely large excess of basic amino acids.

A comparison of the data from the inner calcite layer with that of the nacreous layer shows that the matrix of the inner calcite layer is

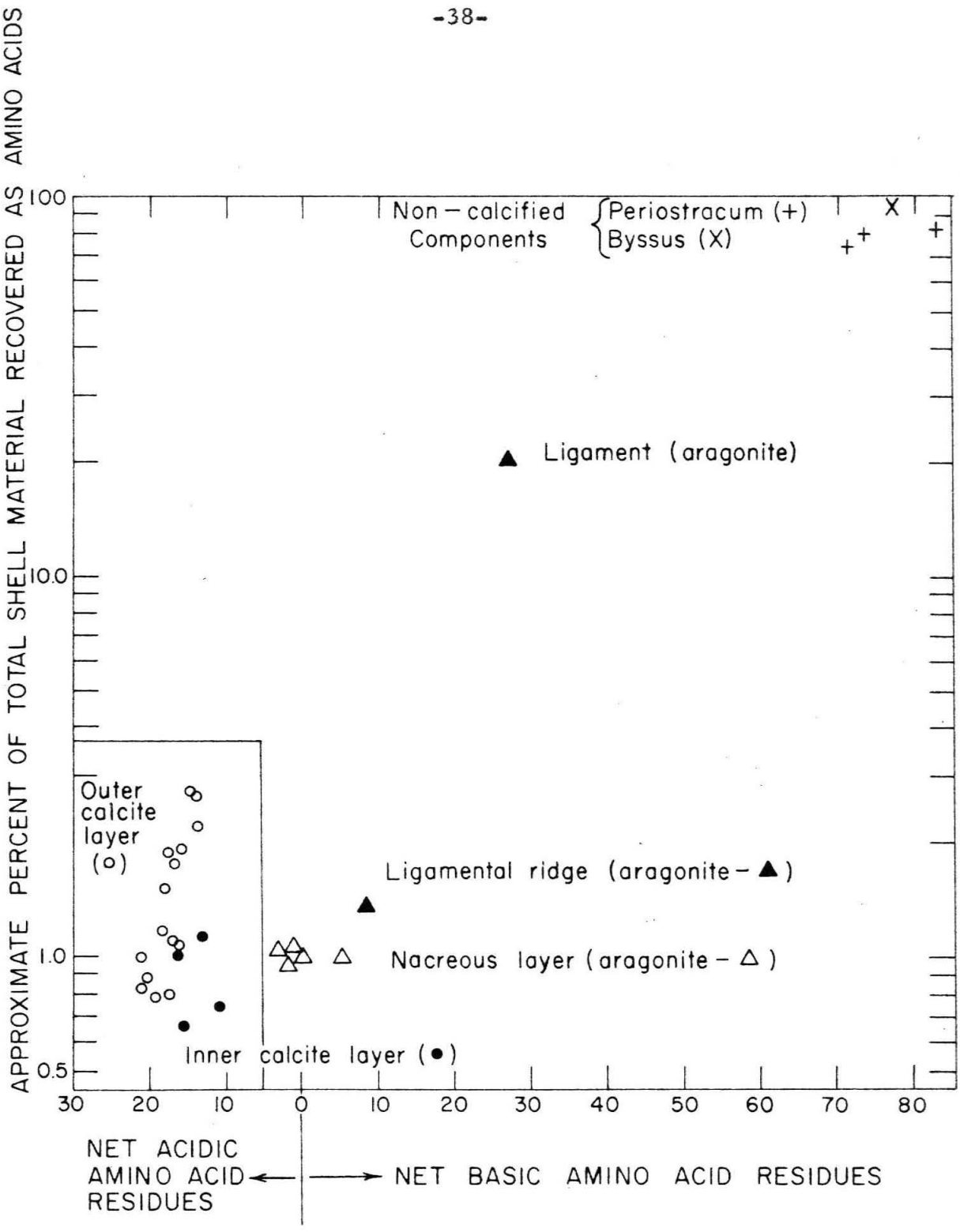


FIGURE 13. A plot of the approximate percentage of organic material against the net number of excess acidic or basic amino acid residues. Net acidic residues are calculated from $\Sigma(ASP + GLU - NH_3) - \Sigma(LYS + HIS + ARG)$.

very similar to that of the outside calcite layer with respect to the net excess of acidic amino acids. Since the inner prismatic and nacreous layers are deposited at times by the same part of the mantle (Dodd, 1961), the data suggest that the difference in the crystallographic form deposited may possibly be influenced by the net excess of acidic or basic amino acids in the protein. A pH effect in the freshly elaborated organic matrix may be one of the factors in aragonite-calcite deposition in shell materials. The data of Zeller and Wray (1956) suggest that pH is one of the factors which may influence the inorganic precipitation of calcite and aragonite. A higher pH may favor aragonite deposition due to the coprecipitation of trace amounts of ions larger than calcium.

The organic matrix present in the calcified shell material has very possibly undergone some changes in physical and chemical properties from the organic material first elaborated during the initial stages of calcification. Data on this possibility are not available at present.

VARIATIONS IN THE AMINO ACID COMPOSITION OF SHELL PROTEINS OF MYTILUS

In this chapter, the compositions of the organic matrix proteins from specimens of M. californianus and M. edulis diegensis from different environments are compared to determine the possible effects of temperature and salinity. That temperature and salinity are important factors in the aragonite-calcite ratios of these species has been shown by Lowenstam (1954a, b, c) and Dodd(1961). Therefore, if the organic matrix is a factor in determining the crystallographic form of $CaCO_3$ deposited, it might be expected that the composition of the organic matrix of the aragonite and/or calcite would reflect changes in temperature and salinity.

Another aspect considered is the differences existing in the organic matrices of related species of the same genus. This would seem to be of particular significance in biochemical evolution and may well shed some light on some of the factors involved when morphological changes take place in time. For this purpose M. californianus and M. edulis diegensis from similar environments are compared.

In the previous chapter, only contemporaneously deposited organic material from the growing edge of the periostracum and the outer prismatic calcite layer were considered in discussing the organic composition of these particular structural units. The older material forming the bulk of the shell has, since its formation, been subjected to successively longer periods of repeated exposure to air (for intertidal specimens) and submergence in sea water. What effect, if any, does this treatment have on the shell organic material? In the perio-

stracum, successive increments from the posterior edge of the shell toward the beak represent successively older material and hence material subjected to possible degradative activity for progressively longer periods of time. A study of these increments should reveal the effects of short term exposure. However, in interpreting the results of such a study, it is first necessary to consider the effects of shell size (or age) by comparing a graded series of shells collected from the same locality at the same time.

Effect of Shell Size on Periostracum Composition

The samples for this study were specimens of Mytilus californianus taken from an open-coast environment near Newport Beach, California. They range in length from 20 mm to 150 mm.

The periostracum was taken from similar positions from each specimen (approximately 50 to 60 per cent on Figure 1). Samples of pigmented periostracum were taken from larger specimens at varying distances from the growing edge. Therefore, in the pigmented periostracum series the samples have been subjected to possible degradative action for a varying period of up to three years for the 150 mm specimen (Coe and Fox, 1942, 1944; Fox and Coe, 1943).

It is of interest to compare relatively recently pigmented periostracum with transparent periostracum at the same relative position in the shell. This can be done by comparing the largest specimen in both series (see Table III-9, p.102). The most readily apparent differences seem to be decreases in the amounts of tyrosine and lysine and increases in alanine, glycine, aspartic acid, and ammonia.

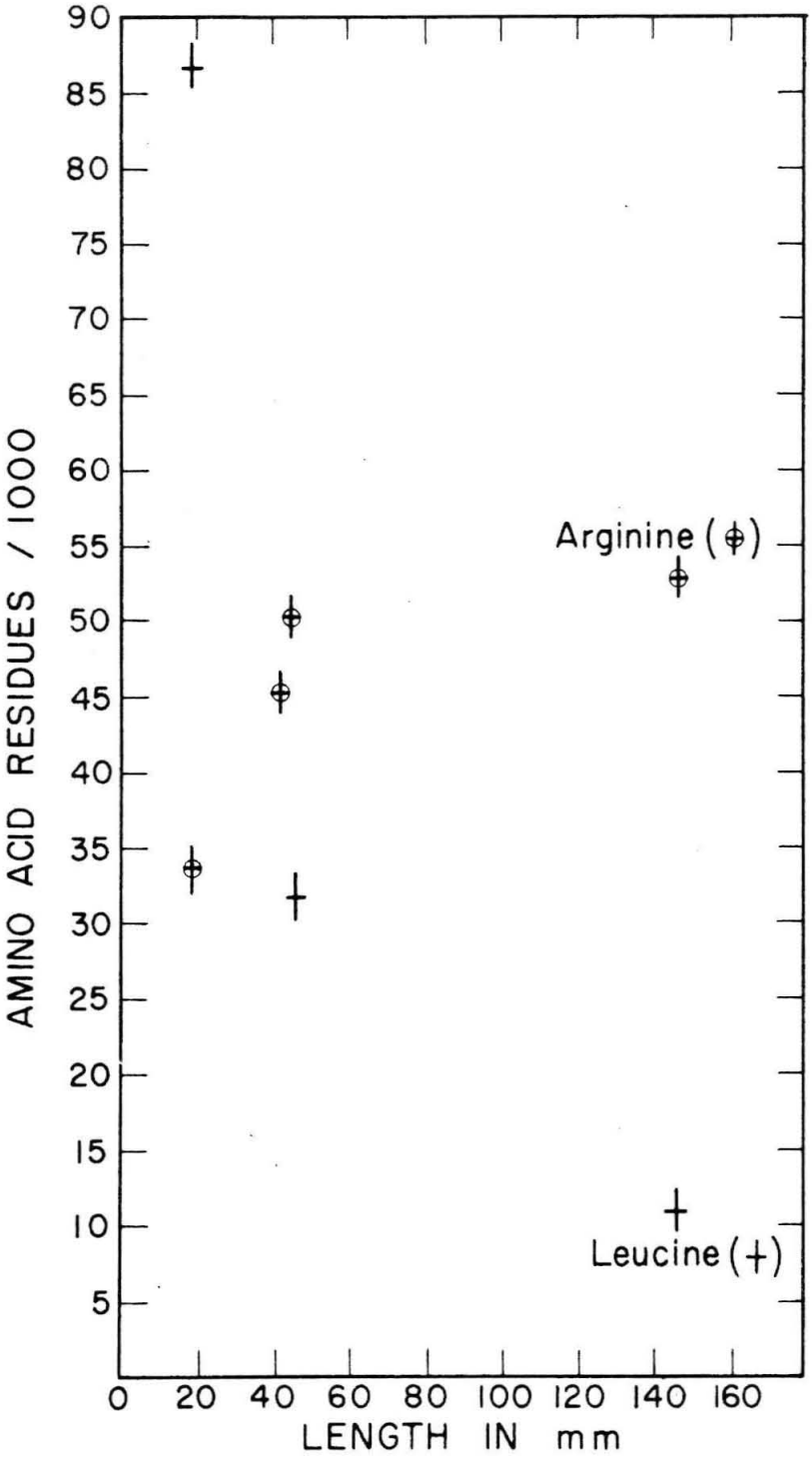


FIGURE 14A. Effect of shell size on the composition of transparent periostracum of Mytilus californianus from Corona del Mar, California.

Data from Table III-9, p. 102.

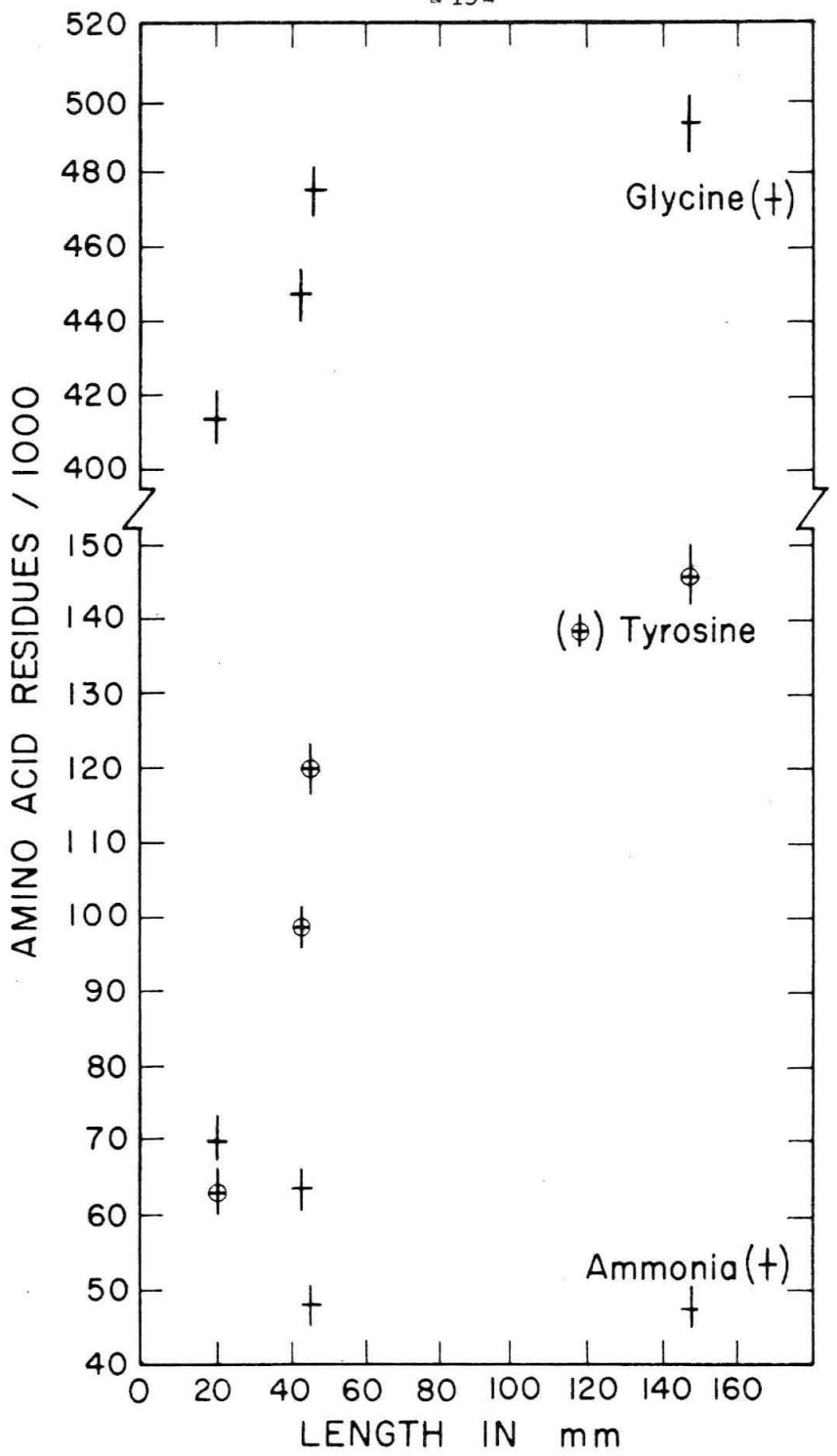


FIGURE 14B. Effect of shell size on the composition of transparent periostracum of Mytilus californianus from Corona del Mar, California.

Data from Table III-9, p. 102.

The data for the transparent periostracum from the graded series of specimens are shown in Figs. 14A, B. Profound changes occur in the composition of the periostracum as the individual increases in size. After about a length of 70 or 80 mm is attained, relatively little further change is noted. These profound variations in the periostracum composition were unexpected. Possibly they reflect physiological changes associated with the relatively faster growth rates of smaller specimens.

Figures 15A and 15B show a similar trend from older pigmented periostracum taken from larger individuals. Clearly, the similar trends would seem to indicate that the periostracum after pigmentation preserves its compositional integrity and does not alter to any large extent in the few years it is exposed to sea water and atmosphere.

Effect of Shell Size on the Composition of the Organic Matrix from the Outer Prismatic Calcite Layer

The outer prismatic calcite layer of Mytilus shells contains only about one per cent protein. This makes it difficult to obtain samples of adequate size from very small specimens. Consequently, instead of sampling only a small fraction of the outer prismatic layer as can be done with larger specimens, it is necessary to sample a relatively large fraction of the growing edge. Data in Table III-11 , page 104, are given for a 20 mm shell compared to a 150 mm shell. There is little difference. This is in marked contrast to the periostracum composition of comparably sized shells. This may indicate a very specific nature for calcified proteins. It may be possible to vary the composition of the calcified proteins only within certain narrow limits,

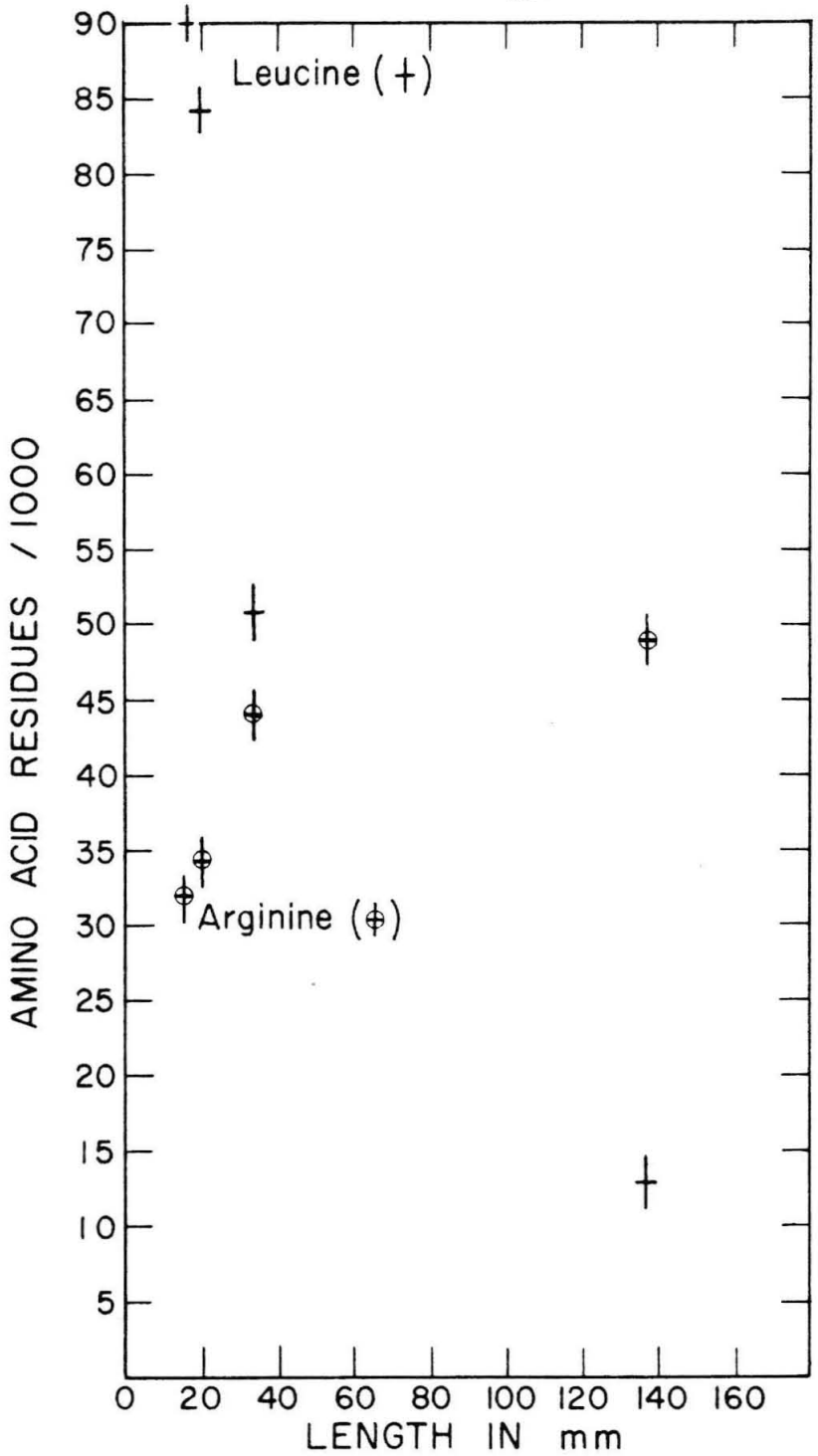


FIGURE 15A. Effect of shell size at time of deposition on the composition of pigmented periostracum of Mytilus californianus from Corona del Mar, California.

Data from Table III-9, p. 102.

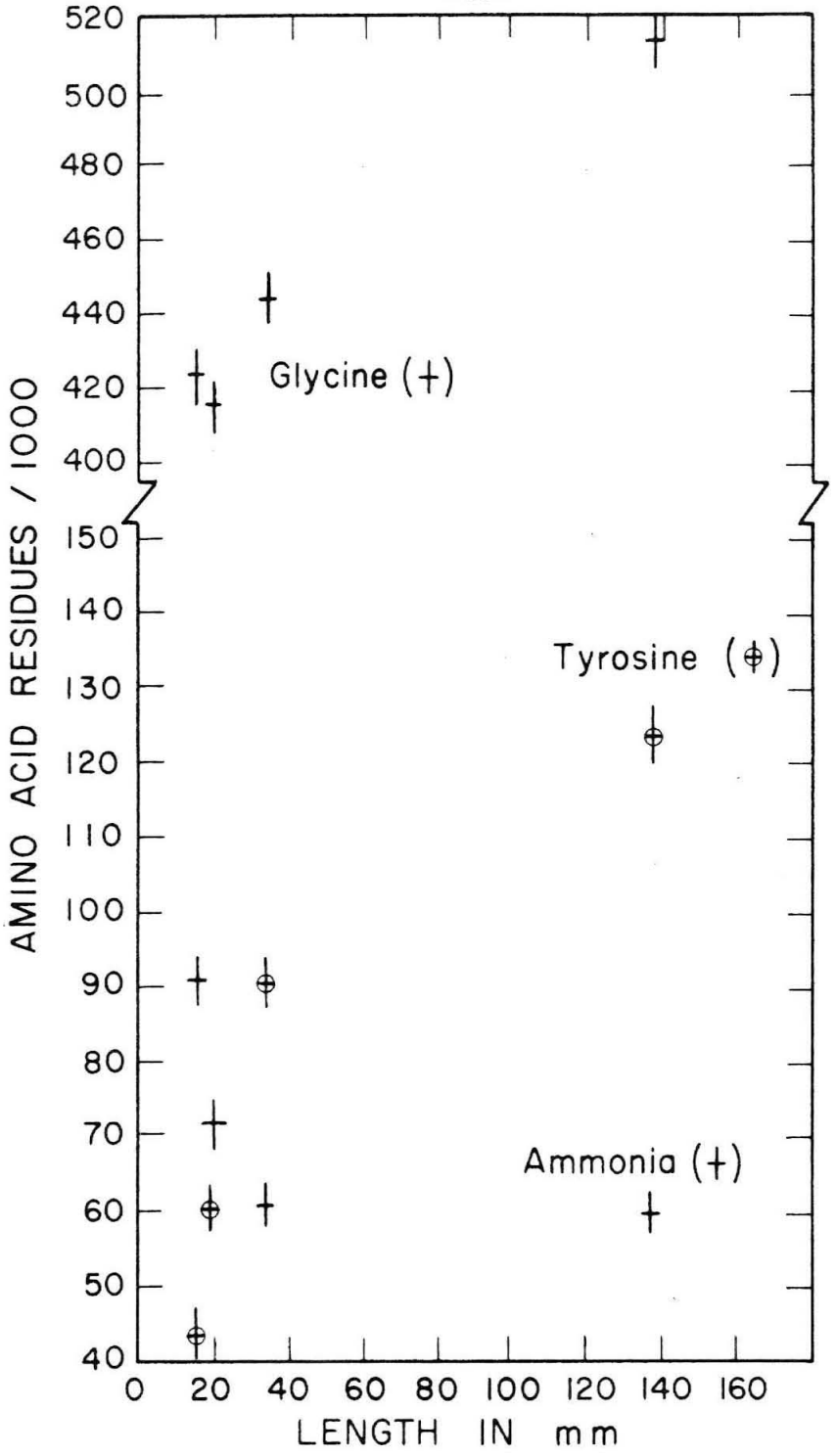


FIGURE 15B. Effect of shell size at time of deposition on the composition of pigmented periostracum of Mytilus californianus from Corona del Mar, California.

Data from Table III-9, p. 102.

outside of which calcification cannot occur (see Glimcher, 1960, on the specificity of organic matrices in mineralization).

In Table III-10 is given the data for a series of specimens from Sonoma, ranging in size from 48 mm to 155 mm. The sums of the deviations from the average individual amino acid concentration values vary from 12 to 23 parts per thousand, or between 1 and a little over 2 per cent. No definite trends seem to exist.

Comparison of Successive Increments of Outer Calcite Material

For this study, a 150 mm specimen from Corona del Mar was sampled first around the growing edge from the ligamental ridge contact to the beak (see Figure 1). Next, successive increments were taken starting at the posterior edge and working toward the beak.

The variation with position of the most recently deposited material is shown in Figures 16A and B. The trends are similar to those from Sonoma (see Figure 10A,B), although some differences exist in details.

The position at the posterior end of the shell (37 - 46 per cent in Figure 1) was selected for the traverse, and a total of eight samples were prepared extending 80 mm toward the beak from the posterior edge. From the data of Coe and Fox (1942, 1944) this should represent about two years of growth. The results are shown in Figures 17 A and B . Most of the amino acids do not show any definite periodic trends. There seems to be a periodic variation in glutamic acid, glycine, and possibly alanine. Lysine appears to generally decrease in amount, but superimposed on this trend seems to be a second, perhaps periodic, variation. If the variations in glutamic acid and glycine do

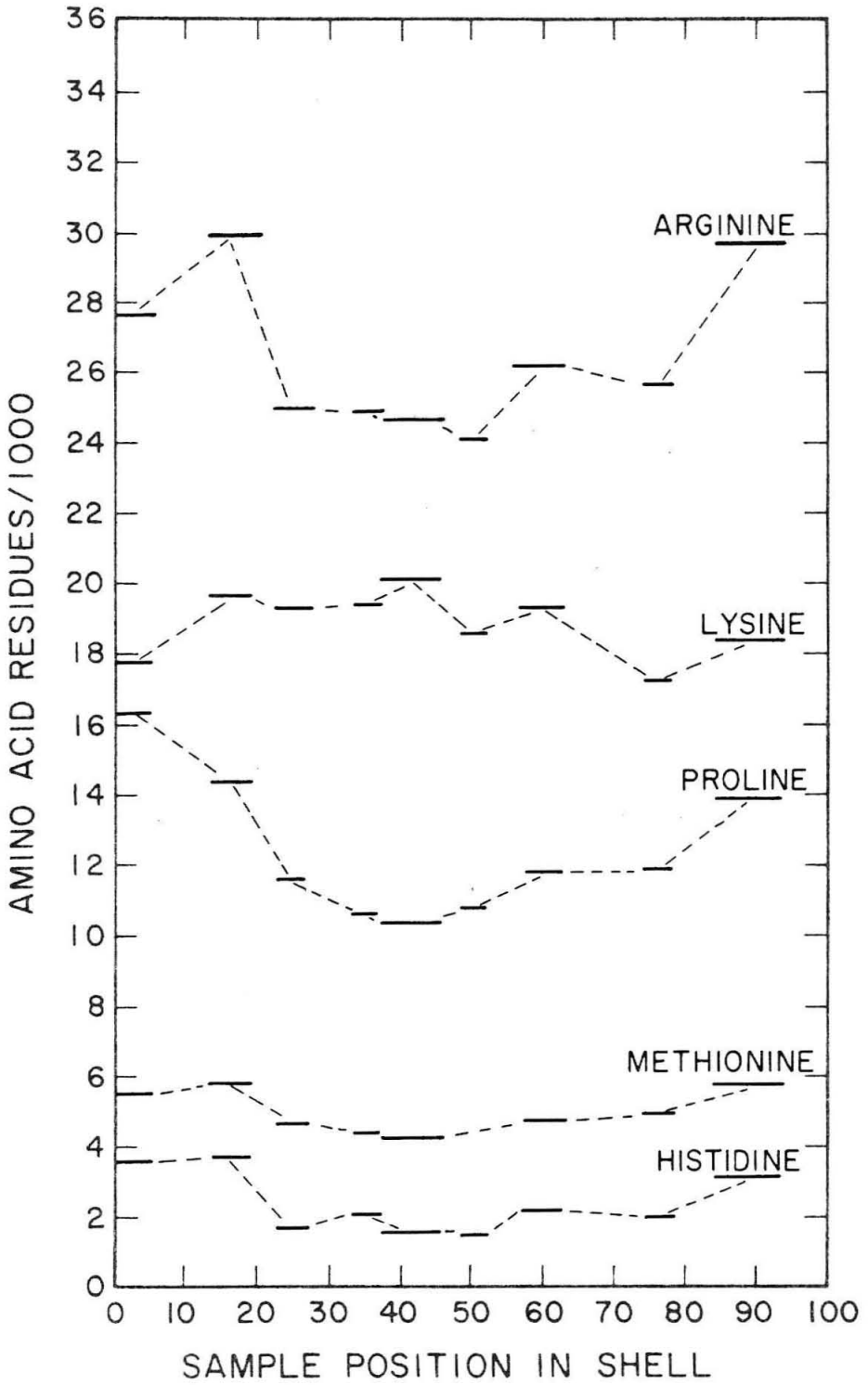


FIGURE 16A. Variations in certain amino acids with position in the outer prismatic layer of a specimen of Mytilus californianus from Corona del Mar, California.
Data from Table III-11, p. 104.

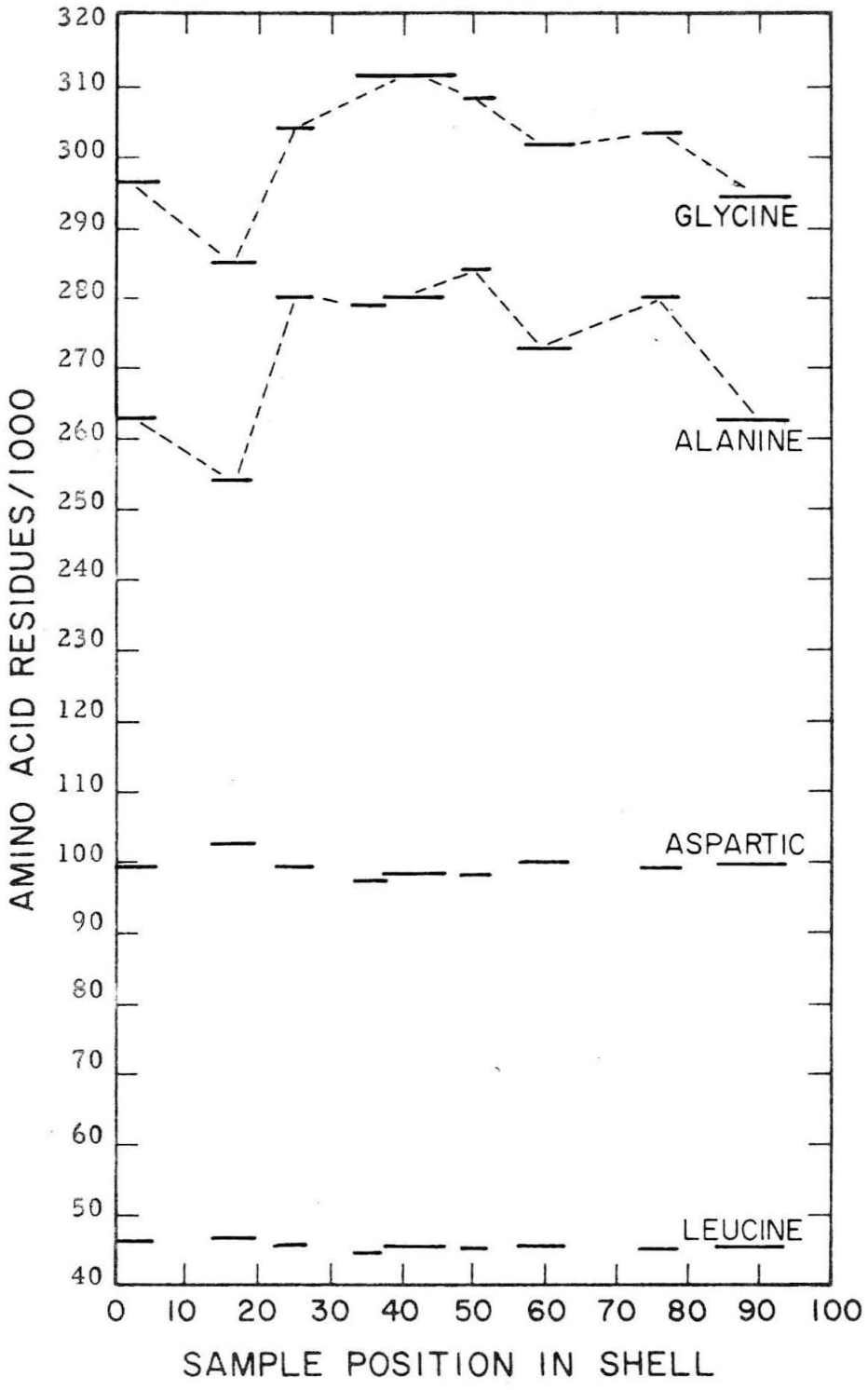


FIGURE 16B. Variations in certain amino acids with position in the outer prismatic layer of a specimen of Mytilus californianus from Corona del Mar, California.
Data from Table III-11, p. 104.

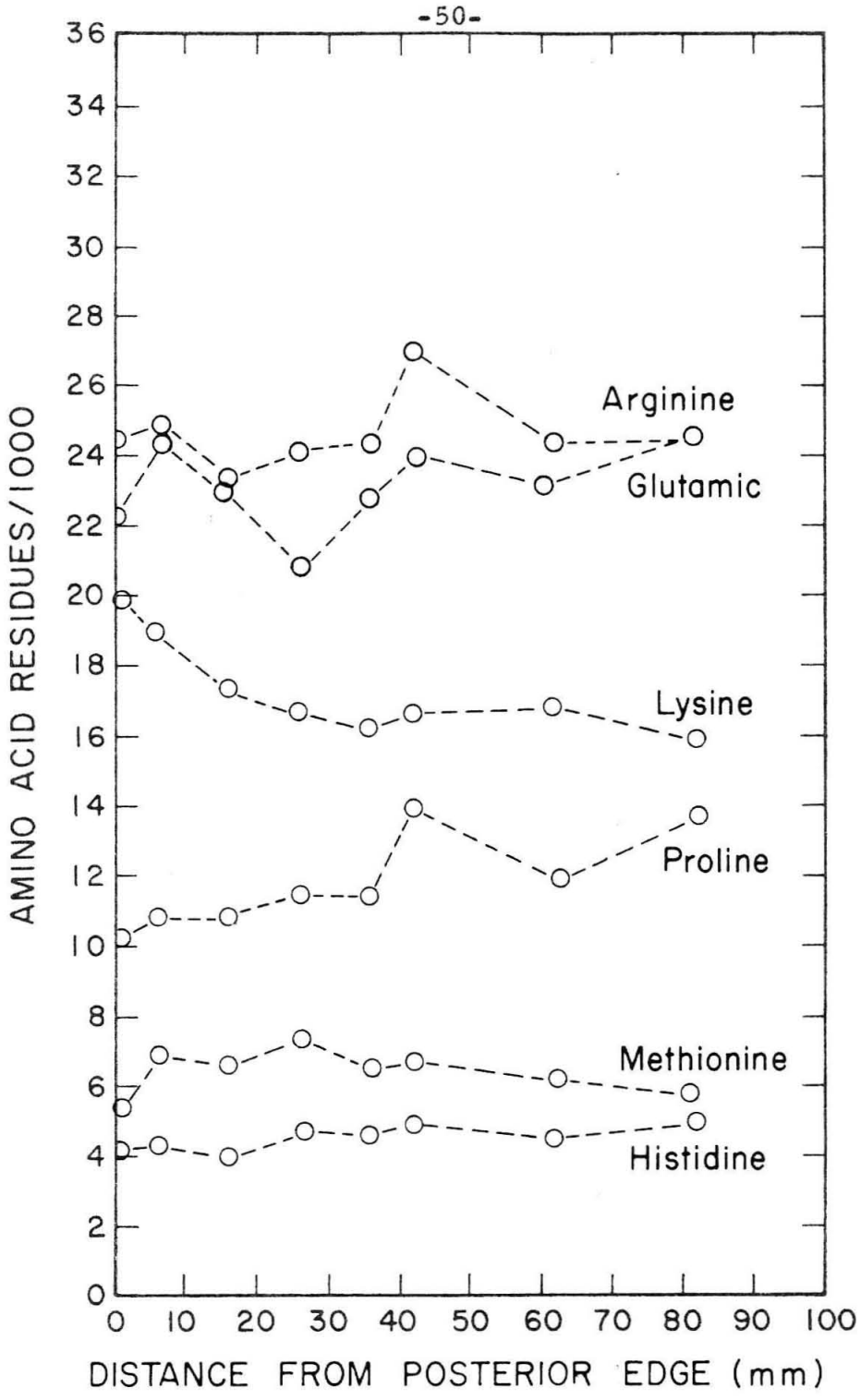


FIGURE 17A. Variations in composition in longitudinal section of outer prismatic calcite layer of Mytilus californianus from Corona del Mar, California.

Data from Table III-12, p. 105.

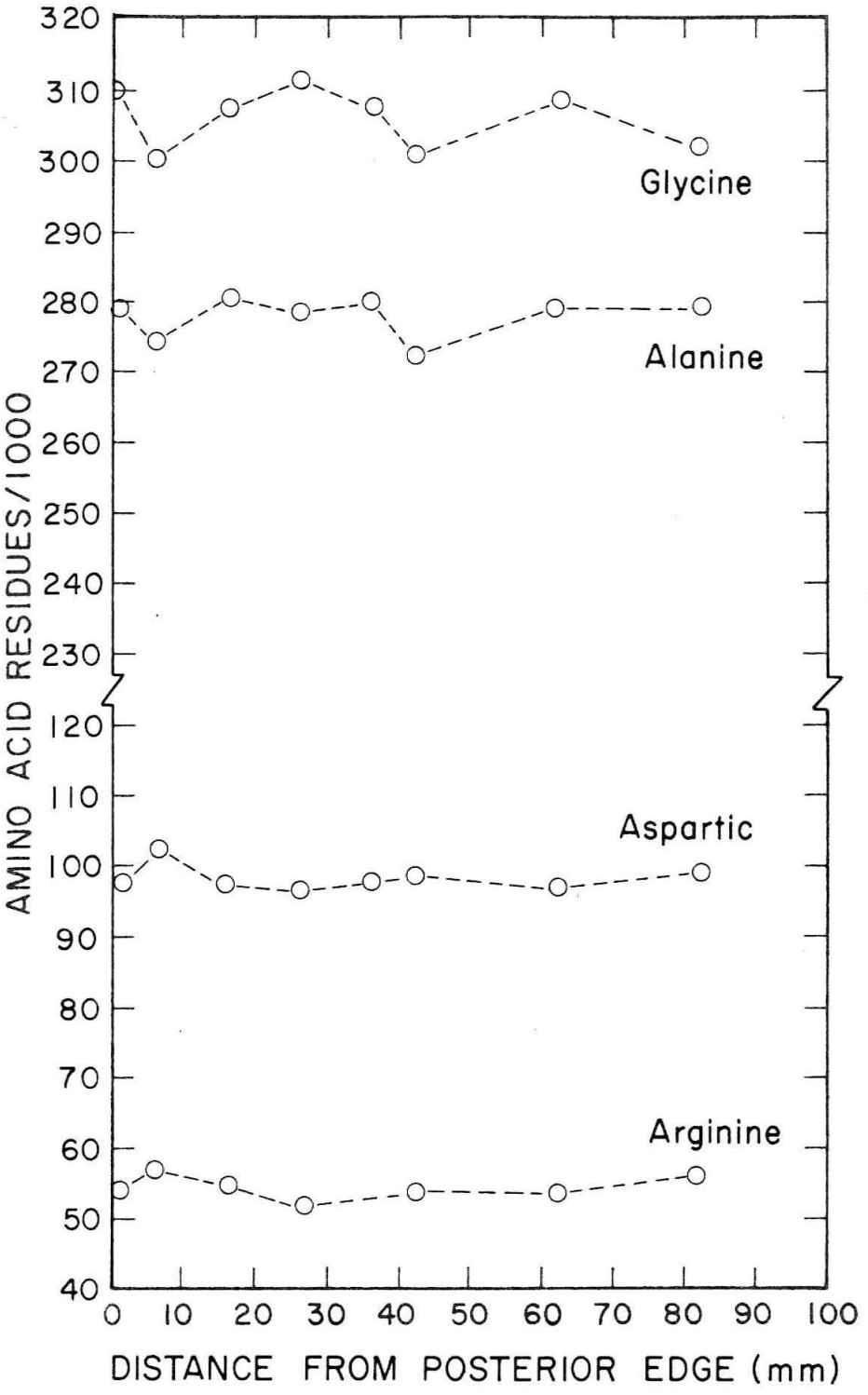


FIGURE 17B. Variations in composition in longitudinal section of outer prismatic calcite layer of Mytilus californianus from Corona del Mar, California.

Data from Table III-12, p. 105.

indeed reflect seasonal changes, it appears that about 1.5 to 2.0 years are represented by the 80 mm of growth. This is in good agreement with the data of Coe and Fox (1942, 1944).

Some of the variations observed may be merely sampling error in view of the fact that considerable variation exists in the composition around the periphery of the shell (Figures 16A and B). Successive samples may include more or less material from slightly different positions of the shell. The range of variation found in longitudinal section is no greater than that found in simultaneously deposited outer calcite from several specimens from a single locality (Table III-10, page 103).

It seems that exposure of the shell to sea water and air for periods of a few years does not appreciably affect the protein composition. Lysine may decrease, but this trend may be due to other factors such as sampling.

Comparison of Geographically Separated Specimens of M. californianus

If temperature influences the organic matrix composition of aragonite and/or calcite by regulating certain physiological reactions of the animal, then one might expect specimens of a single species from widely separated but similar environments to have similar matrix compositions. Punta Banda in Baja California (latitude $31^{\circ}45'$) is an unusually good place for studying effect of temperature. The north east side is protected from upwelling cold water and consequently has a moderately warm water temperature. On the south side, cold water wells up and keeps the temperature several degrees below that of the north east side (Hubbs, 1960). Dr. C. L. Hubbs (1960) collected

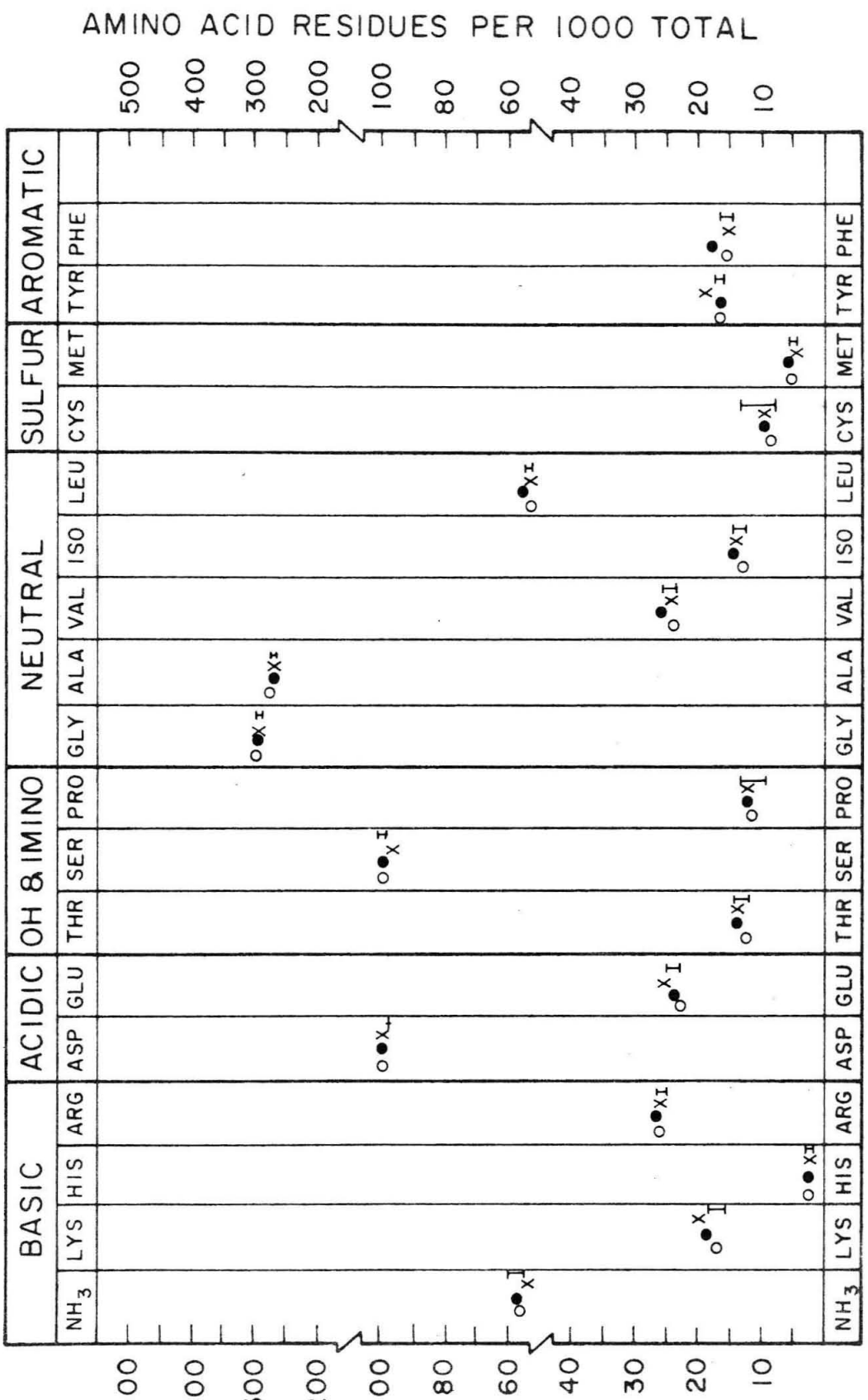
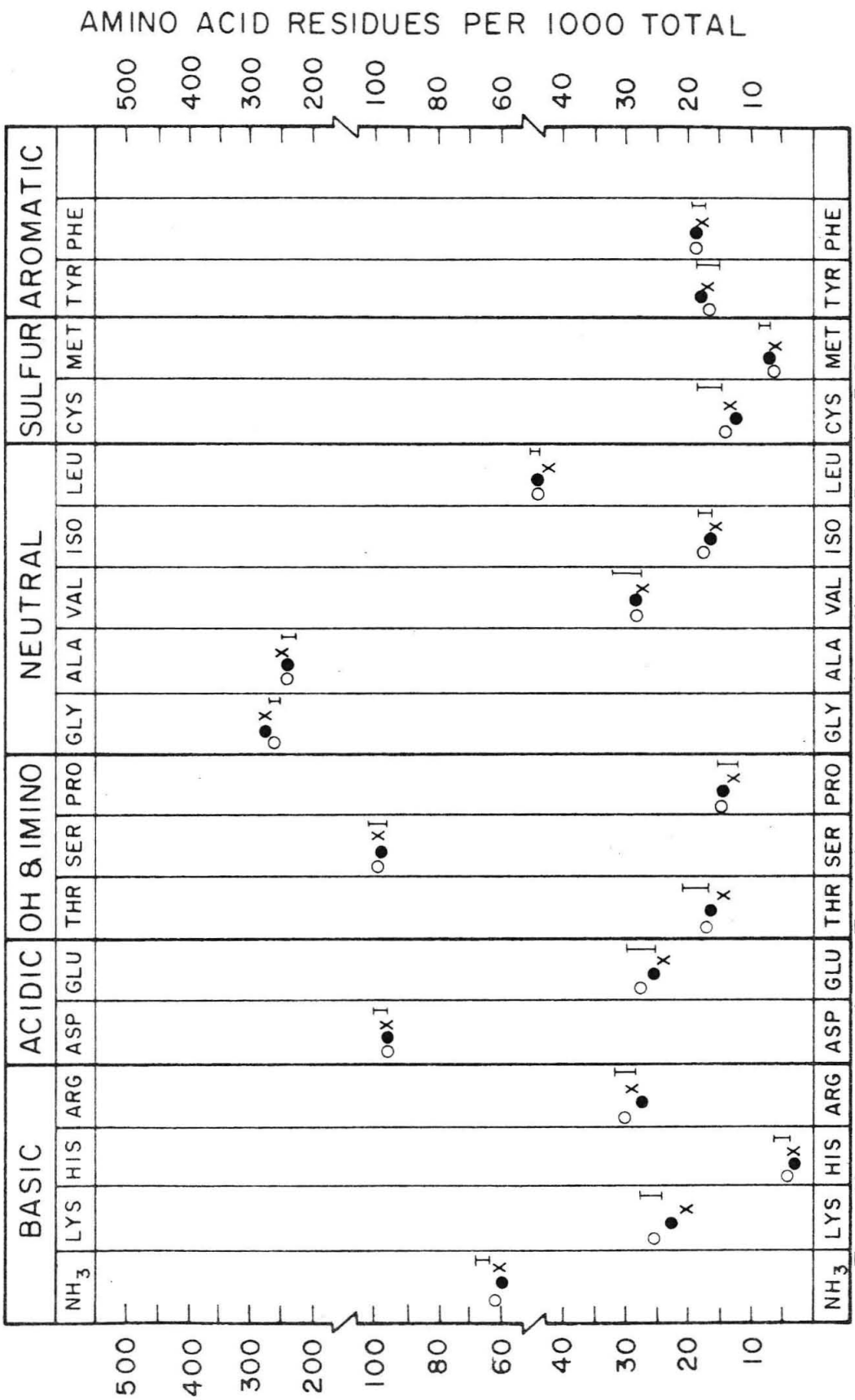


FIGURE 18. Comparison of outside calcite protein of Mytilus californianus from various ecological environments. Samples taken from approximately 50-60% position (Fig. 1, p. 13). Data from Table III-13, p. 106.



o Tres Hermanas, south; • Tres Hermanas, north; x Newport Beach; I Sonoma

FIGURE 19. Comparison of nacreous aragonite of Mytilus californianus from various ecological environments.

Data from Table III-14, p. 107.

specimens from both localities, and the data for the aragonite and calcite fractions together with data from analogous samples from Sonoma and Corona del Mar are included in Tables III-13,14. The results from the calcite layer shown in Figure 18 seem to show some variation in the amount of lysine, which may possibly be related to temperature effects. In general, however, the calcite organic matrix composition does not seem to vary significantly with temperature. Considering the average of the Sonoma samples as the standard, the sums of the individual deviations from this "standard" are 25 residues per thousand for the Newport Beach sample; 17 residues per thousand for Tres Hermanas, North; and 21 residues per thousand for Tres Hermanas, South. These are all near the values for experimental and biological variability for the outer prismatic layer of specimens from a single location (see Table III-10 , page 103).

The results from the aragonite organic fraction shown in Figure 19 and Table III-14 seem more pronounced. The number of net acidic or basic amino acid groups apparently varies with temperature. Both specimens from the cooler environments have fewer net acidic amino acids. In each specimen, the matrix from calcite has significantly more net acidic residues than does the aragonite, which seems to confirm the data in Figure 13. However, for the specimens from the warmer temperatures, the difference between the aragonite and calcite matrices decreases. For example, specimens from Sonoma had roughly from 18 to 21 net acidic residues in the outer prismatic calcite (see Figure 13), while the nacreous aragonite from this same location averaged around 0 (no net acidic or basic residues). The actual

range of differences between the calcite and aragonite matrices is from 17 to 20 acidic residues for Sonoma. Similar data from the other locations give: Tres Hermanas (South) 15 acidic residues; Corona del Mar, 8 to 15 acidic residues; and Tres Hermanas (North), 6 acidic residues. These data are plotted against the mean surface water temperature in Figure 20. Also included are values for two specimens of Mytilus edulis diegensis. The trend is toward smaller differences between the calcite and aragonite matrices with increase of temperature. Extrapolation to zero difference between calcite and aragonite proteins would give a temperature in the neighborhood of 22° to 24°C. If the net acidic or basic residues of the matrix are a possible factor in determining the crystallographic form of $CaCO_3$ deposited in the nacreous and prismatic layers, then when the difference in the net acidic residues between the respective matrices becomes zero, it may be possible to deposit the same mineral form in both layers. This seems to be in agreement with the results of Lowenstam (1954a, b) where shells of Mytilus species with aragonite in both the inner and the outer layers were confined to environments with mean temperatures of over 22°C. This may reflect only one of the factors related to aragonite precipitation at various temperatures. It appears that as the temperature of the environment increases, the aragonite "stability" field (Figure 13) expands into the more acidic matrix type which at lower temperatures is only occupied by calcite.

Comparison of Two Mytilus Species, californianus and edulis diegensis

It is interesting to determine to what extent differences between well-defined species are reflected in the compositions of the shell

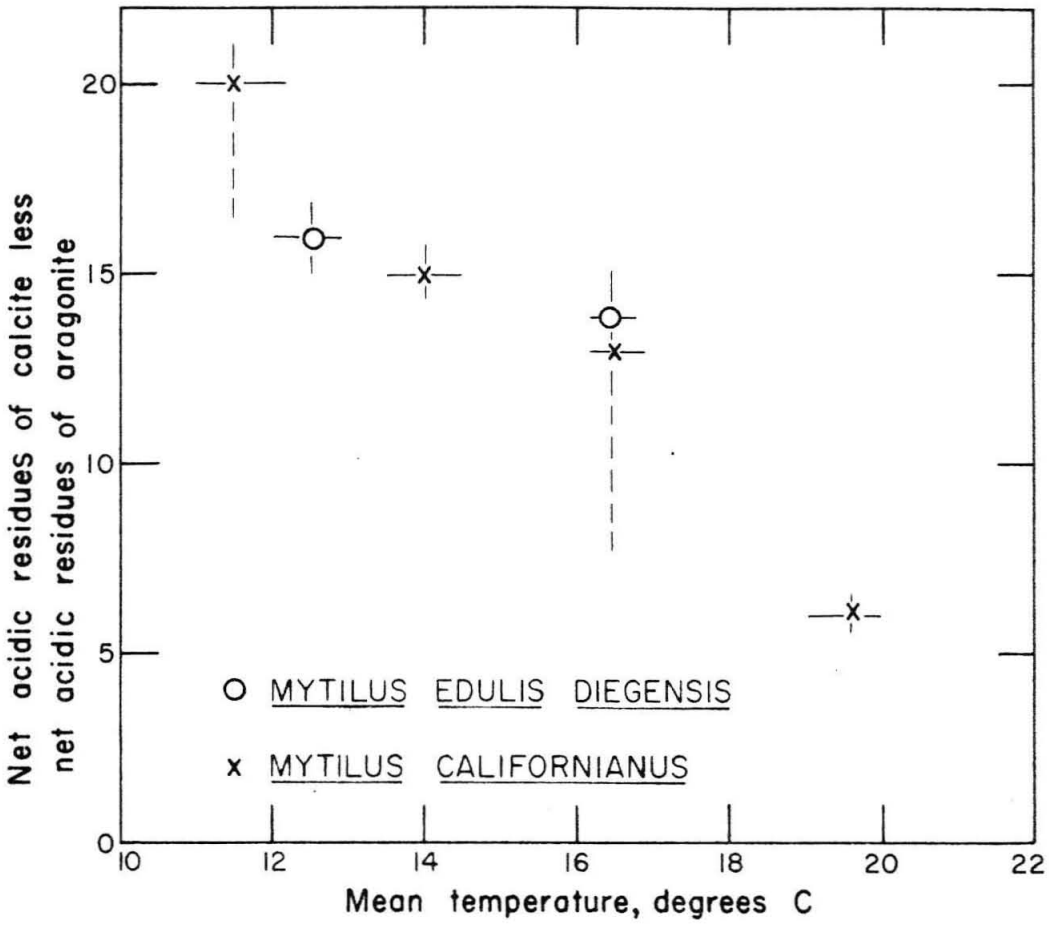


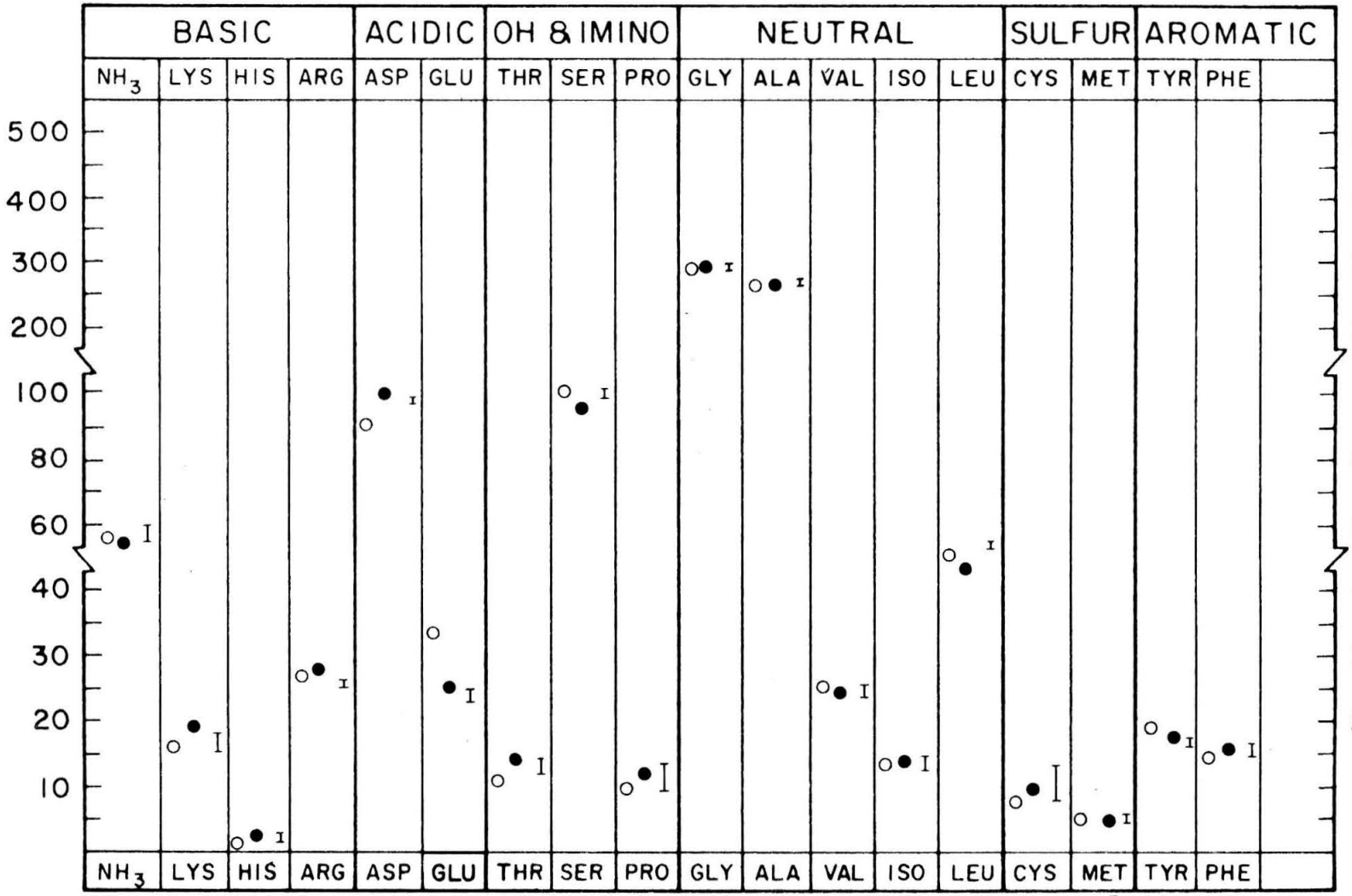
FIGURE 20. Difference in net acidic residues between calcite and aragonite plotted against mean temperature of environment.

net acidic residues = Σ(ASP+GLU-NH_3) - Σ(LYS+HIS+ARG)

proteins of the two species. M. edulis diegensis differs from M. californianus generally by having a higher, flatter form, but great variation in form is present in both species. There is a noticeable difference in the structure of the outside prismatic layer, with M. californianus having well-defined radial ribs, and M. edulis diegensis having a relatively smooth surface broken only by growth lines.

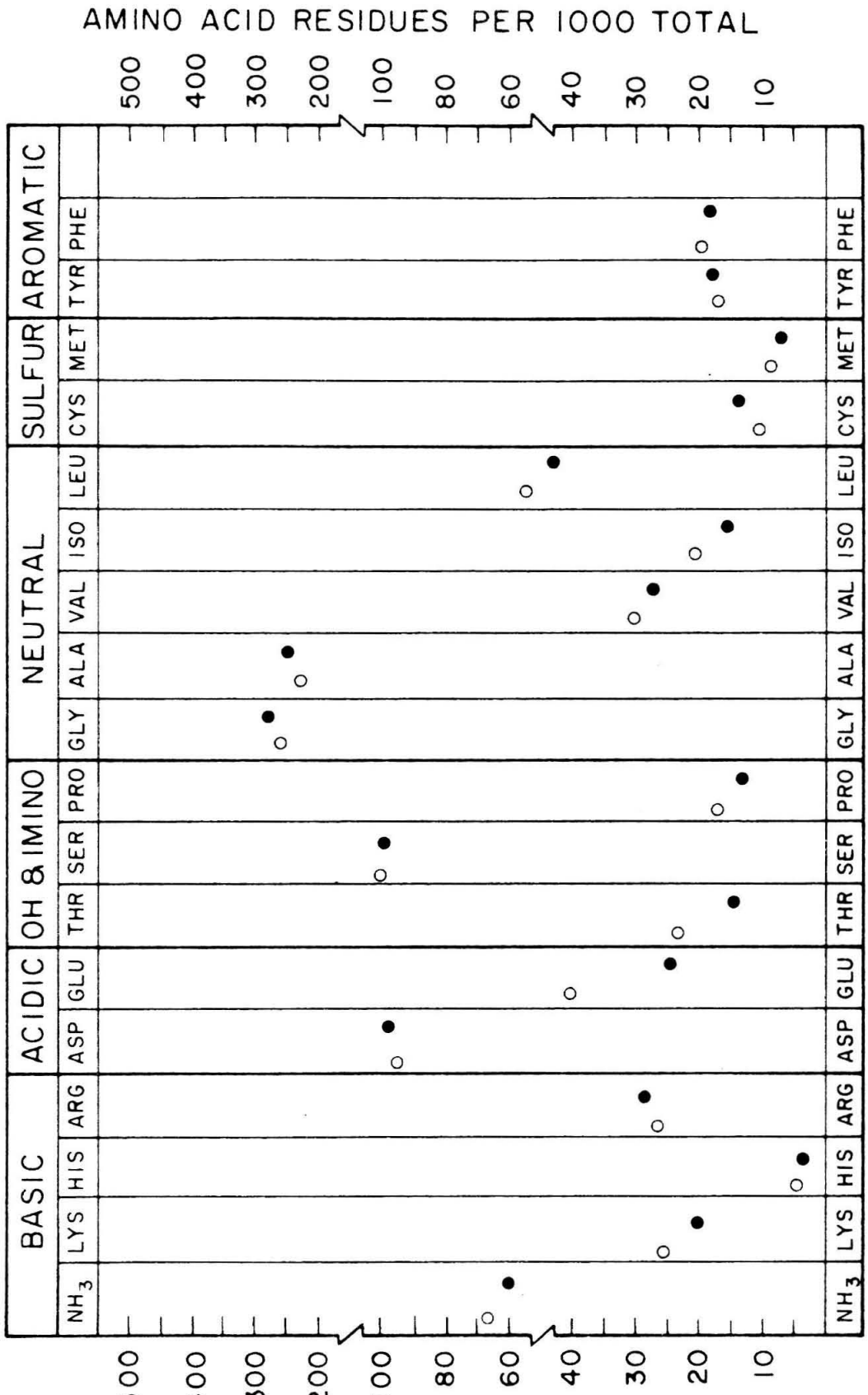
Specimens of the two species from the same environment (Corona del Mar) were taken for comparison of the organic matrix composition of the outer prismatic calcite layer and the nacreous aragonite layer. The results for the prismatic layers are shown in Figure 21 (and Table III-15). The sum of the deviations is about 50 residues per thousand, which seems significantly more than merely experimental or biological variability within a species from a single environment (sum of deviations for outer calcite protein is usually not much over 25 residues per thousand). The lower amount of aspartic acid and the higher amount of glutamic acid seem significant. In every specimen of M. californianus, regardless of locality, shell position, or size of shell, the number of aspartic acid residues has always been close to 100 residues per thousand (97.6 to 102.5 are the extreme values). Interestingly, and perhaps significantly, the net number of acidic residues is similar in both species (around 24 acidic residues per thousand).

Figure 22 shows the composition of the matrix from the aragonite layer of the same two specimens which were shown in Figure 21. The most significant difference here seems to be in the glutamic acid content with M. edulis diegensis having 41.5 residues per thousand



○ *Mytilus edulis diegensis*; ● *Mytilus californianus*, Corona del Mar; I *M.c.*, Sonoma

FIGURE 21. Composition of protein from outer prismatic layer (calcite) of Mytilus edulis diegensis from Corona del Mar compared to Mytilus californianus from Corona del Mar and Sonoma, California. Data from Table III-15, p. 108.



○ *Mytilus edulis diegensis*; ● *Mytilus californianus*

FIGURE 22. Composition of protein from aragonite layer of Mytilus edulis diegensis compared to Mytilus californianus both from Corona del Mar, California. Data from Table III-15, p. 108.

compared to 24.6 for the specimen of M. californianus. Again, the net number of acidic residues is similar for the two species (10.7 and 9.6), and in both cases it is significantly lower than for the calcite matrix of the same shell.

Comparison of Specimens of M. edulis diegensis from Different Temperature Environments

Specimens of M. edulis diegensis were collected from an open-coast environment near Cliff House in San Francisco. Table III-15 gives data on the composition of the matrices of calcite and aragonite for this specimen compared to the specimen from Corona del Mar. The high value for aspartic acid from the calcite protein from the northern California specimen lies near the range of values found for M. californianus. In M. californianus, aspartic acid varied only between narrow limits, while in M. edulis diegensis it varied between 90 residues per thousand for Corona del Mar to 97 for Cliff House. It is conceivable that the Cliff House specimen represents another genetically distinct subspecies, but further data are needed to evaluate this possibility.

A possible temperature effect seems to be reflected in the net acidic residues for the aragonite protein with the warmer water specimen containing 11 net acidics compared to 7 net acidics for the cooler water form. These data are included in Figure 20.

Effect of Changes in Salinity on the Composition of the Organic Matrices of Aragonite and Calcite from M. edulis diegensis

Specimens of M. edulis diegensis were collected in June 1961 from two localities of widely different salinities near San Francisco. The salinity at Cliff House, an open-coast environment, was approxi-

mately 34 o/oo, while at Glen Cove on the Sacramento River near Benecia, the salinity was only 20 o/oo.

Table III-15 compares the composition of the aragonite matrix from these two specimens. For the low-salinity environment, the differences between the composition of the aragonite and calcite matrices are less pronounced than at the normal salinity environment. This is especially true for lysine. The net acidic residues in the aragonite matrix increases from 7 at normal salinity to over 14 at 20 o/oo salinity. The effect is in the same direction as that observed for increasing temperature. Dodd (1961) showed that decreasing salinity for M. edulis diegensis from the San Francisco Bay area increased the percent aragonite in the shell from about 17 per cent at a salinity of 32 o/oo to about 34 per cent aragonite at 20 o/oo.

Discussion

The systematic variations in the organic matrix composition of the calcitic and aragonitic shell layers of Mytilus suggest a relationship between matrix composition and the mineralogic form of $CaCO_3$.

It is conceivable that the observed variations of organic matrix composition with environmental temperature and salinity may be due to protein solubility effects. In all probability, we are dealing with mixtures of an unknown number of proteins and other components, each of which may have a different solubility. As the organic matrix is elaborated during mineralization, the more soluble components may be dissolved and thus not be preserved in the shell itself. These soluble components may have had an essential role in the nucleation of $CaCO_3$ onto the organic matrix. A study of this factor would require a careful

comparison of the materials present during and after mineralization.

Temperature and salinity affect the solubility of a protein, but not in a predictable manner (Schmidt, 1938). Some proteins are more soluble at higher temperatures (below denaturation) while others are less soluble. The presence of salt increases the solubility of some proteins and decreases the solubility of others (Schmidt, 1938). The general class of proteins to which shell proteins belong is not known, and very little work has been done on the solubility characteristics of shell proteins. Gregoire (1955) separated a soluble component from some mollusk shells which differed (but not systematically) in composition from the insoluble residue. In the present study, the organic matrix from the outer prismatic calcite layer was boiled in water for two hours and the solution analyzed for soluble protein. Its composition was strikingly different from the insoluble residue remaining after boiling. The soluble fraction was enriched in lysine, ammonia, aspartic acid, and glutamic acid. Furthermore, it had a net excess of acidic amino acids. The residue remaining after boiling amounted to over 90 per cent by weight of the original material, with only aspartic acid showing a significant decrease.

It is possible that temperature and salinity variations are responsible for leaching or retaining certain organic components during the mineralization of shell materials. This can only be evaluated when more data are available on the actual chemistry of shell formation.

COMPOSITION OF ORGANIC MATRIX PROTEINS FROM RADIOCARBON-DATED SHELL MATERIAL

The previous chapters have considered the types and magnitudes of the variations which exist in the composition of the shell proteins of recent specimens of Mytilus. This background information is essential when considering the fate of the organic material in shells of known age.

Abelson (1956) showed significant differences in the amino-acid content and composition between recent and Miocene specimens of Mercenaria mercenaria. Using paper chromatography, he detected 15 amino acids (only 13 are shown) in the protein from the recent specimen. In the present study using ion-exchange chromatography, 17 amino acids plus ammonia were found in the protein of a recent specimen of Mercenaria mercenaria. In addition to the 13 amino acids which Abelson listed, arginine, cystine, histidine, and methionine were detected.

Abelson (1956) also determined the amino acid content of shells of the same species which were 1000 years old. Although the protein had turned brown and lacked mechanical strength, no differences in the amount of protein or in the amino acids present were detected when compared to recent specimens. In contrast, the present study revealed significant differences in protein composition of shells of Mytilus californianus which were dated by radiocarbon (Hubbs, et al., 1960) at 400 years. Most probably the automatic recording ion-exchange technique is able to detect small differences which are not easily detected by

paper chromatography.

In Pleistocene shells of Mercenaria mercenaria Abelson (1956) found no intact protein but rather a black tarry substance which gave amino acids upon hydrolysis. In Miocene specimens no residue was left after decalcification; and the filtrate contained only individual amino acids. The amino acids found in the filtrate from the Miocene specimen include alanine, glutamic acid, glycine, leucine, isoleucine, proline, and valine. It seems clear that these surviving amino acids are inherently more stable or more resistant than those no longer present in the older specimens.

There are probably several mechanisms by which amino acids in shell proteins are ultimately destroyed or converted to simpler materials. Abelson (1956) suggested a sequence of steps in the decay of shell protein consisting initially of hydrolysis to peptides and amino acids by water penetrating into the shell followed by possible leaching of soluble components. It was also suggested that the determining factor in the stability of a particular amino acid was its thermal stability as determined by high temperature pyrolysis. For alanine it was shown that decarboxylation to an amine was the predominant reaction (at least at high temperatures) and that the activation energy was approximately 41, 000 calories, which means that at room temperature alanine could survive for periods of time of the order of 10^{10} years. Thermal degradation studies on other amino acids seemed to correlate with the results found in the fossil shell materials. The amino acids most stable to thermal degradation were the same ones found in the older fossils.

The results of Jones and Vallentyne (1960) are somewhat different from Abelson's earlier work. They reasoned that if thermal decomposition were responsible for the survival of amino acids in fossil material that it should be possible to treat artificially a Pleistocene specimen so that it would resemble a natural Miocene specimen. Heating samples in an open flask under a nitrogen atmosphere resulted in a mixture of only aspartic, leucine(s), phenylalanine, and lysine. Of these amino acids, only isoleucine and leucine were detected by Abelson in the Miocene specimen, and the "stable" amino acids which were found in the Miocene sample did not survive the pyrolysis treatment of Jones and Vallentyne.

Possibly one of the reasons for the discrepancy between the experimental and natural results is the presence of small amounts of oxygen in fossil materials. Conway and Libby (1958) calculated that at room temperature the half-life for the oxidation of alanine was approximately 20,000 years compared to the extremely long half-life of 10 billion years for decarboxylation at room temperature.

Another possible reason for the discrepancy between Abelson's findings and those of Jones and Vallentyne could be the microbiological activity in natural environments. This factor has not been evaluated in the present study. It may be that since fossil materials have been exposed to a variety of conditions, no single laboratory treatment would be adequate to produce the results found in fossil specimens.

The present study includes a limited number of specimens of Mytilus californianus from horizons in Indian shell mounds which have been dated by radiocarbon. Samples were supplied by Dr. C. L. Hubbs.

Sample descriptions have been given elsewhere (Hubbs, et al., 1960). A summary of sample number, location, and age is given below. With the exception of Sample LJ-111 from Oregon, the specimens come from comparable depositional environments.

TABLE 1. RADIOCARBON DATED SHELL MATERIAL
(Hubbs, et al., 1960)

LJ-33	Rancho Cuevas, Baja California	32° 17' N.	400 ± 200 yrs.
LJ-84	Valle de Rosario, Baja California	30° 3.3' N.	1060 ± 150 yrs.
LJ-111	Cape Arago, Oregon	43° 20' 27" N.	1500 ± 100 yrs.
LJ-78	Malibu Beach, California	34° 2' 16" N.	3460 ± 200 yrs.
LJ-110	Scripps Estates, Site 1,	32° 52' 18" N.	5460 ± 100 yrs.

Method

The shell-mound samples were treated similarly to the recent specimens as described in Appendix II. In samples older than a thousand years, the organic matrix disintegrated into numerous flakes during the decalcification step, which made it difficult to quantitatively recover the insoluble residue. The free and combined amino acids which were soluble in the acetic acid decalcifying solution were analyzed for total amount by taking an aliquot of the solution, hydrolyzing it, and calculating the total concentration of amino acids, exclusive of ammonia, from column 3 on the Beckman Model 120 amino acid analyzer. The acidic and neutral amino acids are not completely resolved, but it is possible to get an estimate of the total amount present. This was done for the 400-year and 5460-year old samples.

Results

The free and combined amino acids from the 400-year old shells, (LJ-33), which were soluble in the acetic acid decalcifying solution, showed no significant increase in amount over that found for recent specimens of Mytilus californianus. In modern shells, only 5 to 10 per cent of the total amino acids present were soluble in the acetic acid solution used for decalcification. In the oldest sample tested (LJ-110, 5460 years), about 75 per cent of the total amino acids in the aragonitic shell material were soluble in the decalcifying solution. Of this soluble material a large fraction was present as peptides, since there was a 50 per cent increase in the amino acid content when an aliquot was first hydrolyzed (to break down the peptides) before analysis. In the calcite fraction of the 5460-year shells, about 30 per cent of the total amino acids present were soluble.

As shown in Figure 23, the composition of the insoluble organic residue from the above sub-fossil series (exclusive of LJ-111) shows marked changes from analogous recent specimens. In general, most of the amino acids decrease. The relative amount of alanine appears to increase by more than a factor of two for the oldest specimen. The ratio of alanine to glycine may be a useful parameter which varies with time as shown in Figure 24.

It seems clear that sample LJ-111, which comes from Cape Arago, Oregon, does not fit the trend apparently valid for the group of specimens from southern California and Baja California. Physically, the Oregon shell appeared chalky and has clearly been subjected to a different post-depositional environment than the other specimens

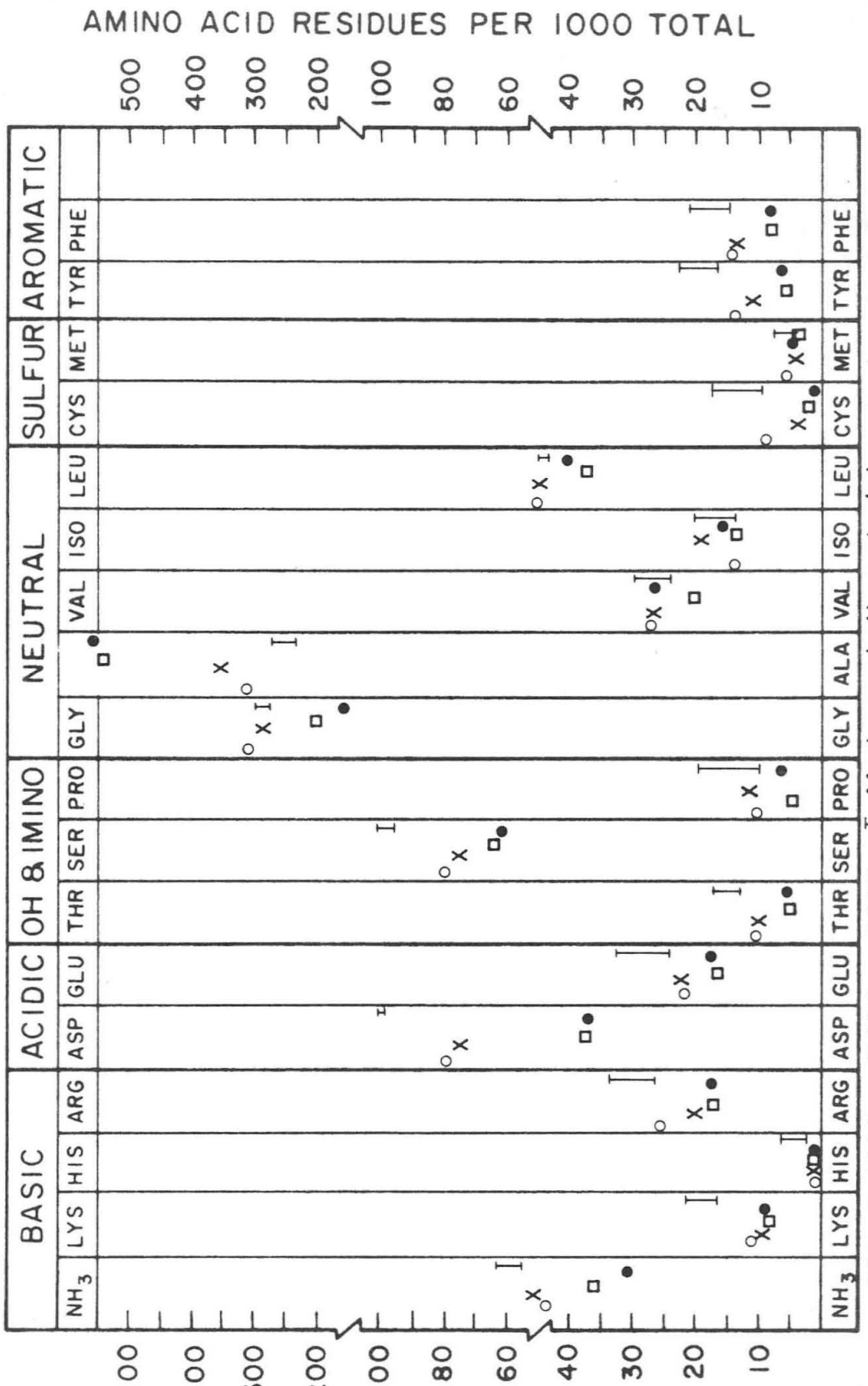


FIGURE 23. Composition of organic matrix from calcite of radiocarbon-dated shells. Data from Table III-16, p. 109.

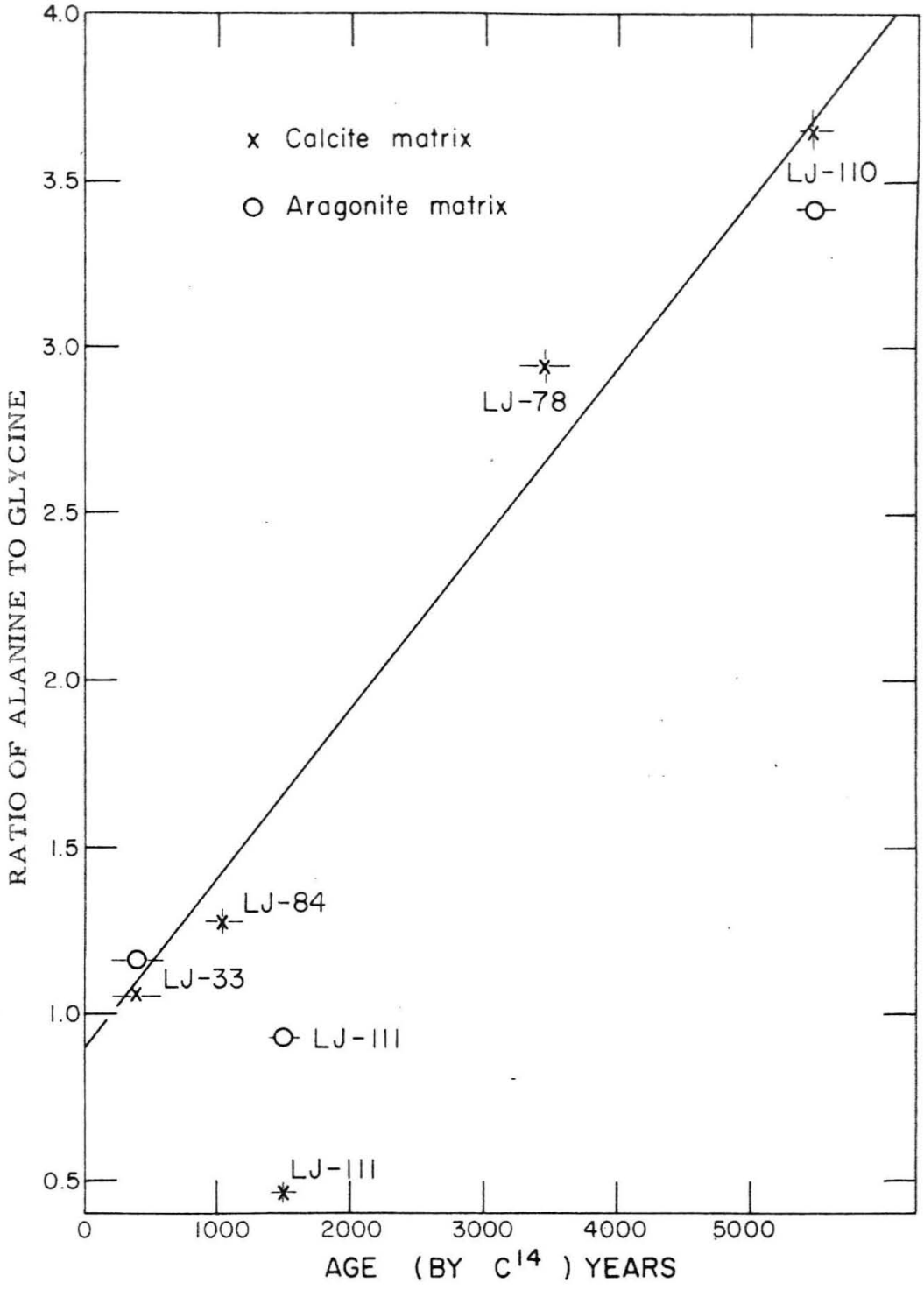


FIGURE 24. Ratio of alanine to glycine in the organic matrix residue of calcite and aragonite as a function of age of shell. See Table 1, p. 66 for location of samples. Data from Table III-16, p. 109.

studied. It is possible that the composition of the organic residue of sample LJ-111 reflects the different environment. However, until a series of dated shell materials from a similar environment can be studied, no conclusions are possible. Schoute-Vanneck (1960) found that the amount of insoluble residue after decalcification was greatly influenced by environment. (See also Cook and Heizer, 1953; Abelson, 1958.)

In the sub-fossil specimens studied, there were some peaks on the chromatographic record which did not appear for analogous modern shell material. One of these occurs between aspartic acid and threonine (see Figure II-1, page 90). In a comprehensive study of nearly 50 compounds by Spackman, et al. (1958), only methionine sulfone, an oxidation product of methionine, occurs at this position between aspartic acid and threonine. Furthermore, there is a reciprocal relationship between methionine and this compound in the series of sub-fossil materials studied. The ratio of methionine sulfone to methionine is shown in Figure 25. More data are certainly needed before even tentative mechanisms can be suggested.

Other peaks tentatively identified which are in relatively greater concentration in older materials compared to modern shell materials are; allo-isoleucine between methionine and isoleucine; glucoseamine, galactoseamine, and an unidentified compound, all three of which occur between tyrosine and lysine on column 2, and cysteic acid, which occurs early in the run before aspartic acid.

Discussion

The probable presence of methionine sulfone seems to imply

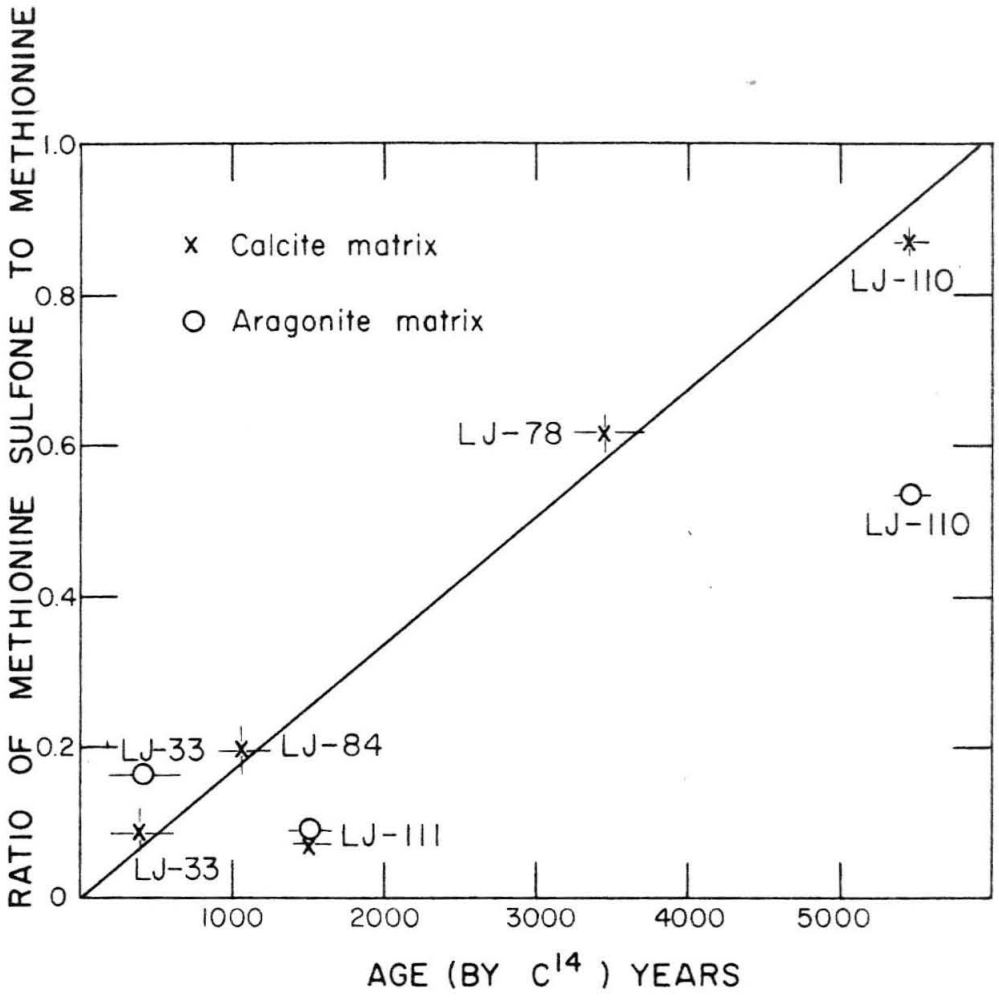


FIGURE 25. Ratio of methionine sulfone to methionine in the organic matrix residue of calcite and aragonite as a function of age of shell. See Table 1 , p. 66 for location of samples. Data from Table III-16, p. 109.

the presence of oxygen during diagenesis. When protein samples are hydrolyzed in air rather than nitrogen, small amounts of methionine sulfone [as well as the sulfoxide which is less stable (Dawson, et al., 1959)] are formed. The occurrence of cysteic acid in higher proportion in older specimens also implies the presence of oxygen. If small amounts of oxygen indeed were present during the diagenesis of these shell materials, it would seem to indicate that probably thermal decomposition in the absence of oxygen, as experimentally determined by Jones and Vallentyne (1960), is not responsible for the decomposition of these shell proteins under natural conditions.

From the results of this study on sub-fossil materials it seems clear that it would be rather difficult, if not impossible, to "work backwards" and derive the original protein composition of even relatively recent fossil material. However, unusual situations sometimes occur which may enable one to evaluate, at least approximately, the original composition of even an extinct form. If shortly after deposition a shell or bone deposit is saturated by petroleum seepage and the shells are encased in an asphalt residue, then a near-ideal situation exists for the preservation of the original organic matrix. For example, in the La Brea tar pits, the organic matrix of the bones encased in the asphalt has undergone very little decomposition (Abelson, 1956).

CONCLUSION

The amino acid compositions of the organic matrix proteins of the shells of Mytilus californianus are distinctive for the various structural units of the shell. The uncalcified byssus fiber by which the shell attaches to the substrate contains over 300 glycine residues per thousand. It also contains hydroxyproline, the only unit studied which had this amino acid. The periostracum, also uncalcified, has 400 to 500 glycine residues per thousand and also 150 tyrosine residues per thousand. The ligament matrix is characterized by a high methionine content of over 300 residues per thousand, and the ligamental ridge matrix has a relatively even distribution of amino acid concentrations with just over 100 residues per thousand for aspartic acid, serine, and glycine. Both the ligament and the ligamental ridge are aragonitic with about 30 per cent and $1\frac{1}{2}$ per cent respectively by weight of organic material.

The organic matrices from the outer prismatic (calcite), nacreous (aragonite), and inner prismatic (calcite) layers have relatively similar amino acid compositions characterized by around 270 - 300 glycine residues per thousand and a similar but always smaller number of alanine residues. A somewhat systematic variation in amino acid composition exists around the periphery of the outer prismatic calcite layer with fewer net acidic amino acid residues occurring in portions of the shell which have a higher organic content.

Differences in the relative amounts of basic and acidic amino acids present in the matrices of aragonite and calcite suggest a relationship between the net number of acidic or basic residues and the mineralogic form of $CaCO_3$ present. There are relatively more net

acidic residues present in calcite matrices compared to aragonite matrices.

There also seems to be a relationship between the net acidic or basic residues present and the percentage of organic material in the particular structural unit. For specimens from a moderately cool water environment the uncalcified components, periostracum and byssus fiber, have a relatively large excess of net basic amino acids (70 - 80 residues per thousand) while the aragonitic components have relatively fewer net basic residues (ligament, 27 net basics; ligamental ridge, 8 net basics; nacreous aragonite, 5 net basics to 2 net acidics). The calcitic layers have from 12 to 21 net acidic residues per thousand.

Temperature seems to affect the composition of the matrix from the nacreous aragonitic layer of Mytilus more than it does the corresponding outer prismatic calcitic layer. This effect is most apparent in the relative excess of acidic amino acid residues. At warmer temperatures, the nacreous aragonitic layer matrix has a relatively higher excess of net acidic residues. There seems to be little effect on the calcite matrix. Thus the difference in the net acidic residues between the calcite and aragonite matrices becomes progressively less as temperature increases (range: $12^{\circ}C$ to $20^{\circ}C$ mean temperature). If this trend continues to still warmer temperatures, one would expect that at some temperature this difference in net acidic residues between the prismatic and nacreous layer would be zero, and that if this is a factor in determining the mineralogic form, perhaps only one form of $CaCO_3$ would be found in these two layers above this temperature. On this point, it may be significant that in a study of the species of

Mytilus, Lowenstam (1954a, b, c) found 100 per cent aragonite in both the inner and outer layers in species confined to waters of mean temperature greater than 22°C.

Differences between Mytilus californianus and Mytilus edulis diegensis from the same locality seem to be reflected in the respective organic matrix compositions. In the matrices from both the calcitic and aragonitic layers there was significantly more glutamic acid in M. edulis diegensis and less aspartic acid. The number of net acidic residues was similar in both species, which may be a further indication of its relationship to mineralogic form. The data at present indicate that taxonomic classification of mollusks by means of biochemical characterization of shell proteins should be feasible. This may elucidate certain taxonomic relationships which morphology alone could not detect.

Changes in salinity, like temperature changes, apparently affect the nacreous aragonite matrix to a greater degree than the corresponding calcitic matrix. In Mytilus edulis diegensis, the aragonite matrix had 7 net acidic residues in a normal salinity environment and 14 net acidics in a low salinity environment of 20 o/oo.

To determine to what extent the organic matrix of shell materials is affected by early diagenetic activity, a series of radiocarbon-dated sub-fossil shells of Mytilus californianus ranging from 400 to 5460 years were studied. These came from archeological sites along the west coast, all but one sample coming from southern California and Baja California. Some differences, though small, could be detected in the 400 year old shell as compared to the recent shell material. The ratio of alanine to glycine seems to vary linearly with time for samples

from a similar post-depositional environment. This is also true of the ratio of methionine sulfone to methionine. This may prove of some value in the correlation of marine terraces whose age is beyond the range of Carbon-14.

The presence of small amounts of oxygen seems indicated by the progressive increase in the amounts of methionine sulfone, an oxidation product of methionine. This makes it difficult to apply the results of thermal decomposition studies made in the absence of oxygen (Jones and Vallentyne, 1960), to the occurrence of organic material in fossils.

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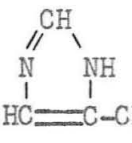
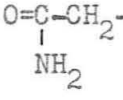
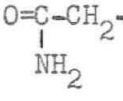
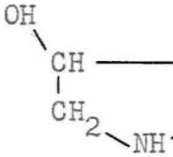
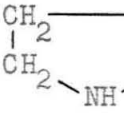
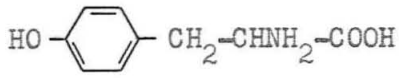
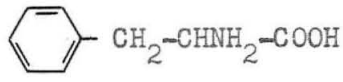
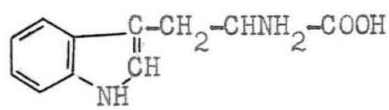
APPENDIX I

L-Amino Acids Commonly Found in Protein Hydrolysates

Name	Formula		Solubility (g/100 ml, H_2O, 25°C)
Basic Amino Acids			
OH-LYSINE	NH_2CH_2-CHOH-CH_2-CH_2-$CHNH_2$-COOH	162.2	?
LYSINE	NH_2CH_2-CH_2-CH_2-CH_2-$CHNH_2$-COOH	146.2	?
HISTIDINE		155.2	4.19 (1)
ARGININE		174.2	?
Acidic Amino Acids			
ASPARTIC ACID	HOOC-CH_2-$CHNH_2$-COOH	133.1	0.5 (2)
GLUTAMIC ACID	HOOC-CH_2-CH_2-$CHNH_2$-COOH	147.1	0.86 (2)
Amide Amino Acids (hydrolysis yields NH_3 + corresponding acidic amino acid)			
ASPARAGINE		132.1	2.46 (2)
GLUTAMINE		146.1	?
Imino Acids			
OH-PROLINE		131.1	36.1 (1)
PROLINE		115.1	162. (2)

APPENDIX I. (Continued)

Name	Formula	Molecular Weight	Solubility (g/100 ml, H_2O, 25°C)
Hydroxyamino Acids			
THREONINE	$CH_3-CHOH-CHNH_2-COOH$	119.1	?
SERINE	$CH_2OH-CHNH_2-COOH$	105.1	5.0 (2)
Neutral Amino Acids			
GLYCINE	$CHNH_2-COOH$	75.1	25.0 (2)
ALANINE	CH_3-CHNH_2-COOH	89.1	16.5 (2)
VALINE	$CH_3-CH(CH_3)-CHNH_2-COOH$	117.1	8.9 (2)
ISOLEUCINE	$CH_3-CH_2-CH(CH_3)-CHNH_2-COOH$	131.2	4.12 (2)
LEUCINE	$CH_3-CH(CH_3)-CH_2-CHNH_2-COOH$	131.2	2.2 (2)
Sulfur Amino Acids			
CYSTINE	$(S-CH_2-CHNH_2-COOH)_2$	240.3	0.01 (2)
METHIONINE	$CH_3-S-CH_2-CH_2-CHNH_2-COOH$	149.2	5.75 (2)
Aromatic Acids			
TYROSINE		181.2	0.05 (2)
PHENYLALANINE		165.2	3.0 (2)
TRYPTOPHAN (largely destroyed in acid hydrolysis)		204.2	1.15 (2)

APPENDIX I. (Continued)

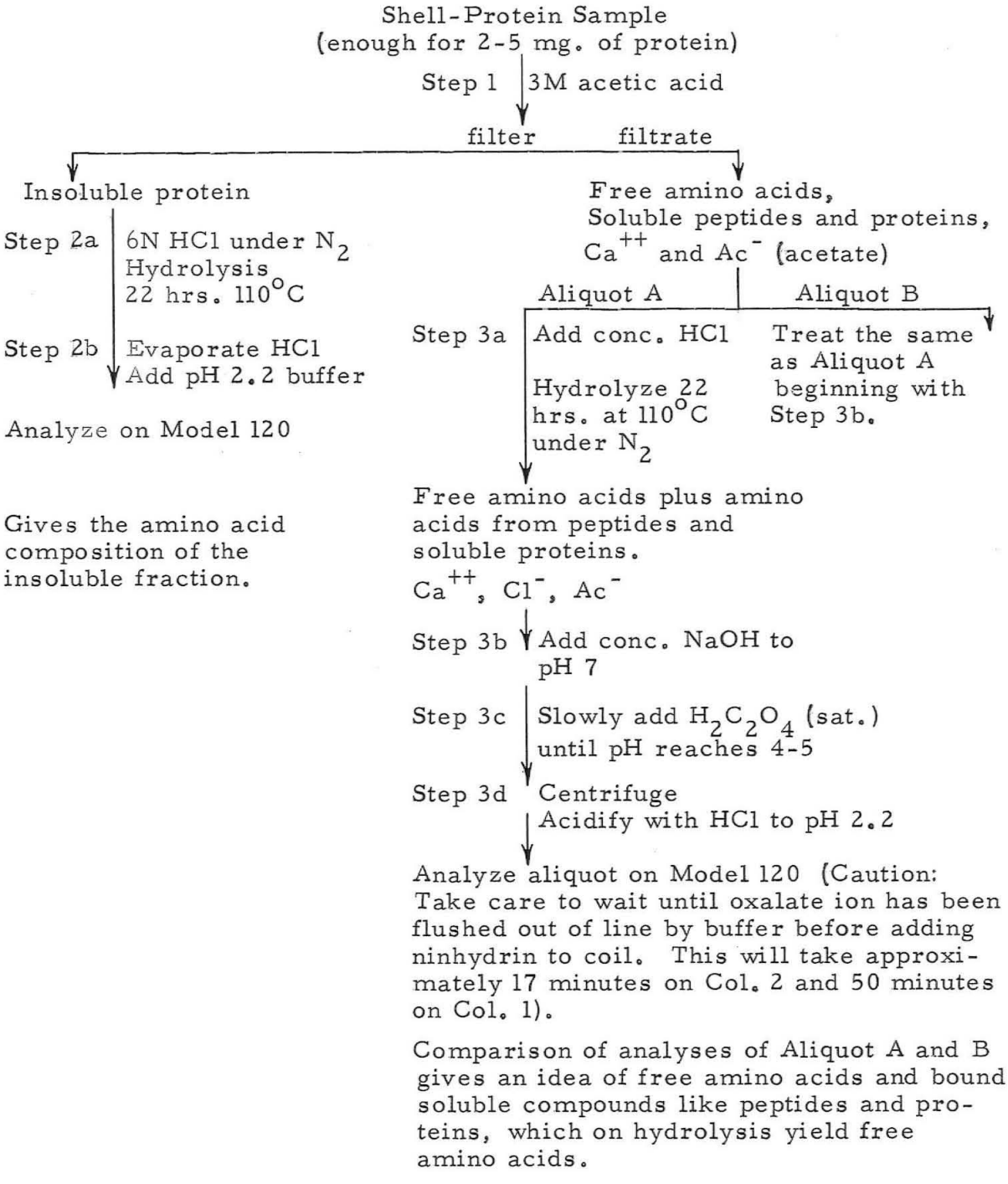
(1) From Dunn, M. S., F. J. Ross, and M. P. Stoddard in Handbook of Chemistry and Physics (34th Edition, 1952-3) p. 1571, Chemical Rubber Publishing Co., Cleveland, Ohio.

(2) From Technical Data supplied with L-amino acid kit by Nutritional Biochemical Corporation, Cleveland, Ohio.

APPENDIX II

Sample Preparation of Amino Acids from Shell Protein, Analysis of Amino Acids, and Presentation of Data

A. Flow Diagram of Sample Preparation



Notes on Sample Preparation

Step 1 Hydrochloric acid, acetic acid, and EDTA (ethylenediaminetetraacetic acid) were all tried as decalcifying reagents for $CaCO_3$. EDTA probably affects the organic components to the least extent and is widely used where structural studies of the organic matrix are being made. However, EDTA is ninhydrin positive and reacts as an amino acid; therefore, it is not suitable for a study of the soluble amino acid compounds in shell materials.

Acetic acid was chosen because it decalcifies rapidly and at a relatively moderate pH (2-4) compared with hydrochloric acid and hence should be expected to attack the organic components less. Actual comparison of the amino acid compositions of proteins separated from comparable samples of shell material decalcified by EDTA and acetic acid showed no significant differences.

The reaction was carried on in a vacuum desiccator attached to a water aspirator. This decreased the partial pressure of CO_2 above the solution and increased the reaction rate substantially.

Step 2a Hydrolysis under nitrogen was accomplished by flushing the tube containing sample and 1 ml. of 6N HCl with a stream of purified nitrogen prior to sealing the tube. The results of samples hydrolyzed under nitrogen were superior to those hydrolyzed under air and at least as good as those sealed under vacuum.

Step 2b HCl was evaporated by placing an opened hydrolysis tube in a desiccator containing NaOH pellets and evacuating with a water aspirator overnight.

Step 3c The purpose of adding oxalic acid ($H_2C_2O_4$) is to precipitate the Ca^{++}. The CaC_2O_4 precipitate does not selectively adsorb any of the amino acids. A mixture containing known amounts of each common amino acid was added to an acetic acid solution containing Ca^{++}. The Ca^{++} was precipitated with oxalic acid. The recovery of each amino acid from an aliquot of the filtrate was better than 95% for each amino acid with no selectivity noted for any amino acid.

B. Analysis of Amino Acid Mixtures

The method used in the present study involved the use of ion-exchange chromatography. In acidic solutions the following reaction takes place with all amino acids:

$$\underset{\text{free form}}{R\text{-}CH(NH_2)COOH} + H^+ = \underset{\text{cationic form}}{R\text{-}CH(NH^+_3)\text{-}COOH}$$

It follows that

$$K = \frac{(\text{cationic form})}{(\text{free form})\,(H^+)}$$

or at constant pH

$$K' = K(H^+) = \frac{(\text{cationic form})}{(\text{free form})}$$

This is known as the distribution coefficient and is largely a function of pH, ionic strength, and temperature of the solution. Each amino acid has its own characteristic distribution coefficient which affords the basis for their separation. The cationic form of the amino acids is strongly attracted to the polysulfonic ion-exchange resin while the free form, being neutral, stays dissolved in the buffer solution. An aliquot

containing a mixture of amino acids in buffer at pH 2.2 is added to the top of a resin column 150 cm. x 0.9 cm. At this pH the cationic form of the amino acids greatly predominates and the amino acids are quantitatively retained at the top of the resin. Then 0.2N sodium citrate buffer at pH 3.25 ± 0.01 is pumped past the bound amino acids and through the column. At pH 3.25 the distribution coefficients of most of the amino acids are such that nearly complete separation is achieved at a flow rate of 30 mls./hr. As the buffer is pumped past the sample at the top of the column the equilibrium between cationic and free amino acids is temporarily disrupted. However, as the free form is carried down the column more free form is produced from the cationic form bound to the resin. To re-establish equilibrium some of the free form carried in buffer reacts with hydrogen ions to form the cationic species, which is again immediately bound to the resin. This disruption and re-establishment of equilibrium occurs continuously so that each amino acid, with its unique distribution coefficient, travels down the column in a discreet, fairly narrow band separated from all other amino acids.

To speed up the separation of amino acids on the ion-exchange resin, a second buffer of higher pH (4.25) is introduced about halfway through the analysis. The basic amino acids lysine, histidine, and arginine, as well as ammonia have such strong affinities for the resin at pH 3.25 and 4.25 that they do not come off the long column in a reasonable length of time. Therefore, they are analyzed in a duplicate sample on a 15 cm. column at pH 5.28.

The effluent from the columns is forced through capillary tubing (to prevent remixing) and mixed with ninhydrin reagent, which reacts with the amino acids to form a blue compound (imino acids form a yellow reaction product). The intensity of the color produced is monitored in a colorimeter, which consists of three photometer units each consisting of a light source, a lens, a narrow wave-length band color filter, a slit, and a photovoltaic cell. The solution flows successively through each photometer unit generating an electrical current in the photovoltaic cell proportional to the density of color.

The output from the photovoltaic cells drives a three-point recorder, which then gives a simultaneous record of all three photometer units. Two of the units monitor the blue color (filter of 570 mu), while the third unit monitors the yellow color for imino acids (filter of 440 mu). The two units monitoring the blue color record the optical density of two different solution path-lengths in a ratio of approximately three to one. This enables a wide range of amino acid concentrations to be used and satisfactorily calculated.

C. Presentation of Data

Figure II-1 shows a typical record from a 24-hour run. The peak positions represent specific amino acids, while the areas under the peaks are related to concentrations. Typically a machine is calibrated under normal working conditions with a calibration mixture containing known amounts of amino acids, usually one micromole quantities. The peaks are integrated by multiplying the net height (in optical density units) by the width at half-height as measured in dots (since the dots are 15

seconds apart this provides a highly accurate measure of the width of the peak). A set of constants for calculating amino acid concentrations from peak areas is thus obtained from the calibration mixture.

After the concentration of each amino acid in a protein has been calculated in micromoles, it remains to express these concentrations in such a way that comparisons between samples may be readily made. The total number of micromoles of amino acids recovered (which depends on sample size, purity, moisture content of sample, etc.) is considered as 1000 total residues (exclusive of trytophan and ammonia). Each amino acid concentration is then recalculated as per 1000 total residues. The results expressed in this way are independent of sample size and also the presence of non-protein material which may have been in the original sample. This method of expressing protein composition is in wide use in current literature on comparative biochemistry (see Piez and Likins, 1960).

In Table II-1, page 90, is a sample calculation from the same record from which Figure II-1 was made. The protein material was periostracum from a specimen on Mytilus edulis diegensis taken from Corona del Mar, California. In Figure II-2, page 92, the data from Table II-1 are plotted as amino acid residues per 1000. This provides a convenient method for comparing the amino acid concentrations of various proteins.

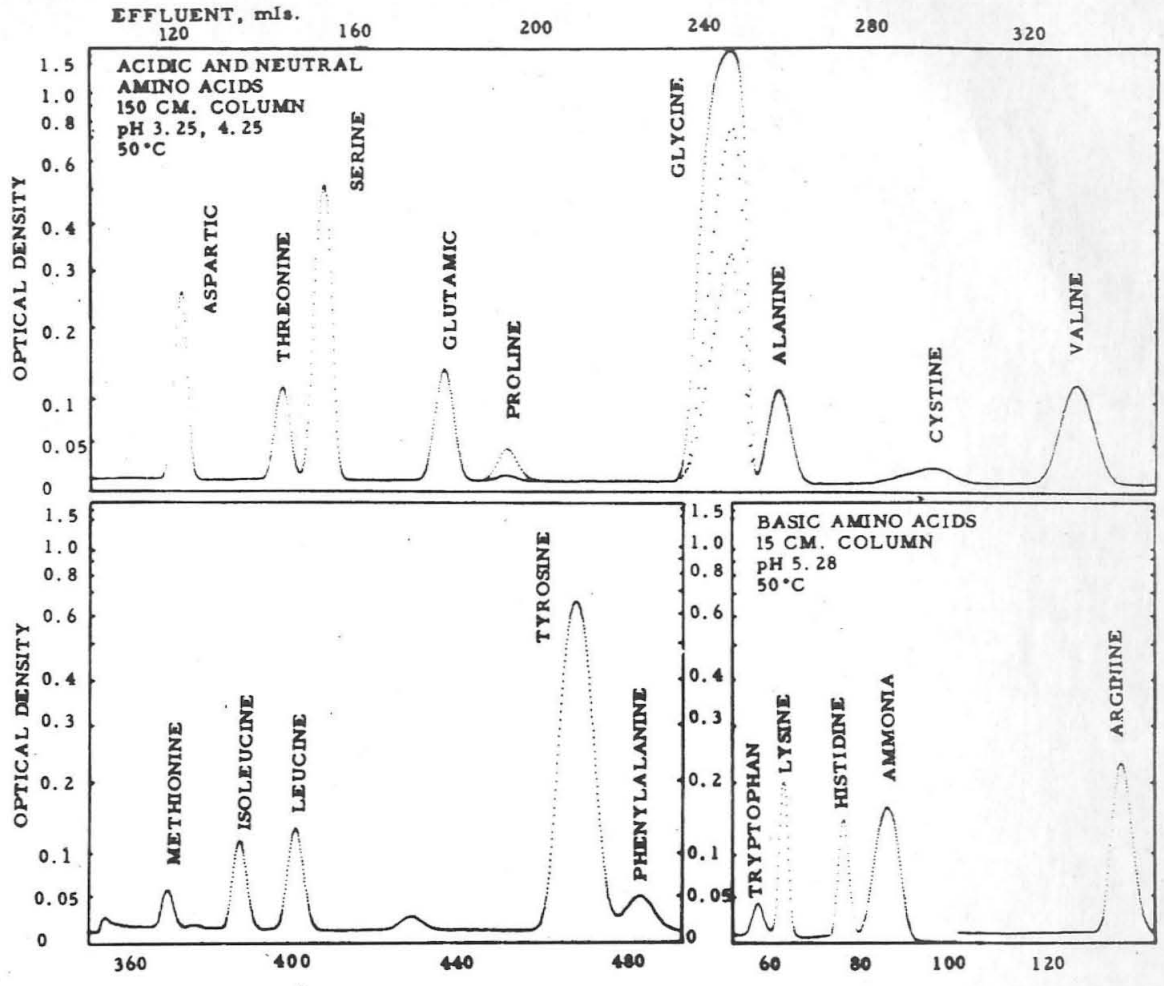


FIGURE II-1. Typical Chromatographic Record from Beckman Model 120 Amino Acid Analyzer. Sample: Periostracum from Mytilus edulis diegensis from Corona del Mar, California.

APPENDIX II

TABLE II-1. Sample Calculation Based on Record Shown in Figure A-1

Species: Mytilus edulis diegensis Size: 5.0 cm.
Location: Corona Del Mar, California

Amino Acid	Base Line (O.D.)	Peak Height (O.D.)	H=Net Height (O.D.)	Half Height on Chart (O.D.)	W=Half Height Width (Dots)	H x W x Volume Factor	Analyzer Constant	Micro-moles (H x W/C)	Resi-dues/ 1000
Lysine	0.005	0.191	0.186	0.098	17.1	3.18 x 4/3	26.6	0.159	16.7
Histidine	0.004	0.138	0.134	0.071	22.1	2.96 x 4/3	24.1	0.164	17.2
Ammonia	0.002	0.155	0.153	0.079	50.5	7.72 x 4/3	23.2	(0.444)*	(46.6)*
Arginine	0.010	0.222	0.212	0.116	41.3	8.77 x 4/3	23.7	0.494	51.3
Aspartic Acid	0.011	0.254	0.243	0.133	25.5	6.20	21.5	0.288	30.2
Threonine	0.010	0.113	0.103	0.062	30.7	3.16	21.5	0.147	15.4
Serine	0.010	0.498	0.488	0.254	30.6	14.91	22.3	0.680	71.0
Glutamic Acid	0.009	0.137	0.128	0.073	38.0	4.86	22.0	0.221	23.2
Proline	0.010	0.042	0.032	0.026	41.5	1.28	5.35	0.238	25.0
Glycine**	0.010	0.732	0.722	0.371	53.8	38.8/102.0	22.1	4.572	480.0
Alanine	0.007	0.111	0.104	0.059	45.5	4.74	22.6	0.210	22.0
Half Cystine	Calculated by summation of O.D.					1.37	12.4	0.110	11.5
Valine	0.008	0.118	0.110	0.063	67.4	7.40	22.1	0.335	35.0
Methionine	0.014	0.052	0.039	0.327	25.0	0.94	22.7	0.040	4.2
Isoleucine	0.012	0.109	0.097	0.061	28.1	2.74	23.5	0.116	12.1
Leucine	0.011	0.126	0.115	0.069	31.4	3.61	23.5	0.154	16.1
Tyrosine	0.012	0.616	0.604	0.313	57.1	34.45	22.7	1.520	160.0
Phenylalanine	0.012	0.048	0.036	0.030	60.5	2.19	22.8	0.095	10.0
								9.544	1000.9

*Not included in total.

**Alternative curve used because of high concentration.

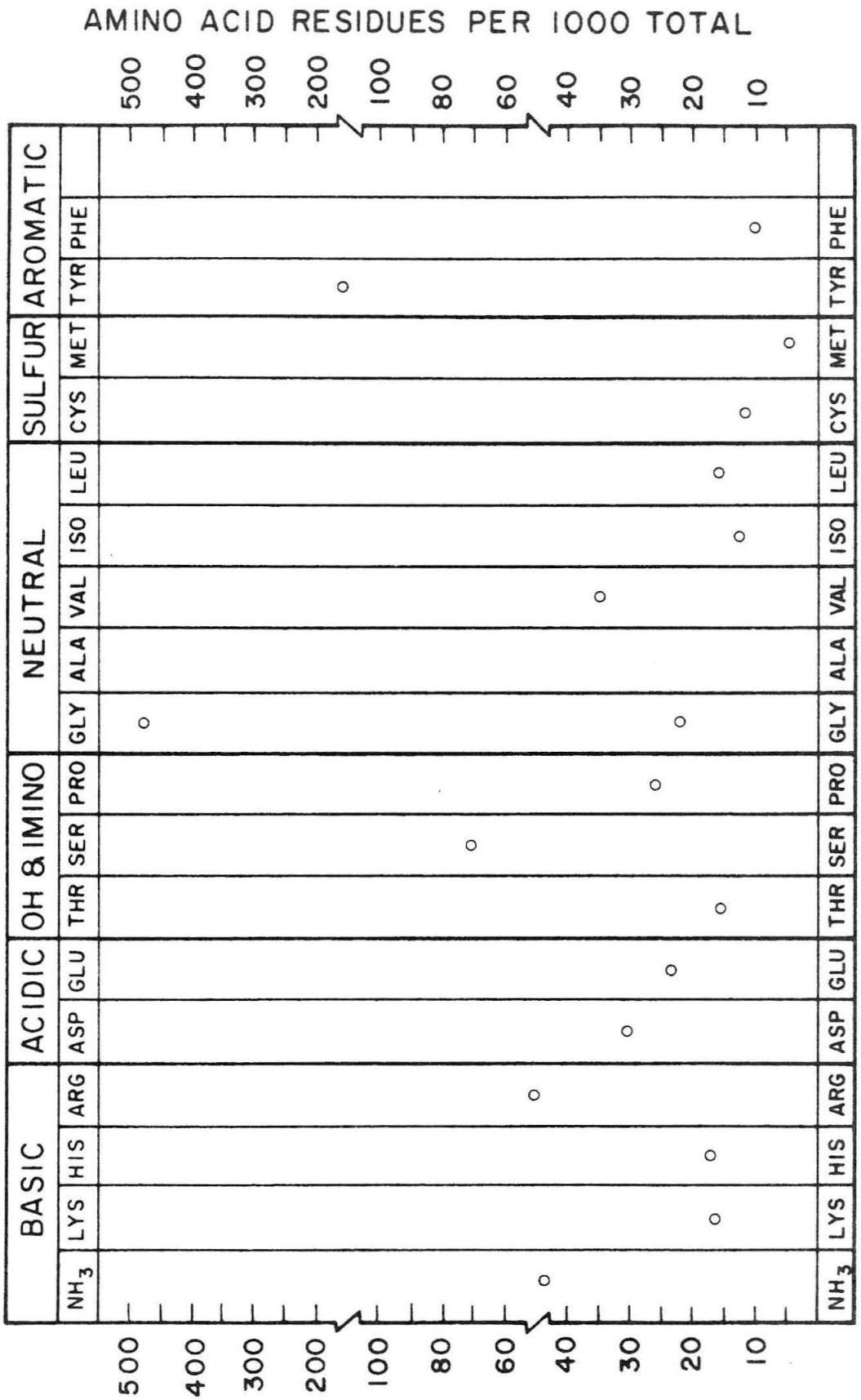


FIGURE II-2. Data from Table II-1 plotted as amino acid residues per 1000 total.
Sample: Periostracum from Mytilus edulis diegensis.
Location: Corona del Mar, California

APPENDIX III

This appendix contains tables of data of amino acid compositions of shell proteins. All amino acid compositions are given in residues per thousand total as discussed in Appendix II-C.

The sum of deviations shown in Table III-10 is calculated by summing for each sample the absolute values of the deviations of the individual amino acid residues from the individual averages.

$$\text{sum of deviations} = \sum_{n=1}^{18} | X_n - \overline{X}_n |$$

where X is each amino acid concentration is residues per thousand, $\overline{X}$ is the average individual amino acid concentration in residues per thousand, n is each amino acid from lysine to phenylalanine.

Temperature data for Tables III-13, 14 come from the data reports, Surface Water Temperatures at Shore Stations, United States West Coast and Baja California, 1955, 1956-1959, and 1960 (SIO References 57-28, 60-27, and 61-14) published by the University of California, Scripps Institution of Oceanography; and from the U. S. Department of Commerce's U. S. Coast and Geodetic Survey Special Publication No. 280, Surface Water Temperatures at Tide Stations, Pacific Coast, North and South America and Pacific Ocean Islands, Fifth Edition (with data through 1955).

TABLE III-1. Distribution of Organic Material in the Various Structural Units of *Mytilus californianus* from Sonoma Coast, California

Structural unit	Byssus fibers	Periostracum	Ligament	Ligamental ridge	Outer prismatic layer	Nacreous layer	Inner prismatic layer
Mineral form	none	none	aragonite	aragonite	calcite	aragonite	calcite
% Insoluble residue after decalcification (crude organic fraction)*	100%	100%	30%	1.5%	1-3%	1%	1%
Micromoles of amino acids/ gram total material	9600	7300-8300	2000	135	80-260	100	67-115

*Variable ash content (5-15%) probably indicative of incomplete decalcification.

Other components present include carbohydrates and trace amounts of lipids. The anthrone-sulfuric acid test for carbohydrates was positive for each fraction indicating up to 2 or 3% carbohydrate in the organic matrices from the prismatic and nacreous layers and up to 5% in the periostracum and ligamental ridge. No detailed analyses of these fractions have been attempted.

APPENDIX III

TABLE III-2. Determination of Reproducibility of Identical Samples

Species: Mytilus californianus Size: 13.2 cm. x 5.3 cm.
Location: Sonoma Coast State Park, California

Position	45-50% L.V.	45-50% R.V.	% Deviation from mean	70-75% L.V.	70-75% R.V.	% Deviation from mean
Lysine	16.2	16.4	.6	17.4	17.3	.3
Histidine	2.0	1.9	2.6	3.2	3.4	3.0
Ammonia	(57.5)	(58.1)	.5	(57.5)	(57.0)	.4
Arginine	25.5	25.3	.4	28.1	28.9	1.4
Aspartic Acid	100.0	99.5	.3	100.0	100.0	-
Threonine	12.4	12.5	.4	14.0	14.4	1.4
Serine	104.5	104.5	-	102.5	102.5	-
Glutamic Acid	23.5	23.4	.2	25.8	26.1	.6
Proline	9.9	9.3	3.1	12.4	12.1	1.6
Glycine	299.0	299.0	-	293.5	293.0	.1
Alanine	276.0	276.0	-	266.5	264.0	.5
Half Cystine	12.5	12.9	1.6	12.1	12.7	2.4
Valine	24.8	24.4	.8	25.3	25.8	1.0
Methionine	4.4	4.6	2.2	5.0	5.3	2.9
Isoleucine	12.3	12.5	.8	13.8	13.9	.4
Leucine	46.6	46.7	.1	46.6	47.0	.4
Tyrosine	15.5	15.6	.3	16.8	17.3	1.5
Phenylalanine	14.6	15.5	3.0	15.9	16.8	2.8
Micromoles/ gram of shell	105.0	101.0		125.0	113.0	

L.V.--left valve R.V.--right valve

TABLE III-3. Determination of Reproducibility on Identical Samples with Different Treatments

Species: Mytilus californianus Size: 13.2 cm. x 5.3 cm.
Location: Sonoma Coast State Park, California

Position	0-5% L.V.*	0-5%[1] R.V.**	% Deviation from mean	60-65% L.V.	60-65%[2] R.V.	% Deviation from mean
Lysine	18.5	18.2	.8	16.6	16.3	.9
Histidine	3.8	3.7	1.3	2.1	2.2	2.3
Ammonia	(59.6)	(60.0)	.3	(57.8)	(57.2)	.5
Arginine	28.4	28.0	1.1	25.6	25.4	.4
Aspartic Acid	101.5	99.0	1.3	98.2	99.0	.4
Threonine	14.7	13.8	3.2	12.2	12.4	.8
Serine	100.0	98.8	.6	103.8	102.8	.5
Glutamic Acid	27.8	26.7	2.0	23.2	23.1	.2
Proline	15.0	14.4	2.0	9.6	10.3	3.5
Glycine	287.0	291.0	.7	301.0	300.0	.2
Alanine	262.0	267.0	1.7	278.0	279.0	.2
Half Cystine	11.8	10.7	5.0	12.2	10.1	9.4
Valine	26.3	25.8	1.0	23.8	23.5	.6
Methionine	5.7	5.7	-	4.8	4.8	1.0
Isoleucine	14.9	14.5	1.4	12.6	12.6	-
Leucine	46.7	46.7	-	46.5	46.6	-
Tyrosine	17.7	18.1	1.1	15.7	15.7	-
Phenylalanine	17.3	17.4	.3	15.1	15.2	.3
Micromoles/ gram of shell	144.0	148.0		74.5	79.0	

*L.V.--left valve **R.V.--right valve [1]Heated by cutting tool. [2]Left in decalcifying solution 30 hours longer.

TABLE III-4. Amino Acid Composition of Proteins from Miscellaneous Structural Units of Shells of Mytilus californianus

Species: Mytilus californianus Size: Various but between 10 and 15 cm.
Location: Sonoma Coast State Park, California

Position	Byssus Fibers	Periostracum 0-10% (?)	Periostracum 25-35%	Periostracum 60-80%	Ligament	Ligamental Ridge
Lysine	41.6	17.7	17.6	21.7	91.6	61.4
Histidine	23.2	19.2	16.5	16.7	1.8	14.3
Ammonia	(83.1)	(39.6)	(40.5)	(41.4)	(35.2)	(100.0)
Arginine	33.7	43.7	50.8	55.2	7.8	48.7
Hydroxyproline	60.0	-	-	-	-	-
Aspartic Acid	54.5	26.2	28.2	26.3	38.6	126.0
Threonine	37.7	6.8	8.4	8.7	23.8	59.2
Serine	48.0	57.6	65.2	69.0	26.8	115.0
Glutamic Acid	51.0	20.3	26.2	25.0	70.9	89.5
Proline	57.9	24.1	23.4	23.7	139.0	37.8
Glycine	326.0	527.0	515.0	508.0	197.0	139.0
Alanine	133.0	6.4	9.0	8.0	15.9	52.5
Half Cystine	9.7	11.2	13.5	14.4	25.3	13.1
Valine	31.2	37.6	34.4	32.1	7.4	55.3
Methionine	4.9	1.6	2.7	3.9	327.5	25.1
Isoleucine	22.0	11.8	12.9	13.1	10.1	48.2
Leucine	26.3	7.6	9.7	8.9	13.7	57.3
Dopa*	0.8	12.5	14.0	5.5	-	-
Tyrosine	22.5	171.0	155.0	155.0	-	26.2
Phenylalanine	17.6	10.3	10.5	10.6	4.8	33.0
Mineral Form	none	none	none	none	aragonite	aragonite
Micromoles/ gram of total material	9600.0	8000.0	7300.0	8300.0	2000.0	135.0

*Dihydroxyphenylalanine identification tentative.

TABLE III-5. Amino Acid Composition of Proteins from Various Parts of the Outer Prismatic Calcite Layer (Sonoma Specimen No. 1)

Species: Mytilus californianus Size: 14 x 5.5 cm.
Location: Sonoma Coast State Park, California

Position	0-4%	8-13%	15-19%	27-30%	30-34%	34-37%	37-40%
Lysine	18.5	18.7	16.8	18.6	18.9	16.7	17.2
Histidine	3.4	3.5	1.9	4.1	3.8	2.3	2.5
Ammonia	(58.6)	(58.0)	(54.5)	-	(59.1)	(56.9)	(56.5)
Arginine	29.8	28.2	26.3	33.6	32.8	27.6	27.3
Aspartic Acid	100.0	98.5	97.6	99.3	99.6	98.6	99.6
Threonine	15.2	15.1	12.8	16.9	16.3	13.2	13.0
Serine	102.5	101.3	103.0	103.1	105.2	104.0	104.2
Glutamic Acid	27.1	26.7	23.4	28.2	26.7	23.7	23.5
Proline	13.3	12.7	10.7	14.8	12.7	11.4	9.5
Glycine	289.0	288.0	297.0	283.0	285.5	298.0	300.0
Alanine	259.0	262.5	276.0	247.0	246.5	271.0	270.0
Half Cystine	12.6	13.9	11.0	14.3	12.3	9.4	11.6
Valine	26.7	27.0	24.6	29.3	29.0	25.6	25.6
Methionine	6.4	6.4	5.4	5.8	5.7	5.1	4.9
Isoleucine	15.7	15.0	13.2	18.1	20.0	13.9	13.7
Leucine	47.6	48.0	47.0	49.2	50.0	48.1	47.3
Tyrosine	17.3	17.0	16.4	18.6	18.8	16.1	16.1
Phenylalanine	17.4	16.9	16.0	16.6	15.9	15.3	14.9
Micromoles/ gram of shell	191.0	81.1	106.0	194.0	-	156.0	-

Position	41-47%	49-53%	57-62%	66-70%	76-87%	96-100%*
Lysine	17.2	17.1	16.6	16.1	17.9	18.7
Histidine	2.5	2.6	1.9	2.2	3.7	5.0
Ammonia	(58.4)	(59.5)	(55.8)	(57.5)	(58.5)	(60.0)
Arginine	27.5	28.4	26.3	25.7	30.6	32.7
Aspartic Acid	99.6	99.5	98.2	98.2	100.1	101.6
Threonine	13.9	14.5	12.7	13.1	14.3	16.4
Serine	104.0	102.0	103.0	101.9	100.0	98.5
Glutamic Acid	25.0	26.0	23.4	24.7	28.1	32.0
Proline	10.5	12.3	10.6	11.2	13.6	16.4
Glycine	294.5	293.0	300.0	297.5	285.0	277.0
Alanine	269.5	269.0	277.0	276.1	260.0	244.6
Half Cystine	11.6	11.7	11.2	10.9	15.1	17.6
Valine	26.1	25.9	23.7	24.9	26.8	28.3
Methionine	4.4	5.3	4.9	5.1	5.8	6.5
Isoleucine	14.7	14.3	13.0	13.6	16.5	18.6
Leucine	48.0	47.8	46.6	46.9	47.6	48.1
Tyrosine	16.4	16.5	16.6	16.6	17.2	18.0
Phenylalanine	15.7	15.9	15.4	15.5	17.9	19.5
Micromoles/ gram of shell	80.0	110.0	84.0	-	191.0	262.0

*Corrected for Periostracum contamination.

TABLE III-6. Amino Acid Composition of Proteins from Various Parts of the Outer Prismatic Calcite Layer (Sonoma Specimen No. 2)

Species: Mytilus californianus Size: 15.2 cm. x 5.7 cm.
Location: Sonoma Coast State Park, California

Position	0-4%	15-20%	30-37%	45-50%	66-70%	79-83%	98-100%
Lysine	18.9	16.5	18.7	16.5	16.3	16.8	21.2
Histidine	4.4	2.3	4.2	2.5	3.0	4.1	5.8
Ammonia	(62.8)	(57.5)	-	(58.0)	(57.5)	-	(61.3)
Arginine	28.6	25.9	30.4	29.0	26.7	29.4	32.5
Aspartic Acid	101.0	98.5	100.8	99.8	98.6	100.6	102.5
Threonine	15.9	13.5	16.7	13.4	13.7	15.4	16.8
Serine	97.5	100.0	100.6	101.0	99.9	100.2	95.0
Glutamic Acid	30.5	23.8	28.1	24.4	24.5	26.2	32.1
Proline	17.4	12.6	14.9	12.7	13.7	15.0	19.3
Glycine	281.0	297.0	285.0	298.0	297.5	291.0	273.0
Alanine	258.0	275.0	254.0	271.0	272.0	258.0	246.0
Half Cystine	14.4	13.2	14.2	9.3	11.1	13.7	14.1
Valine	27.0	24.7	28.1	25.4	24.9	27.2	28.1
Methionine	6.6	5.2	6.2	5.1	5.0	5.7	7.1
Isoleucine	16.3	13.4	16.5	13.6	13.6	15.3	17.7
Leucine	46.6	46.0	47.6	46.8	46.4	47.2	47.4
Tyrosine	18.2	16.2	17.8	16.2	16.8	17.4	20.4
Phenylalanine	18.6	16.2	17.2	15.5	16.1	17.3	21.1
Micromoles/ gram of shell	175.0	87.5	160.0	118.0	80.0	94.0	221.0

TABLE III-7. Amino Acid Composition of Proteins from the Nacreous Aragonite Layer (5 Sonoma Samples)

Species: Mytilus californiamus Size: Various but between 10 cm. and 15 cm.
Location: Sonoma Coast State Park, California

Sample	A	B	C*	D**	E***	Average
Lysine	28.1	26.9	28.0	25.6	25.0	26.7
Histidine	5.5	5.7	6.7	4.6	5.2	5.5
Ammonia	(67.1)	(69.5)	(68.5)	(66.0)	(67.2)	(67.6)
Arginine	30.7	29.5	31.2	32.5	30.4	30.9
Aspartic Acid	100.0	103.2	105.0	99.2	101.5	101.7
Threonine	18.7	20.6	21.1	17.4	18.0	19.2
Serine	107.0	99.2	99.7	100.8	99.0	101.0
Glutamic Acid	26.3	29.1	30.3	28.9	28.6	28.6
Proline	12.9	14.3	15.9	15.1	15.4	14.7
Glycine	266.0	272.0	262.5	274.0	274.0	269.5
Alanine	250.0	242.0	238.0	251.0	249.0	246.0
Half Cystine	16.3	18.3	15.2	16.8	15.9	16.5
Valine	28.3	29.0	32.1	28.8	28.9	29.4
Methionine	7.1	8.6	8.0	7.0	7.2	7.6
Isoleucine	17.6	17.0	16.8	17.3	16.6	17.1
Leucine	50.1	49.0	50.0	50.0	48.7	49.5
Tyrosine	16.9	16.8	16.6	15.6	18.7	16.9
Phenylalanine	19.0	18.7	19.3	18.1	18.6	18.7
Micromoles/ gram of shell	98.6	100.8	100.8	-	108.0	-

*Dissolved in EDTA. **Composite sample from a single specimen. ***Includes fragments from three specimens.

TABLE III-8. Amino Acid Composition of Proteins from Inner Prismatic Calcite Layer (5 Sonoma Samples)

Species: Mytilus californianus Size: Various but between 10 and 15 cm.
Location: Sonoma Coast State Park, California

Sample	A	B	C	D	E	Average
Lysine	22.6	22.5	22.5	23.0	23.0	22.7
Histidine	2.7	2.4	3.0	2.6	2.5	2.6
Ammonia	(64.5)	(58.6)	(61.2)	(59.6)	(59.6)	(60.7)
Arginine	27.5	24.9	27.8	27.2	25.4	26.6
Aspartic Acid	100.0	99.0	100.0	101.0	100.2	100.0
Threonine	15.7	15.3	14.9	15.5	15.6	15.4
Serine	99.2	100.0	100.0	99.6	101.0	100.0
Glutamic Acid	28.1	25.2	26.6	28.8	25.8	26.9
Proline	12.2	12.0	12.7	13.4	12.7	12.6
Glycine	287.0	294.0	288.0	286.0	290.5	289.1
Alanine	265.5	268.0	266.0	263.0	268.0	266.1
Half Cystine	12.4	12.9	13.7	14.2	10.4	12.7
Valine	26.6	22.9	25.0	25.0	24.2	24.7
Methionine	6.0	5.8	5.9	6.0	5.7	5.9
Isoleucine	16.0	14.2	15.5	15.4	14.3	15.1
Leucine	48.6	46.5	46.8	47.5	47.5	47.4
Tyrosine	16.7	16.5	17.2	16.9	17.1	16.9
Phenylalanine	16.7	15.6	16.9	16.8	17.4	16.7
Micromoles/ gram of shell	76.8	68.4	116.5	100.2	67.0	-

TABLE III-9. Effect of Shell Size on Amino Acid Composition of Periostracum

Species: Mytilus californianus
Location: Corona Del Mar, California
Position: Variable but approximately 50-70%

	Transparent Edge				Lightly Pigmented			
Shell length (mm)	20	43	45	147	14-17*	18-21*	32-36*	135-140*
Lysine	16.3	15.8	16.4	22.6	12.3	15.7	14.3	11.8
Histidine	12.5	15.6	16.7	18.4	4.5	10.5	14.3	14.2
Ammonia	(70.1)	(63.5)	(48.0)	(48.0)	(93.0)	(75.4)	(63.4)	(61.8)
Arginine	33.9	44.5	49.5	52.0	31.5	33.9	43.6	48.3
Aspartic Acid	52.9	47.2	38.2	32.7	66.7	54.2	48.4	43.5
Threonine	23.2	17.1	13.5	10.5	22.0	22.9	17.5	11.0
Serine	66.5	61.4	64.4	66.4	67.3	65.1	62.0	60.0
Glutamic Acid	22.4	24.1	24.5	25.6	22.5	22.7	25.0	24.2
Proline	44.6	47.6	33.6	27.6	44.5	45.6	39.4	30.6
Glycine	414.0	447.0	475.0	493.0	424.0	417.0	455.0	514.0
Alanine	27.8	21.9	16.1	10.0	27.7	29.7	22.6	19.0
Half Cystine	20.8	20.2	16.8	18.5	19.2	22.0	14.9	12.8
Valine	61.3	50.4	45.7	34.7	61.6	60.4	51.9	39.8
Methionine	1.8	2.3	2.4	4.3	1.1	2.0	2.3	3.5
Isoleucine	47.0	32.9	26.2	14.6	48.5	46.3	34.3	16.3
Leucine	85.1	44.7	31.2	10.8	90.2	83.2	50.1	12.8
Tyrosine	63.0	99.5	120.5	146.0	48.5	62.1	93.9	128.0
Phenylalanine	7.0	9.15	10.3	10.6	7.2	6.9	9.7	9.7
Micromoles/gram of total material	6400.	6500.	7500.	7900.	6700.	7600.	6800.	7300.

*Estimated size when periostracum was secreted based on growth lines of present shell.

TABLE III-10. The Amino Acid Composition of Proteins from Similar Positions in the Outer Prismatic Calcite Layer of Various Sized Shells from Sonoma

Species: Mytilus californianus
Location: Sonoma Coast State Park, California

Position Shell length (mm)	50-70% 48	60-67% 63	55-65% 80	50-67% 99	56-66% 122	60-65% 131	57-62% 143	66-70% 155	Average
Lysine	18.6	16.3	16.1	17.3	15.8	16.6	16.6	16.3	16.7
Histidine	2.5	3.0	2.6	2.0	2.5	2.1	1.9	3.0	2.5
Ammonia	(57.4)	(58.5)	(60.0)	(58.0)	(57.3)	(57.8)	(55.8)	(57.5)	(57.9)
Arginine	26.6	27.0	26.4	25.4	25.1	25.6	26.3	26.7	26.2
Aspartic Acid	98.0	98.6	99.0	99.0	98.6	98.2	98.2	98.6	98.6
Threonine	12.8	14.5	13.9	13.1	13.8	12.2	12.7	13.7	13.4
Serine	100.8	101.3	99.9	104.8	103.0	103.8	103.0	99.9	102.1
Glutamic Acid	23.6	25.4	25.6	24.1	24.3	23.2	23.4	24.5	24.4
Proline	10.5	12.1	12.1	10.5	11.7	9.6	10.6	13.7	11.4
Glycine	302.5	293.0	295.0	298.0	297.0	301.0	300.0	297.5	298.5
Alanine	272.0	269.0	271.5	275.5	277.0	278.0	277.0	272.0	274.0
Half Cystine	7.7	13.3	12.9	11.7	10.9	12.2	11.2	11.1	11.4
Valine	24.6	25.6	25.7	23.7	24.0	23.8	23.7	24.9	24.6
Methionine	4.9	5.6	5.4	4.6	5.1	4.8	4.9	5.0	5.0
Isoleucine	13.7	14.3	14.0	13.3	13.3	12.6	13.0	13.6	13.5
Leucine	(47.0)	47.6	47.1	47.4	47.2	46.5	46.6	46.4	47.0
Tyrosine	15.5	16.8	17.3	16.0	15.9	15.7	16.6	16.8	16.3
Phenylalanine	14.4	16.6	16.9	15.1	15.9	15.1	15.4	16.1	15.7
Sum of Deviations from Average	19.1	22.7	19.4	14.7	12.0	18.4	14.0	11.9	-
Micromoles/ gram of shell	104.0	100.0	114.0	80.0	86.4	74.5	84.0	80.0	(90.4)

TABLE III-11. Amino Acid Composition of Proteins from the Outer Prismatic Calcite Layer from Different Sizes of Shells
(2 Corona Del Mar specimens)

Species: Mytilus californianus
Location: Corona Del Mar, California

Size of Shell:	20mm	150mm							
Position:	20-70%	0-5%	13-19%	22-27.5%	33-37%	37-46%	48-52%	56-63%	74-78%	84-94%
Lysine	21.0	17.7	19.6	19.2	19.3	20.0	18.5	19.2	17.2	18.3
Histidine	3.2	3.7	3.8	1.8	2.2	1.7	1.7	2.3	2.1	3.2
Ammonia	(57.5)	59.5	(59.4)	57.0	55.2	55.6	54.6	(54.3)	56.1	(55.6)
Arginine	26.9	27.4	29.7	24.8	24.7	24.5	23.9	26.0	25.5	29.5
Aspartic Acid	97.4	99.0	102.6	99.7	97.6	98.5	98.5	101.1	99.5	99.9
Threonine	15.4	14.1	16.4	12.4	11.7	12.2	11.9	13.8	12.8	15.0
Serine	99.4	98.0	99.9	101.1	99.9	97.2	101.2	97.5	100.1	96.5
Glutamic Acid	26.5	26.9	29.2	23.1	22.5	22.3	22.4	25.3	23.6	26.4
Proline	13.1	16.2	14.3	11.6	10.6	10.4	10.8	11.8	11.9	13.9
Glycine	298.1	295.0	284.0	302.5	310.0	310.0	307.0	300.0	302.0	293.0
Alanine	257.0	262.0	255.5	279.0	278.0	279.0	293.0	272.0	279.0	262.0
Half Cystine	10.3	9.7	14.5	6.9	7.2	5.5	6.3	9.1	8.2	14.7
Valine	26.6	26.0	27.5	23.0	23.4	22.8	23.0	24.5	23.2	26.0
Methionine	5.1	5.6	5.9	4.8	4.5	4.4	4.5	4.8	5.0	5.8
Isoleucine	15.7	15.4	16.3	13.0	12.5	12.9	12.4	13.7	13.2	15.2
Leucine	49.0	46.6	47.3	46.2	45.3	46.0	46.0	46.3	46.1	46.3
Tyrosine	20.0	19.2	18.4	16.5	18.4	16.8	16.1	18.4	16.6	18.4
Phenylalanine	15.7	17.7	17.1	14.6	14.4	14.3	13.5	15.2	15.1	16.6
Micromoles/ gram of shell	86.2	159.0	189.0	101.8	104.6	105.0	90.1	94.5	81.6	163.0

TABLE III-12. Amino Acid Composition of Outside Calcite Proteins from Successive Increments from Posterior Edge Toward Beak

Species: Mytilus californianus — Collection Date: May, 1961
Location: Corona Del Mar, California — Size: 150mm

Distance from posterior edge in mm:	0-2	5-8	15-18	25-28	35-38	40-45	60-65	80-85
Lysine	20.0	18.9	17.4	16.8	16.3	16.7	16.9	16.1
Histidine	1.7	2.3	1.6	1.6	1.8	2.2	1.9	2.0
Ammonia	55.6	58.5	56.4	53.0	54.5	55.5	55.0	57.2
Arginine	24.5	24.8	23.3	24.1	24.3	26.9	24.3	24.5
Aspartic Acid	98.5	103.1	98.9	97.9	98.5	99.5	98.1	100.4
Threonine	12.2	13.2	12.1	11.9	12.2	14.0	12.3	13.0
Serine	97.2	101.2	102.0	101.0	100.5	96.0	99.5	99.5
Glutamic Acid	22.3	24.4	23.1	21.9	22.8	24.0	23.1	24.6
Proline	10.4	11.0	11.0	11.6	11.6	14.0	11.0	13.7
Glycine	310.0	300.0	307.0	311.0	307.0	302.0	308.0	303.0
Alanine	279.0	274.0	281.0	278.5	280.0	272.0	279.0	279.0
Half Cystine	5.5	7.1	6.8	7.5	6.7	6.9	6.4	6.0
Valine	22.8	24.2	23.7	22.6	23.2	25.4	23.0	23.4
Methionine	4.4	4.5	4.3	4.9	4.9	5.1	4.7	5.2
Isoleucine	12.9	13.4	12.5	12.8	12.9	15.3	12.8	13.0
Leucine	46.0	46.0	46.3	46.0	46.8	47.1	47.2	45.5
Tyrosine	16.8	18.1	16.6	15.9	16.0	17.5	17.3	15.9
Phenylalanine	14.3	14.8	14.8	14.7	14.4	15.5	14.4	13.9
Micromoles/ gram of shell	105.0	133.0	106.0	109.0	100.0	118.0	108.0	-

TABLE III-13. Amino Acid Composition of Outer Calcite Protein from Various Ecological Environments: Effect of Changing Temperature

Species: Mytilus californianus					
Date Collected:	September, 1955		May, 1961	February, 1961	
Approximate Temperature at Collecting Time:	15°C	25°C	15-16°C	12°C	
Position in Shell:	50-60	50-60	56-63	50-60	
Location:	Tres Hermanas, Baja California		Corona Del Mar, California (From Table III-11)	Sonoma Coast, California (Ave. taken from Table III-10)	
	South	North			
Latitude:	31°42'N	31°44'N	33°36'N	38°23'N	Range
Lysine	16.9	18.7	19.2	16.7	(15.8-18.6)
Histidine	2.1	2.0	2.3	2.5	(1.9-3.0)
Ammonia	57.4	58.0	54.3	57.9	(55.8-60.0)
Arginine	26.0	26.4	26.0	26.2	(25.1-27.0)
Aspartic Acid	97.5	96.8	101.1	98.6	(98.6-99.0)
Threonine	12.4	13.8	13.8	13.4	(12.2-14.5)
Serine	100.6	100.0	97.5	102.1	(99.9-104.8)
Glutamic Acid	23.0	24.0	25.3	24.4	(23.2-25.6)
Proline	11.5	12.1	11.8	11.4	(9.6-13.7)
Glycine	302.0	297.0	300.0	298.5	(293.0-302.5)
Alanine	279.0	273.0	272.0	274.0	(271.5-277.0)
Half Cystine	8.5	9.5	9.1	11.4	(7.7-13.3)
Valine	23.6	25.6	24.5	24.6	(23.7-25.7)

Methionine	5.0	5.7	4.8	5.0	(4.6-5.4)
Isoleucine	12.6	14.0	13.7	13.5	(12.6-14.3)
Leucine	46.5	47.6	46.3	47.0	(46.4-47.6)
Tyrosine	16.0	16.2	18.4	16.3	(15.6-17.3)
Phenylalanine	15.4	17.5	15.2	15.7	(14.5-16.9)
Micromoles/gram of shell	124	67	94.5	90.4	(74.5-114.0)

TABLE III-14. Amino Acid Composition of Aragonite Protein from Various Ecological Environments
Effect of Changing Temperature

Species: Mytilus californianus					
Date Collected:	Sept. '55/Sept. '55		May, 1961	February, 1961	
Mean Temperature:	14-15°C	19-20°C	16-17°C	11-12°C	
Location:	Tres Hermanas, Baja California		Corona Del Mar California	Sonoma Coast, California	
	South	North		Ave.	
Latitude:	31°42'N	31°44'N	33°36'N	38°23'N	Range
Lysine	26.2	23.6	20.3	26.7	(25.0-28.1)
Histidine	4.5	3.7	3.5	5.5	(4.6-6.7)
Ammonia	62.8	60.1	60.6	67.6	(66.0-69.5)
Arginine	31.2	28.0	29.9	30.9	(29.5-32.5)
Aspartic Acid	99.0	98.4	99.3	101.7	(99.2-105.0)
Threonine	18.0	17.1	14.6	19.2	(17.4-21.1)
Serine	102.4	101.2	101.5	101.0	(99.0-107.0)
Glutamic Acid	28.6	26.8	24.6	28.6	(26.3-30.3)
Proline	15.9	15.2	13.3	14.7	(12.9-15.9)
Glycine	272.0	282.5	289.0	269.5	(262.5-274.0)
Alanine	251.0	251.0	259.0	246.0	(238.0-251.0)
Half Cystine	14.3	12.6	13.5	16.5	(15.2-18.3)
Valine	28.6	28.6	27.0	29.4	(28.3-32.1)

Methionine	6.8	7.3	6.6	7.6	(7.0-8.6)
Isoleucine	17.8	16.7	15.4	17.1	(16.6-17.6)
Leucine	49.4	49.7	46.7	49.5	(48.7-50.1)
Tyrosine	17.3	18.5	17.2	16.9	(15.6-18.7)
Phenylalanine	19.1	19.6	18.3	18.7	(18.1-19.3)
Net Acidics (A) or Basics (B)	3.0A	9.8A	9.6A	0.4B	(5B-2.3A)

TABLE III-15. Amino Acid Composition of Aragonite and Calcite Proteins from Various Ecological Environments: Effect of Changing Salinity

Species:	Mytilus californianus		Mytilus edulis diegensis					
Location:	Corona Del Mar, California		Corona Del Mar, California		Cliff House, California (San Francisco)		Glen Cove, California	
Approximate Salinity:	34 °/oo		34 °/oo		34 °/oo		20 °/oo	
Date Collected:	May, 1961		April, 1961		June, 1961		June, 1961	
Layer:	ARAG.	CAL.	ARAG.	CAL.	ARAG.	CAL.	ARAG.	CAL.
Lysine	20.3	19.2	26.2	16.0	28.1	15.8	18.4	14.8
Histidine	3.5	2.3	4.9	1.6	4.0	1.2	3.2	1.5
Ammonia	(60.6)	(54.3)	(69.0)	(56.2)	(75.9)	(65.1)	(72.0)	(67.0)
Arginine	29.9	26.0	27.0	25.4	30.2	25.7	29.0	24.2
Aspartic Acid	99.3	101.1	96.3	90.4	98.9	96.5	96.6	96.6
Threonine	14.6	13.8	23.3	10.8	20.9	11.1	16.2	13.0
Serine	101.5	97.5	103.8	106.0	104.0	109.0	105.3	99.5
Glutamic Acid	24.6	25.3	41.5	33.4	46.5	34.4	40.8	35.0
Proline	13.3	11.8	17.2	9.9	20.2	9.4	16.2	13.7
Glycine	289.0	300.0	263.0	298.0	260.5	289.5	275.0	306.0
Alanine	259.0	272.0	236.0	271.0	234.0	273.0	252.0	256.0
Half Cystine	13.5	9.1	10.2	7.8	7.1	6.7	8.6	8.3
Valine	27.0	24.5	30.2	25.3	28.4	24.2	26.5	27.0

Methionine	6.6	4.8	8.9	4.5	9.5	5.0	8.5	6.1
Isoleucine	15.4	13.7	20.6	13.5	18.5	11.8	15.0	12.7
Leucine	46.7	46.3	55.5	51.7	50.4	47.1	49.9	46.6
Tyrosine	17.2	18.4	16.8	19.0	16.7	18.3	16.4	19.0
Phenylalanine	18.3	15.2	19.3	14.7	22.8	18.1	23.3	19.7
Net Acidics	9.6A	24.6A	10.7A	24.6A	7.2A	23.1A	14.8A	24.1A

TABLE III-16. Composition of Organic Matrix Proteins from Radiocarbon-Dated Shell Material

Sample No. C-14 Age	LJ-33C* 400(±200)	LJ-84C* 1060(±150)	LJ-111C* 1500(±100)	LJ-78C* 3460(±200)	LJ-110C* 5460(±100)	LJ-33A* 400(±200)	LJ-111A* 1500(±100)	LJ-110A* 5460(±100)
Lysine	12.1	9.0**	51.7	8.1	9.2	27.3	59.1	21.1
Histidine	1.3	0.66	4.8	0.53	0.63	5.3	17.9	1.6
Ammonia	(49.8)	(53.5)	(235.0)	(35.7)	(30.6)	(65.5)	(193.0)	(51.3)
Arginine	25.4	19.8	21.1	17.3	18.7	30.6	7.1	18.8
Aspartic Acid	80.0	75.0	199.0	37.9	37.5	97.5	207.0	76.0
Threonine	11.2	10.8	39.5	5.1	5.8	30.2	51.6	13.6
Serine	80.1	76.0	74.1	63.0	61.3	79.5	51.7	58.5
Glutamic Acid	22.5	23.8	81.4	16.9	19.2	41.1	70.1	32.0
Proline	10.9	12.0	41.4	5.6	7.6	18.8	33.6	13.1
Glycine	302.0	282.0	217.0	192.0	159.0	231.0	143.5	144.0
Alanine	319.5	360.0	101.0	567.0	581.0	268.0	134.5	492.0
Half Cystine[1]	7.7	3.2	15.0	2.8	1.2	5.5	11.8	6.1
Valine	25.1	24.8	30.1	19.0	23.1	38.0	48.6	26.4
Methionine[1]	5.31	4.6	11.3	4.1	5.0	10.7	12.9	7.8
Isoleucine	13.7	20.0	24.7	11.7	14.5	27.4	39.1	24.1
Leucine	51.4	50.6	45.0	35.4	40.7	56.2	57.1	48.0
Tyrosine	14.4	11.1	20.2	4.8	5.2	10.8	25.5	5.0
Phenylalanine	16.4	15.4	23.4	8.6	11.0	22.2	29.8	14.4
Micromoles/ gram of shell	59.8	43.4	-	-	11.6	-	-	3.8
Meth. Sulfone/ Methionine	0.090	0.198	0.072	0.62	0.87	0.165	0.093	0.53

*Letter following Sample No. designates: C-calcite, A-aragonite.

**Basic amino acids from a 2nd sample.

[1] Includes oxidation products.